UNIVERSIDAD AUTÓNOMA DE MADRID

FACULTAD DE MEDICINA

Tesis doctoral

La anisocitosis eritrocitaria como marcador pronóstico de la sepsis y de la infección grave por COVID-19

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A Valentín y a Carmen, por la oportunidad

A Juan Antonio, Raquel, Miguel, Susana, Pablo y Pedro, por la acogida

A Guillermo y a los chicos de Cruces, por hacerme sentir como uno más del equipo

A Antonio, por su constante apoyo y preocupación

A Elena, por hacer que esto haya sido un poco más divertido

A Ana, por su eterna paciencia y profesionalidad

A Alejandro, por la excelencia y el cariño

A Lara, por su valentía

A Zaira, Yago e Irene, por la ilusión

A Paqui y a Marvin, por la adopción

A Adrián, Cristina y Dani, por el ejemplo

A Pablo, por enseñarme lo que de verdad supone ser un hombre

A mi madre, por la ayuda en todos y cada uno de los pasos que me han traído hasta aquí

A Bimba, coautora de este trabajo

A Patricia, sin la que todo esto hubiese sido absolutamente imposible y con la que siempre estaré en deuda.

Y a los que vendrán…

ÍNDICE

Abreviaturas

ADE: Ancho de distribución eritrocitaria.

ADM: Adrenomedulina.

AP: Actividad de Protrombina.

APACHE-II: Acute Physiology and Chronic Health Evaluation-II.

AST: Aspartato amino-transferasa

AUC-ROC: Área bajo la curva operativa característica del receptor.

BAC: Bacteriemia asociada a catéter.

BLEE: Betalactamasas de espectro extendido.

BUN: Nitrógeno ureico en sangre.

CHCM: Concentración hemoglobina corpuscular media.

CPAP: Presión positiva continua.

CPE: Células progenitoras endoteliales.

DD: D-Dímero

DE: Desviación estándar.

DTR: Organismo con resistencia de difícil tratamiento.

EEG: Ecuación de estimación generalizada.

FC: Frecuencia cardíaca.

FR: Frecuencia respiratoria.

FiO2: Fracción inspirada de oxígeno.

GA-a O2: Gradiente Alveolo-arterial de oxígeno.

GCS: Escala de coma de Glasgow.

GN: Gafas nasales.

GNAF: Gafas nasales de alto flujo.

HT: Hipertensión.

ICC: Índice de comorbilidad de Charlson.

IL-1: Interleukina 1.

IL-1b: Interleukina 1b.

IL-2: Interleukina 2.

IL-6: Interleukina 6.

IL-7: Interleukina 7.

IL-10: Interleukina 10.

IL-33: Interleukina 33.

INR: Índice internacional normalizado.

IPAP: Presión positiva fase inspiratoria.

JAK: Kinasa Janus.

LDH: Lactato dehidrogenasa.

MDR: Organismo multi-resistente.

MR-Pro-ADM: Región medial de la Pro-adrenomedulina.

NEWS2: National Early Warning Score 2.

LODS. Logistic Organ Dysfunction System.

LPS-LPB: complejo lipopolisacárido-proteína ligadora de lipopolisacáridos.

PAFI. Ratio Presión arterial de oxígeno-Fracción inspirada de oxígeno.

PaO2: Presión arterial de oxígeno.

PaCO2: Presión arterial de dióxido de carbono.

PDFG: Factor de crecimiento derivado de plaquetas.

PCR: Proteína C reactiva.

PCT: Procalcitonina.

PyPB: Piel y partes blandas.

qSOFA: quick-SOFA.

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

SAFI: Ratio saturación periférica de oxígeno-Fracción inspirada de oxígeno.

SAMR: Staphylococcus aureus meticilin-resistente

SAMS: Staphylococcus aureus meticilin-sensible.

SAPS-II. Simplified Acute Physiology Score-II.

SatO2: Saturación de oxígeno (pulsioximetría).

sCD14-ST: subtipo del marcador soluble CD14.

SDMO: Síndrome de Disfunción Multiorgánica.

SDRA: Síndrome de Distrés Respiratorio Agudo.

SIDA: Síndrome de inmunodeficiencia adquirida.

SIRS: síndrome de respuesta inflamatoria sistémica.

SOFA: Sequential Organ Failure Assessment.

TAS: Tensión arterial sistólica.

TAD: Tensión arterial diastólica.

TAM: Tensión arterial media.

TNF: Factor de necrosis tumoral.

TTPA: Tiempo de tromboplastina parcial activado.

UCI: Unidad de Cuidados Intensivos.

UCQP: Unidad de Cuidados Críticos Postquirúrgicos.

VCM: Volumen corpuscular medio.

VEGF: Factor de crecimiento vascular endotelial.

VIH: Virus de la inmunodeficiencia humana.

VM: Ventilación mecánica.

VMI: Ventilación mecánica invasiva.

VMNI: Ventilación mecánica no invasiva.

1. RESUMEN

1.1. INTRODUCCIÓN Y OBJETIVOS

La sepsis se define como una disfunción orgánica que puede comprometer la vida y que está causada por una respuesta alterada de un huésped a una infección. A su vez, el desarrollo de complicaciones en la infección por el SARS-CoV-2 ("severe acute respiratory syndrome coronavirus"), entre las que se incluye el Síndrome de Distrés Respiratorio Agudo (SDRA), se deben a un estado hiperinflamatorio tras la fase replicativa del virus que se conoce como la "tormenta de citoquinas". La fisiopatología de ambos procesos puede mostrar ciertas homologías, ya que es la respuesta inmunitaria exagerada y no controlada, tras un estímulo como la infección, la que condiciona el daño orgánico a diversos niveles y las elevadas tasas de mortalidad que pueden conllevar ambas patologías.

El ancho de distribución eritrocitaria (ADE) es un parámetro que se obtiene de forma rutinaria con un hemograma. En los últimos años, se ha estudiado como un marcador asociado a la inflamación y como un factor pronóstico de gravedad y mortalidad en escenarios clínicos muy diversos, incluyendo enfermedades de base inmunitaria, el SDRA, la infección por COVID-19 y la sepsis. Por consiguiente, el ADE sería un parámetro fácilmente disponible y accesible que podría ayudar a determinar la intensidad de la respuesta inflamatoria, independientemente de la causa, y tener por lo tanto un papel pronóstico en la sepsis y en la infección grave por COVID-19.

De acuerdo con estas premisas, el objetivo del presente estudio fue evaluar la capacidad del ADE como marcador de gravedad y mortalidad, tanto en pacientes ingresados en la UCI por sepsis como en pacientes con infección grave por COVID-19.

1. 2. MATERIALES Y MÉTODOS

El estudio se diseñó como un análisis observacional y retrospectivo de tres grupos poblacionales, con tres objetivos distintos. Las tres poblaciones proceden del Hospital Puerta de Hierro Majadahonda y el estudio fue aprobado por el Comité Ético de Investigación Clínica de dicha institución.

En primer lugar, se analizó la capacidad predictiva de mortalidad del ADE en la sepsis en los pacientes que ingresaron por este motivo en la unidad de cuidados intensivos (UCI) entre 2018 y 2019. Los pacientes se incluyeron si ingresaban en la UCI por sepsis o si cumplían los criterios diagnósticos de sepsis al ingreso. Se analizó la capacidad discriminatoria de mortalidad de las distintas escalas, así

como la capacidad pronóstica de los biomarcadores, proteína C reactiva (PCR), Procalcitonina (PCT) y el ADE. Posteriormente, se analizó el valor pronóstico añadido que supone el ADE sobre las mencionadas escalas, mediante la comparación del área bajo la curva operativa característica del receptor (AUC-ROC).

En segundo lugar, se analizó el rol pronóstico del ADE y su correlación con otros marcadores inflamatorios en pacientes con neumonía grave por COVID-19. En este grupo se estudiaron los pacientes del estudio TOCICOV que se habían reclutado en nuestro centro y se evaluaron la dinámica, la asociación con la mortalidad, y la capacidad predictiva de mortalidad de los marcadores inflamatorios. Posteriormente, se consideró el posible efecto del bloqueo del receptor de la Interleukina 6 (IL-6) en estos parámetros y se estudió la correlación entre los distintos marcadores inflamatorios.

En tercer lugar, se analizó la prevalencia, los factores de riesgo de infecciones bacterianas y su impacto en la mortalidad, en los pacientes que ingresaron en la planta de hospitalización por neumonía por COVID-19, confirmada o sospechada, durante la primera ola de la pandemia, independientemente de su gravedad. Este último análisis se realizó con el objetivo de dilucidar si la presencia de infecciones bacterianas podía ser un potencial factor de confusión de la capacidad pronóstica del ADE en los pacientes con neumonía por COVID-19. De acuerdo con las variables estudiadas en el análisis univariante, se realizaron dos análisis multivariantes mediante regresión logística binaria para identificar los factores de riesgo de desarrollo de infecciones bacterianas y para identificar los factores relacionados con la mortalidad, respectivamente.

1.3. RESULTADOS

1.3.1. Pacientes ingresados en la UCI por sepsis

1.3.1.1. Características de la población y del ingreso en UCI

En el periodo de estudio, 203 pacientes adultos ingresaron en la UCI por sepsis. El foco respiratorio fue el más frecuente (38,9%), seguido del foco abdominal (36%). La mortalidad global fue del 31,6%, en un 81,3% de los casos debido a la sepsis. El análisis univariante de la mortalidad con las variables epidemiológicas, comorbilidades, datos clínicos y de laboratorio (**Tablas 1-7**), demostró asociación estadísticamente significativa, entre otras, con el Índice de comorbilidad de Charlson (ICC) y la hemoglobina, que fueron las que se incluyeron posteriormente en el análisis multivariante.

1.3.1.2. Escalas diagnósticas y pronósticas.

La capacidad de discriminación de mortalidad de las distintas escalas se comparó en función de su aplicación, diseño y según las variables que se utilicen para su cálculo: la escala National Early Warning Score 2 (NEWS2) presentó mejor capacidad discriminatoria que el quick-SOFA (qSOFA), (AUC-ROC=0,615 vs 0,536, p=0,039) así como la escala Sequential Organ Failure Assessment (SOFA) en comparación con la escala Logistic Organ Dysfunction System (LODS) y el Síndrome de Respuesta Inflamatoria Sistémica (SIRS), (AUC-ROC= 0,776 vs 0,693 vs 0,521, respectivamente, p<0,01), (**Tablas 8 y 9, Figuras 1-2**). A pesar de que la escala Simplified Acute Physiology Score-II (SAPS-II) mostró mayor AUC-ROC que la escala Acute Physiology and Chronic Health Evaluation-II (APACHE-II), (AUROC=0,738 vs AUROC=0,673), esta diferencia no alcanzó la significación estadística (p=0,08), (**Figura 3**).

1.3.1.3. Biomarcadores

El análisis longitudinal no observó una asociación de la mortalidad con los valores de la PCR (OR=1, IC 95% 0,99-1,00. p=0,957) y la PCT (OR=1,01, IC 95% 0,99-1,02, p=0,130) durante la primera semana de ingreso (**Tablas 10 y 11, Figuras 4-7**). A su vez, los mayores valores promedio de ADE se dieron a las 48 horas (media 16,09%) y 72 horas (media 16,02%) del ingreso (**Tabla 11, Figura 8**). Los pacientes fallecidos presentaron mayores valores de ADE al ingreso (16,95% vs 15,06%, p<0,001, AUC-ROC=0,737), a las 24 horas (17,15% vs 15,35%, p<0,001, AUC-ROC=0,737), a las 48 horas (17,54% vs 15,49%, p<0,001, AUC-ROC=0,750), a las 72 horas (17,55% vs 15,48%, p<0,001, AUC-ROC=0,747) y a los 7 días del ingreso en UCI (17,33% vs 15,40%, p<0,001, AUC-ROC=0,740), (**Figura 9-10**). El análisis longitudinal confirmó que, durante la primera semana de ingreso, el ADE se asociaba con un mayor riesgo de mortalidad (OR=1,05, 95% CI 1,01-1,10, p=0,048) (**Figura 11**).

El análisis multivariante de mortalidad para cada valor del ADE, considerando además el índice de comorbilidad de Charlson, la inmunosupresión, la infección nosocomial, la hemoglobina y las escalas NEWS2, SOFA y SAPS-II, mostró que solo la escala SOFA y el ADE al ingreso, 24 horas, 48 horas, 72 horas y sus valores máximos se asociaban estadísticamente y de forma independiente con la mortalidad (**Tabla 12**). El AUC-ROC de todos estos modelos estuvo por encima de 0,700 (0,827 considerando el ADE al ingreso, 0,821 considerando el ADE a las 24 horas, 0,826 considerando el ADE a las 48 horas, 0,831 considerando el ADE a las 72 horas, 0,812 considerando el ADE a los 7 días y 0,812 considerando el ADE máximo), (**Tabla 13, Figura 12**). Añadidos a las escalas pronósticas, el ADE a las 24 horas y sobre todo el ADE al ingreso, mejoraron la capacidad discriminatoria del SOFA (AUC-ROC= 0,772 vs 0,812 con ADE al ingreso, p=0,041), LODS (AUC-ROC=0,687 vs 0,710, p=0,002), SAPS-II (AUC-ROC=0,734 vs 0,785, p=0,021) y APACHE-II (AUC-ROC=0,672 vs 0,755, p=0,003) (**Tabla 14, Figura 14**).

El modelo resultante de la combinación de la escala SOFA con el ADE al ingreso mostró la mejor capacidad discriminatoria de mortalidad (AUC-ROC=0,812).

1.4.2. Pacientes ingresados por neumonía grave por COVID-19

1.4.2.1. Características de la población y del ingreso hospitalario

Se incluyeron 173 pacientes con neumonía grave por COVID-19 (**Tablas 15 y 16**). La mortalidad global fue del 20,8%, y en un 8,7% se identificaron complicaciones infecciosas. Un 62,4% de los pacientes recibió tocilizumab; el 87,2% en el día de inclusión de estudio (día 0), coincidiendo con el deterioro respiratorio y/o analítico.

1.4.2.2. Dinámica de los parámetros inflamatorios y su relación con la mortalidad.

En comparación con los pacientes supervivientes, los pacientes fallecidos mostraron mayores valores de la PCR en el ingreso (136 vs 105 mg/L, p=0,002, AUC-ROC= 0.625), el día 1 (176 vs 128 mg/L, p<0,001, AUC-ROC=0,662) y el día 7 (55 vs 23 mg/L, p=0,01, AUC-ROC=0,633), así como mayores valores de IL-6 el día 1 (454 vs 243 pg/ml, p=0,046, AUC-ROC=0,681) y ferritina el día 3 (1934 vs 1444 ng/ml, p=0,029, AUC-ROC=0,540), (**Tablas 17 y 18, Figuras 14-17**). Además, los pacientes fallecidos presentaron mayor ADE en el momento del ingreso (14,35% vs 13,52%, p=0,0016, AUC-ROC=0,668), en el día 0 (14,42% vs 13,60%, p=0,026, AUC-ROC =0,680), en el día 3 (14,35% vs 13,43%, p<0,001, AUC-ROC=0,695) y en el día 7 tras la inclusión (14,31 vs 13,41%, p=0,046, AUC-ROC= 0,666), (**Figura 18**).

1.4.2.3. Dinámica de los parámetros según el tratamiento con tocilizumab

El valor de los parámetros se estudió en función del tratamiento con tocilizumab (**Figuras 19-24**). La capacidad pronóstica de los parámetros que habían demostrado asociación con la mortalidad se evaluó mediante un análisis multivariante considerando las cifras de hemoglobina y el tratamiento con tocilizumab, ya que estos pacientes podrían presentar un perfil clínico distinto. Tanto el ADE al ingreso (OR=1,23, IC 95% 1,01-1,49, p=0.041), en el día 0 (OR=1,22, 95% CI 1-1,49, p=0,05), y en el día 3 (OR=1,25, 95% CI 1,01-1,56, p=0.047), como la PCR en el día 0 (OR=1,01, 95% CI 1-1,01, p=0,043) y en el día 1 (OR=1,01 95% CI 1,01-1,02, p=0.001) fueron factores que mostraron capacidad pronóstica de mortalidad, independiente al bloqueo de la IL-6.

1.4.2.4. Correlación entre los marcadores inflamatorios

La IL-6 mostró correlación con ciertas determinaciones de la PCR y la ferritina durante el periodo de estudio, mientras que los valores ADE y la PCR presentaron correlación durante los 7 días

tras la inclusión (**Tabla 19**). No se demostró significación entre los valores de IL-6 y ADE en la cohorte global. Sin embargo, en los pacientes que no recibieron tocilizumab, los valores de IL-6 en el momento de la inclusión (día 0) mostraron una fuerte correlación con el ADE del día 3 (r=0.733, p=0.004) y la PCR del día 3 (r=0,727, p=0,022), (**Figura 25**)**.** En este grupo de pacientes, las cifras de ADE y PCR el día 3 también mostraron una correlación significativa (r=0,358, p=0,005), (**Figura 26**).

1.4.3. Pacientes ingresados por neumonía por COVID-19

1.4.3.1. Características de la población y de las infecciones bacterianas

Durante la primera ola de la pandemia, ingresaron un total de 1.594 pacientes por neumonía por COVID-19, confirmada o sospechada (**Tablas 20 y 21**). En el total, se identificaron 156 infecciones bacterianas en 135 pacientes (8,5% de la población total), con aislamiento microbiológico en un 91,9% de los casos (**Tablas 22-24**). El foco más frecuente fue el urinario (31,6%), seguido de la bacteriemia primaria (31,9%) y de las infecciones pulmonares (31,8%). En el 54,1% de los pacientes se aislaron cocos gram-positivos y en un 29,6% enterobacterias. La mortalidad fue mayor en los pacientes que presentaron complicaciones infecciosas (25,2% vs 14,2%, p<0,001), (**Tablas 25-26**).

1.4.3.2. Factores de riesgo desarrollo de infección bacteriana

En el análisis multivariante, los factores relacionados de forma independiente con las complicaciones infecciosas fueron la edad (OR=1,69, IC 95% 1,01-1,04), la enfermedad neurológica (OR= 1,69, IC 95% 1,01-2,82), la inmunosupresión anterior al ingreso (OR=4,41, IC 95% 2,76-7,06) y el ingreso en UCI (OR=21,36, IC 95% 13,21-34,55), (**Tabla 27**). El tratamiento esteroideo, el tratamiento con tocilizumab o la combinación de ambos no supuso un mayor riesgo de infecciones bacterianas.

1.4.3.3. Análisis multivariante de mortalidad

En los pacientes ingresados por neumonía por COVID-19, la mortalidad vino determinada por las comorbilidades; incluyendo la edad (OR=1,13, IC 95% 1,10-1,16), la enfermedad neurológica (OR=2,77, IC 95% 1,77-4,34), la enfermedad renal (OR=3,46, IC 95% 1,92-6,24) o la inmunosupresión previa (OR= 3,33, IC 95% 1,91-5,82), además de la presencia y la gravedad del SDRA: SDRA leve (OR=4,67, IC 95% 1,50-14,54), SDRA moderado (OR=93,88, IC 95% 29,27-301,08) y SDRA grave (OR=282,10, IC 95% 79,18-1005,33) (**Figura 26**). Las infecciones bacterianas no se asociaron con la mortalidad tras el ajuste (OR=0,85, IC 95% 0,47-1,53), mientras que el tratamiento con esteroides (OR=0,35, IC 95% 0,20-0,60) y el tratamiento combinado de esteroides y tocilizumab (OR=0,56, IC 95% 0,34-0,93) mostraron un efecto protector.

2.INTRODUCCIÓN

2.1. SEPSIS

La sepsis, definida como la disfunción orgánica secundaria a una respuesta inmune de un huésped a la infección, supone una elevada causa de morbi-mortalidad en todo el mundo. En los últimos años ha habido un importante esfuerzo de la comunidad científica que ha supuesto cambios y avances en la terminología, epidemiología, diagnóstico y tratamiento de este síndrome. En este sentido, se ha producido una mejor caracterización e identificación de los factores pronósticos, a la vez que se han puesto en marcha grupos de trabajo que han diseñado guías y protocolos de manejo específicos. Sin embargo, y a pesar de los evidentes progresos en este ámbito, sigue habiendo importantes áreas de incertidumbre y en ciertos escenarios se sigue produciendo un retraso en el diagnóstico y un manejo subóptimo de esta patología de elevada mortalidad.

2.1.1. Definición e implicaciones

La sepsis es un síndrome clínico cuya extensa fisiopatología y variabilidad clínica hace que su definición sea extremadamente compleja. En 2016 se publicó la tercera definición de consenso de la sepsis y del shock séptico (SEPSIS-3) que define la sepsis como una disfunción orgánica que puede comprometer la vida y que está causada por una respuesta alterada de un huésped a una infección [1]. Este documento y definición han supuesto cambios terminológicos y conceptuales muy relevantes que merecen mención. Por un lado, se eliminaron los términos previos del síndrome de respuesta inflamatoria sistémica (SIRS), sepsis grave, hipotensión secundaria a sepsis o síndrome de disfunción multi-orgánica (SDMO) [2-5]. Por consiguiente, conceptos como SIRS o SDMO no tienen por qué estar ligados a la sepsis o a la infección, y por lo tanto se han considerado términos poco sensibles y sobre todo inespecíficos. Además, se asume que la expresión de sepsis grave e hipotensión secundaria a la sepsis son conceptos intrínsecos e inherentes a la definición de sepsis y shock séptico, respectivamente, y que por lo tanto resultan innecesarios. Por otro lado, la definición actual de sepsis enfatiza el papel y los conceptos de lesión orgánica, gravedad, respuesta inmune e infección como mecanismo causal o desencadenante de la misma, según se describe a continuación.

En primer lugar, la lesión orgánica se define por un cambio de dos puntos en la escala SOFA con respecto al basal. Esta escala permite caracterizar e identificar a un paciente con sepsis en base a la afectación y daño de los sistemas u órganos diana, teniendo en cuenta la situación respiratoria, hemodinámica, neurológica y la función renal, hepática y de la coagulación [6]. La elección de esta

escala viene determinada por su validez, capacidad predictiva, disponibilidad, aplicabilidad y por el carácter dinámico del SOFA con respecto a otras como la escala LODS o los criterios SIRS [7-9].

En segundo lugar, y conforme a líneas previas, el concepto de sepsis conlleva gravedad y mortalidad. Este hecho queda reflejado en las tasas de mortalidad que oscilan entre el 15-50% en la sepsis y hasta por encima del 50% en el shock séptico, a pesar de una tendencia a la disminución en las últimas décadas [10-20]. Lógicamente, la sepsis es una causa de muerte potencialmente evitable en un número importante de casos, sobre todo si tenemos en cuenta que el retraso diagnóstico y terapéutico son uno de los principales factores determinantes de la mortalidad [21-23]. Por lo tanto, el documento insiste en la importancia de la detección y tratamiento precoz de la sepsis. Para ello, propone la escala qSOFA como una útil herramienta de *screening*, basada en datos meramente clínicos, tales como la alteración del estado mental, la hipotensión sistólica y la taquipnea. Esta escala permite entonces, con una valoración "a pie de cama", identificar a los pacientes con infección y riesgo de sepsis, con el consiguiente objetivo de alertar e impulsar al clínico a cuantificar la lesión orgánica, identificar la infección, iniciar tratamiento y optimizar los cuidados de un paciente potencialmente grave [8-9].

Otro aspecto clave destacado en la definición actual es la alteración de la respuesta inmune, ya que la sepsis es el resultado de un daño sistémico producido por esta respuesta inmune no controlada y que deja de actuar localmente para controlar una infección. Por lo tanto, y resultado del aumento de citoquinas o mediadores pro-inflamatorios y de la disminución de moléculas antiinflamatorias, se produce un daño tisular y una disfunción de la microcirculación con la consiguiente isquemia, daño citopático y apoptosis, que perpetúan la cascada inflamatoria sistémica y que acaban conduciendo a la lesión de órganos diana que define la sepsis [24-27].

De acuerdo con lo previo, la definición de sepsis implica que la infección es el mecanismo desencadenante, y de hecho se considera un requisito para que se produzca la misma [1]. El documento reconoce la limitación que supone confirmar microbiológicamente la infección, ya que sólo el 30-40% de los pacientes con sepsis tienen cultivos positivos y por lo tanto confirmación microbiológica [28,29]. En consecuencia, y a pesar de que otros estudios recientes han mostrado mejores tasas de identificación microbiológica, con una positividad de cultivos en la unidad de cuidados intensivos (UCI) de hasta el 65-70% [30-32], y teniendo en cuenta que la confirmación etiológica no suele ser posible en el momento agudo o en el de diagnóstico de sepsis, el requisito de infección como desencadenante de la sepsis puede cumplirse con la mera sospecha clínica.

Además de lo expuesto, se redefine el shock séptico como un subconjunto o parte de la sepsis en la que las alteraciones circulatorias y del metabolismo celular son lo suficientemente graves como para producir un impacto significativo en la mortalidad. De esta forma, y conforme con la idea previa de que se trata de una disfunción circulatoria aguda, se propone como criterio hemodinámico la hipotensión que requiere vasopresores para mantener la tensión arterial media (TAM) por encima de 65 mmHg y una concentración de lactato sérico por encima de 2 mmol/L, a pesar de un volumen de resucitación adecuado. [1]. En consecuencia, esta definición implica sepsis, hipotensión, elevación de lactato, el uso de vasopresores y la adecuada resucitación del paciente séptico. De forma similar y de acuerdo con las limitaciones previas, se reconoce la incapacidad para definir y delimitar adecuadamente los criterios de "volumen de resucitación adecuado" y "necesidad de vasopresores", ya que se trata de conceptos o datos muy variables, subjetivos, y que dependen de otros muchos factores. Sin embargo, la aparición de estos datos en el marco de la sepsis supone unas tasas de mortalidad mucho mayores que cuando se producen de forma aislada y son, por lo tanto, un reflejo de disfunción circulatoria y gravedad [1,12,13,16,17,25].

Finalmente, este documento asentó las bases para la creación y desarrollo de una serie de iniciativas y plataformas dirigidas a la detección precoz, tratamiento y manejo estandarizado del paciente con sospecha o confirmación de sepsis, que se han visto plasmadas en las "Surviving Sepsis Campaign" y en las guías de práctica clínica [23,26,31]. Dentro de las medidas que se contemplan y que han demostrado mejorar la supervivencia en el paciente séptico se encuentran la determinación de lactato, la extracción de hemocultivos, la utilización de medidas de soporte y la sueroterapia según la situación hemodinámica, y, principalmente, el tratamiento antibiótico y control del foco infeccioso de forma precoz [32-34].

2.1.2. Epidemiología

Un estudio que analizó a escala mundial la epidemiología de la sepsis basándose en certificados de defunción, estimó la incidencia de sepsis en 48,9 millones de casos en 2017 [10]. Además, identificó que, con una mortalidad del 52,8%, en 2017, se produjeron 11 millones de muertes en relación con la sepsis, lo que supone que el 19,7% de las muertes en todo el mundo en este año pudieron deberse a esta patología. A pesar de los evidentes problemas metodológicos que puede suponer una estimación de la incidencia a esta escala, y de las diferencias según el nivel de desarrollo del país, otros estudios en países desarrollados han confirmado la gravedad y magnitud del problema [11]. Por ejemplo, una cohorte norteamericana identificó, de acuerdo con los criterios actuales, una incidencia del 6% en pacientes hospitalizados, y una mortalidad del 15% [12]. De igual forma, el análisis

de más de un millón de pacientes ingresados en la UCI en Nueva Zelanda y Australia mostró una incidencia del 9.7% con respecto al total de ingresos. A pesar de la mejoría a lo largo del periodo de estudio, comprendido entre los años 2000 y 2012, la mortalidad de la sepsis era del 14,2% y la del shock séptico del 22% [13]. En España, un estudio retrospectivo identificó más de dos millones y medio de ingresos por sepsis entre los años 2000 y 2013, con una mortalidad del 18,4%, cifras similares a lo descrito previamente [19].

Por otra parte, y en ocasiones con datos discordantes, la bibliografía reciente parece mostrar que la incidencia en los últimos años ha aumentado y que la mortalidad o la tasa de letalidad han disminuido [12-20]. Probablemente, el aumento de la incidencia sea consecuencia del aumento de la prevalencia de los factores que pueden favorecer la sepsis, como las enfermedades crónicas, la inmunosupresión, la mayor supervivencia del paciente oncológico o el envejecimiento de la población. Pero, por otro lado, y de forma paralela al descenso de la mortalidad y de la letalidad, la incidencia puede haber aumentado por el protagonismo reciente que se le está otorgando a la sepsis, al diagnóstico precoz y a los avances en el manejo y tratamiento. A pesar de lo anterior, sigue siendo una patología de elevadísima mortalidad y que se contempla como una de las primeras causas de mortalidad hospitalaria [14, 31].

2.1.3. Factores pronósticos.

Entre los factores pronósticos de la sepsis, es decir, aquellos condicionantes que determinan la gravedad de la sepsis y por tanto una peor evolución y una mayor mortalidad, se encuentran por un lado las circunstancias previas a la aparición de la sepsis y por otro los elementos concurrentes a su desarrollo. En primer lugar, los factores previos son las características y el contexto del huésped, así como la localización y etiología de la infección, factores íntimamente relacionados entre sí. Muchos de ellos son los mismos factores de riesgo de desarrollo de infección y de desarrollo de sepsis tras la infección, aunque estos últimos están claramente peor definidos en la literatura. En segundo lugar, una vez instaurada la infección y la sepsis, una serie de determinantes condicionarán la mayor repercusión, la mayor gravedad y, por tanto, la mayor mortalidad de la sepsis como son la intensidad de la respuesta inflamatorio y el daño orgánico. Como se comentó, la rápida identificación y el tratamiento precoz de la sepsis permitirán atenuar el impacto de la mayoría de estos factores [23,26,33,34].

Por un lado, la situación del paciente, previa a la infección, es un factor fundamental en la evolución de la sepsis. La edad es un factor determinante y de peso, ya que no solo traduce la

presencia de comorbilidades sino que la propia edad conlleva cierto grado de inmunosupresión o inmunosenescencia y peor control de la respuesta inmunitaria [13,26,30,34-39]. Al mismo tiempo, las comorbilidades y enfermedades previas, como la insuficiencia cardíaca, la enfermedad pulmonar obstructiva crónica, la enfermedad hepática, la enfermedad renal crónica, la diabetes, el alcoholismo, el cáncer e inmunosupresión, entre otras, suponen un mayor riesgo de mortalidad en el paciente séptico [13,25, 30, 31, 34,35,40-44).

En segundo lugar, el foco de la infección y el microorganismo causal, a su vez en relación con la situación previa del huésped, implican diferentes tasas de mortalidad [45]. Los pacientes mayores y con más comorbilidades tienen más contacto con el medio sanitario y están expuestos a más dispositivos, antibioterapia, catéteres y cirugías que, además de asociar mayores tasas de mortalidad, predisponen al mismo tiempo a la adquisición de infecciones nosocomiales, gérmenes multirresistentes e inmunosupresión [13,19,25,26,30,31,46].

En línea con lo expuesto, un destacado estudio publicado en 2020 analizó la prevalencia, la etiología y la microbiología de las infecciones en las UCIs en 1150 centros de 88 países [32]. Con una mortalidad hospitalaria del 30%, los factores asociados en el análisis multivariante fueron la adquisición de la infección en la UCI, la edad, el antecedente de cáncer metastásico, la insuficiencia cardíaca, la infección por el virus de la inmunodeficiencia humana (VIH), la cirrosis y la presencia de microorganismos resistentes como *Enterococos* resistentes a la Vancomicina, especies de *Klebsiella* resistentes a beta-lactámicos y especies de *Acinetobacter* resistentes a carbapenemasas.

Además de los determinantes de la susceptibilidad a la infección y riesgo de desarrollo de sepsis más grave propios del paciente, la gravedad de la sepsis está determinada por la intensidad de la respuesta inflamatoria sistémica, desproporcionada y no regulada, que condiciona el daño orgánico a prácticamente todos los niveles [24,25,33]. De esta forma, la aparición o intensidad del daño a nivel renal, hepático, neurológico, hemodinámico-cardiovascular, miocárdico o hematológico, es el principal factor determinante del pronóstico y de la mortalidad de la sepsis una vez que esta se ha instaurado [1]. A su vez, el tratamiento precoz permitirá, por otro lado, controlar el desencadenante infeccioso (tratamiento antibiótico y manejo del foco infeccioso) y atenuar o interrumpir el daño orgánico que se perpetúa y retroalimenta tras la cascada inflamatoria (sueroterapia, soporte respiratorio, hemodinámico…) [23,26,33,34]. Por ello, la identificación precoz juega un papel clave y ha demostrado ser un factor pronóstico fundamental sobre el que se puede actuar.

2.1.4. Escalas diagnósticas y pronósticas

A raíz de la identificación de la importancia del reconocimiento y diagnóstico precoz de la sepsis, y de forma paralela al estudio de los factores pronósticos comentados, en los últimos años se han diseñado y perfeccionado diferentes escalas diagnósticas y pronósticas de mortalidad y gravedad en la sepsis y en el paciente crítico. Estas escalas, que cuantifican el daño orgánico, la gravedad y la repercusión sistémica de la sepsis, se diseñaron para identificar o diagnosticar de forma precoz y para orientar el manejo del paciente séptico. Entre ellas destacan las escalas qSOFA, NEWS2, SOFA, NEWS2, SOFA, LOFS, SIRS, SAPS-II y APACHE-II, con funciones y aplicaciones distintas, tal y como se explica a continuación [1,7-9,47-62].

Como ya se ha indicado, el *score* qSOFA permite identificar a pacientes con mayor riesgo de fallecer por la sepsis, y ha demostrado ser más útil en pacientes con sospecha de sepsis fuera de la UCI, siendo por lo tanto una útil herramienta de *screening* y detección precoz [1,8,9,47,48]. Con el mismo objetivo, y diseñadas como parte del *triage* en el Servicio Nacional de Salud del Reino Unido (NHS), las escalas NEWS (*National Early Warning Score*) y su posterior actualización (NEWS2) se han postulado como excelentes herramientas de identificación y monitorización del paciente grave en el ámbito extrahospitalario, en urgencias y en el paciente hospitalizado [49-51]. En segundo lugar, las escalas SOFA, LODS y SIRS son marcadores de daño o disfunción orgánica y han formado parte de las distintas definiciones de sepsis [1-9, 52-57]. Entre ellas, destaca la escala SOFA, actualmente utilizada como criterio clasificatorio para la sepsis según el documento Sepsis-3, ya que como se ha expuesto tiene mejor aplicabilidad y capacidad que las anteriores. Finalmente, las escalas SAPS-II y APACHE-II son medidores del daño orgánico y permiten evaluar, independientemente de la causa, la gravedad y mortalidad hospitalaria y en la UCI [58-62].

Aunque no todas necesariamente se diseñaron con este fin, su uso actualmente se ha extendido a los ámbitos de urgencias, hospitalización y unidades de cuidados intensivos, dado que se trata de escalas de fácil aplicabilidad y carácter dinámico que no sólo permiten identificar o estratificar al paciente con sepsis, sino que pueden ayudar a su monitorización y seguimiento.

2.2. INFECCIÓN POR COVID-19

La enfermedad por el coronavirus (COVID-19) se debe a un patógeno descrito a finales de 2019 en la ciudad de Wuhan, China, al que se le denominó SARS-CoV-2 [63]. Dada la magnitud de la propagación, mortalidad e incertidumbre tras la identificación de esta nueva enfermedad, la Organización Mundial de la Salud la declaró en enero de 2020 una emergencia internacional para la

salud pública; y en marzo de 2020 se definió como pandemia [64]. Desde entonces, esta infección se ha diseminado por todo el mundo y se estima que ha afectado a más de 200 millones de personas y que ha producido casi 5 millones de muertes [65]. Con estas cifras, es lógico entender cuál ha sido la trascendencia mundial de la pandemia y el impacto que ha supuesto en la dinámica y realidad hospitalaria.

2.2.1. Tormenta de citoquinas

Clínicamente, la infección por el SARS-CoV2 es muy heterogénea dado que puede cursar de forma asintomática, como una infección viral leve con síntomas pseudogripales o incluso como un SDRA, principal causa de muerte en estos pacientes [66,67]. Aunque realmente no se sabe con certeza a qué se debe esta diferencia interindividual, parece que fisiopatológicamente la infección por COVID-19 transcurre en dos fases, una primera fase de replicación viral, habitualmente autolimitada en 8-10 días, y una segunda fase de hiperactivación inmune [68]. Este segundo periodo, en el que aparece el SDRA y en el que se dan la mayoría de complicaciones que condicionan la potencial elevada morbi-mortalidad de la enfermedad, se ha atribuido a lo que se ha denominado la 'tormenta de citoquinas"; un estado inflamatorio secundario a la expresión aberrante y no controlada de ciertas citoquinas y mediadores inflamatorios, como la Interleukina 1 (IL-1), la IL-6, la Interleukina 7 (IL-7), la Interleukina 10 (IL-10) o la Interleukina 33 (IL-33), entre otros [69,70]. Este mecanismo de hiperactivación inmune tras la infección grave por el SARS-CoV-2 supone dos aspectos concretos que ayudan a entender la compleja fisiopatología de la enfermedad.

Por un lado, la enfermedad tiene un carácter sistémico e inmunomediado, dado que el daño a distintos niveles (miocarditis, fracaso renal agudo, encefalitis, SDRA o los fenómenos trombo-embólicos) se debe más al mecanismo inmunológico con activación del complemento, daño endotelial, apoptosis, coagulopatía y a la inflamación parenquimatosa que a la lesión citopática directa [68-70].

En segundo lugar, los principales tratamientos que se han estudiado y aprobado para el tratamiento de la infección grave y el SDRA producidos por el SARS-CoV-2 no van dirigidos a tratar la infección viral *per se*, a excepción del *Remdesivir*, si no a bloquear el estado proinflamatorio que lo sigue. De esta forma, los corticoides, un antiinflamatorio clásico con efectos a diversos niveles, así como el tocilizumab y el anakinra, antagonistas de los receptores IL-6 e Il-1, respectivamente, han demostrado ser eficaces en la infección grave por SARS-CoV-2, disminuyendo la mortalidad [71-75, documento suplementario 4]. Igualmente, el tofacitinib, un inhibidor de la kinasa Janus (JAK), utilizado

en el tratamiento de la artritis reumatoide, puede mejorar el pronóstico en pacientes graves con COVID-19 gracias a su efecto inmuno-modulador [76,77].

2.2.2. Factores pronósticos

De forma paralela a lo que se describe en la sepsis, el pronóstico de la infección por SARS-CoV-2 va a venir determinado por la fragilidad y susceptibilidad del paciente y por la probabilidad de desarrollo del SDRA y de los fenómenos inmunomediados que ocurren en el seno de la tormenta de citoquinas. Actualmente se reconoce que la edad, y la presencia de ciertas comorbilidades como la hipertensión, la insuficiencia cardiaca, la diabetes, la neumopatía o la enfermedad renal crónica son determinantes claros de la gravedad y mortalidad por la infección [78-81, documentos suplementarios 5 y 6].

Sin embargo, desde el advenimiento de la pandemia se ha cuestionado si la inmunosupresión, por otra parte, un claro factor de mal pronóstico en la sepsis, realmente condiciona una mala evolución en la infección por COVID-19, o si incluso podría comportarse como un factor protector [40, 82-86]. Por un lado, Giannakoulis *et al.* revelaron en un amplio metanálisis que los pacientes inmunocomprometidos por neoplasias presentaban un mayor número de ingresos y muertes en la UCI, mientras que, por el contrario, Minotti *et al.* identificaron una mejor supervivencia en pacientes inmunosuprimidos, replanteando el debate de si la inmunosupresión puede tener un papel protector dado que condiciona una respuesta inmune más débil. Por otra parte, en un reciente trabajo demostramos que la inmunosupresión sí que es un factor independiente de mortalidad en la infección por COVID-19, aunque es probable que esto se pueda deber sobre todo a los pacientes que presentan neoplasias de órgano sólido, enfermedad hematológica o tratamiento crónico con corticoides, y no tanto enfermedades autoinmunes o trasplante, como ya han inferido otros autores [87-90, documentos suplementarios 7 y 8]. En esta misma línea, otro trabajo de nuestro grupo confirmó que la mayor mortalidad de los pacientes trasplantados de órgano sólido durante la primera ola de la pandemia se justificó por las comorbilidades previas y no tanto por la inmunosupresión o los fármacos inmunosupresores [91, documento suplementario 9].

En definitiva, aunque este debate exceda de los objetivos de este trabajo, sugiere que fisiopatológicamente la sepsis y la infección por COVID-19 probablemente no sean del todo equivalentes, a pesar de que la inflamación sea la auténtica responsable del daño en ambas. Esto no solo se traduce en que pueda haber algunas diferencias en los factores pronósticos si no que el

abordaje terapéutico (tratamiento de la infección frente a la inmunosupresión y control de la respuesta inmune) son prácticamente antagónicos.

2. 3. BIOMARCADORES PRONÓSTICOS DE GRAVEDAD Y MORTALIDAD

Un biomarcador es un parámetro analítico que indica un estado biológico o patológico de forma objetiva y validada [92]. Idealmente, un biomarcador debe ser sensible y específico con el consiguiente alto valor predictivo positivo, y negativo, a la vez que debe ser accesible, de fácil y rápida determinación. Por otra parte, los biomarcadores deben tener un carácter dinámico y reflejar las variaciones del estado patológico y la respuesta a un tratamiento, de forma que puedan ayudar a guiar o monitorizar la intervención terapéutica.

2.3.1 Biomarcadores en la sepsis.

En el contexto de la sepsis, la obtención de un biomarcador fiable y adecuado no ha sido posible aún por varios motivos [93-97]. Entre ellos, la fisiopatología tan amplia y compleja de la sepsis, con la participación de varias vías y cascadas inflamatorias, junto con la variedad y heterogeneidad de la expresión clínica, dificulta mucho la identificación de un parámetro único que participe en todos estos procesos y que sea a la vez específico de sepsis. En este escenario, en los últimos años se han estudiado diversos parámetros como potenciales biomarcadores de la sepsis, pero, como se ha expuesto, habitualmente identifican un proceso único (la infección, la inflamación, la presencia de un microorganismo concreto, el daño orgánico…) y no tienen por lo tanto la suficiente sensibilidad y/o especificidad. Entre ellos, destacan:

2.3.1.A. Citoquinas pro-inflamatorias como marcadores de la fase hiperinflamatoria de la sepsis. Principalmente, el factor de necrosis tumoral (TNF), la interleukina 1b (IL-1b) y la IL-6 son citoquinas que median la respuesta inflamatoria inicial del sistema inmunitario innato a la lesión o la infección. Por lo tanto, y aunque han demostrado un papel pronóstico, no son específicos de infección o sepsis [95-97].

2.3.1.B. Proteínas del complemento. La cascada del complemento estimula la fagocitosis de los microorganismos y es una vía fundamental en la fisiopatología de la sepsis. De nuevo, su papel como biomarcador se ha debatido ya que no son específicos y pueden tener efectos a otros muchos niveles [95].

2.3.1.C. Marcadores de activación neutrofílica, macrofágica o monocitaria. El CD64 es un receptor de alta afinidad de la fracción Fc de las inmunoglobulinas y refleja la activación de los polimorfonucleares en la sepsis; ha demostrado ser un marcador diagnóstico de sepsis y del tratamiento antibiótico adecuado [95,98,99]. Por otra parte, la presepsina es un subtipo del marcador soluble CD14 (sCD14-ST) que se expresa en monocitos o macrófagos [100]. Es el receptor del complejo lipopolisacárido-proteína ligadora de lipopolisacáridos (LPS-LPB) y traduce la señal de las endotoxinas bacterianas mediante el receptor "Toll-Like-4". Desde su identificación en 2005, varios estudios han confirmado su rol diagnóstico y pronóstico y la correlación con el *score* SOFA [100-103].

2.3.1.D. Marcadores relacionados con el daño endotelial y vasodilatación. Dado que el daño y disfunción endotelial son fenómenos claves en la cascada inflamatoria de la sepsis, varios parámetros como las células progenitoras endoteliales (CPE), el factor de crecimiento derivado de plaquetas (PDFG) o el factor de crecimiento vascular endotelial (VEGF), se han utilizado como marcadores pronósticos en pacientes con sepsis, shock séptico o coagulación intravascular diseminada [96,97]. Por otra parte, la Adrenomedulina (ADM) es una molécula con un importante papel en el proceso inflamatorio y en la progresión de la sepsis al shock séptico [104]. Se produce en el endotelio y tiene un papel vasodilatador, inotrópico, diurético, natriurético y broncodilatador. Dado que tiene una vida media muy corta, su detección se realiza mediante un fragmento de la molécula (MR-proADM). En una revisión publicada en 2018, la MR-proADM demostró tener valor pronóstico y diagnóstico de sepsis [105].

2.3.1.E. Marcadores de disfunción orgánica. Diversos parámetros como la creatinina, la bilirrubina, la urea o ciertos valores de la coagulación definen el daño de un aparato o sistema y se han incorporado a las diversas escalas pronósticas o diagnósticas (SOFA, LODS, APACHE-II, SAPS-II...) [47-62]. De hecho, la actual definición de sepsis exige la presencia del daño orgánico, identificado por la puntuación mayor o igual de dos en el SOFA [1]. El lactato, a su vez, es un producto derivado del metabolismo o glicólisis anaerobia que se produce en un contexto de hipoperfusión tisular en órganos hipóxicos [106]. Sin embargo, y aunque en el contexto de la sepsis es un parámetro sinónimo de disfunción hemodinámica y orgánica, el lactato puede elevarse en otras situaciones de inflamación sistémica o insuficiencia hepática [1,8]. Por lo tanto, su especificidad también se ha debatido. A pesar de lo previo, su uso en la sepsis está estandarizado y recomendado por las guías de práctica clínica y su monitorización es una de las herramientas utilizadas para el manejo hemodinámico y la sueroterapia en los pacientes con sepsis en lo que se conoce como terapia dirigida por objetivos (aclaramiento de lactato) [23,107].

Finalmente, hasta el momento los parámetros más estudiados y aplicados en la práctica clínica, además del lactato, son la PCR y la PCT, proteínas producidas en respuesta a la infección o inflamación (reactantes de fase aguda) [93-96]. La PCR es una proteína sintetizada en el hígado tras la acción de la IL-6 cuyo rol fisiopatológico no está claro. Sin embargo, se cree que podría tener un papel en la eliminación de productos de degradación o detritus por los macrófagos tras la unión a los fosfolípidos de microorganismos o las células dañadas del huésped [95]. A pesar de ser un marcador muy inespecífico, puesto que se eleva prácticamente en cualquier contexto inflamatorio, tiene una adecuada capacidad pronóstica dado que su sensibilidad es elevada en el debut de la infección y de la sepsis [94]. Además, siendo un parámetro dinámico cuyas variaciones reflejan el estado inflamatorio, actualmente es uno de los principales marcadores utilizados en la monitorización de los pacientes con infección o sepsis [97]. En segundo lugar, la PCT es el precursor de la calcitonina, una hormona que participa en la homeostasis del calcio [93]. A raíz de la identificación de esta molécula en pacientes con infección bacteriana en 1993, numerosos estudios en las dos últimas décadas han analizado la capacidad diagnóstica y pronóstica de este marcador, concluyendo que tiene un alto valor predictivo negativo de infección, que es un marcador pronóstico de mortalidad y que puede ayudar a monitorizar la duración el tratamiento [95, 108, 109]. Sin embargo, y a pesar de que su utilidad diagnóstica y como guía del tratamiento antibiótico está consolidado en la práctica clínica, es un parámetro que se puede elevar durante otras situaciones ajenas a la sepsis, como traumas o quemados graves, cirugías amplias o pancreatitis agudas [94]; además de que hay estudios previos que han puesto en duda su eficacia real. Por ejemplo, el estudio PASS publicado por Jensen *et. al* en 2013, en el que se aleatorizó a 1200 pacientes a recibir el tratamiento de acuerdo con los niveles de procalcitonina o a recibir el tratamiento estándar, no demostró que el tratamiento antibiótico guiado por la procalcitonina supusiera un impacto en la mortalidad, sino que de hecho implicó mayor estancia media [110]. A pesar de la controversia y gracias a la presencia de otros estudios recientes que sugieren que el tratamiento dirigido por la procalcitonina sí puede ser beneficioso, en las guías de práctica clínica actuales ("Surviving Sepsis Campaign") se considera el uso de la procalcitonina para guiar la reducción de la duración y suspender el tratamiento antibiótico en pacientes con sepsis [23,109,111].

Aun así, y tras todo el esfuerzo de la comunidad científica, a fecha de hoy no existe un biomarcador adecuado o un "gold-standard" para el diagnóstico o manejo de la sepsis [1,23]. De forma anecdótica, en un artículo de 2020 se realizó una revisión sistemática de biomarcadores en sepsis y se comparó con los datos de los que se disponía en 2010, derivados de otra revisión realizada por los mismos autores [96,97]. En este interesante artículo, se concluye que, a pesar de toda la bibliografía disponible, se han realizado pocos progresos en la identificación de biomarcadores de sepsis con

significado clínico real. De hecho, sólo algunos parámetros como el CD64 o la presepsina han conseguido superar a la PCR o a la PCT. Por lo tanto, la bibliografía actual y las guías clínicas no contemplan el uso de ningún biomarcador en la sepsis, aparte de la procalcitonina y del lactato en la sepsis, por las razones ya expuestas; además de ello, se reconoce la limitación del uso de biomarcadores en este escenario [23].

2.3.2. Biomarcadores en la infección por COVID-19

De nuevo en la línea de lo descrito en la sepsis, cuya fisiopatología parece mostrar cierta homología con la infección por SARS-CoV-2, los principales parámetros pronósticos de la enfermedad por COVID-19 serán los marcadores de daño orgánico, entre los que se incluyen las troponinas, las transaminasas, los parámetros de la coagulación, como el Dímero-D, o los parámetros de la oxigenación, como el ratio saturación periférica de oxígeno-fracción inspirada de oxígeno (SAFI), así como las moléculas o mediadores de la cascada inflamatoria como la PCR, la ferritina, la linfopenia, la trombopenia o la IL-10 [89,112-115, documentos suplementarios 7 y 10]. Entre ellos, la IL-6 ha sido considerada un mediador clave de la fisiopatología de la activación inmunitaria, cuyos efectos pleiotrópicos podrían justificar el carácter sistémico de la enfermedad y cuyo bloqueo se ha traducido en una mejora de la supervivencia en pacientes con SDRA grave, tal y como se indicaba anteriormente [69, 73, 74, 114, documento suplementario 4]. Sin embargo, y a pesar de las evidentes similitudes fisiopatológicas, los marcadores de daño de órgano, marcadores de la infección y parámetros pronósticos de gravedad o mortalidad no son los mismos y también pueden traducir diferencias en los mecanismos implicados, como adelantamos previamente.

2.3.2. Ancho de distribución eritrocitaria

El ancho de distribución eritrocitaria (ADE) es un parámetro que se analiza y obtiene de forma rutinaria con un hemograma. Es un coeficiente de variación que describe la dispersión, en términos porcentuales, del tamaño de los hematíes; y se calcula al dividir la desviación estándar del tamaño de los hematíes entre el volumen corpuscular medio [116]. El ADE mide por lo tanto la amplitud del tamaño eritrocitario o anisocitosis, clásicamente utilizado para el diagnóstico diferencial de la anemia ferropénica y la anemia de trastornos crónicos [117].

En los últimos años, y gracias a que se trata de un parámetro de amplia disponibilidad y rápida determinación, se ha estudiado como un factor asociado a la inflamación y como un factor pronóstico de gravedad y mortalidad en escenarios clínicos muy diversos como la insuficiencia cardíaca, la enfermedad renal crónica o en enfermedades de base inmunitaria o inflamatoria como el lupus

eritematoso sistémico [118-120]. En la sepsis, el ADE se ha estudiado como un marcador de gravedad con resultados satisfactorios, postulándose como una posible herramienta para identificar a los pacientes con mayor mortalidad [121,122]; mientras que en el SDRA ha demostrado tener capacidad pronóstica [123,124] y en la infección por COVID-19 se ha evaluado como marcador de la tormenta de citoquinas [125,126].

Muchas de estas patologías tienen, en mayor o menor proporción, una base o sustrato inflamatorio, que es la responsable del daño tisular. En este contexto, la presencia de citoquinas, y principalmente la IL-6, promueven una respuesta atenuada a la eritropoyetina, inhiben la eritropoyesis y la síntesis de hemoglobina de forma directa e incluso reducen la vida media de los eritrocitos [127,128]. La IL-6, además de participar en otros muchos procesos como la diferenciación de células T, maduración de células B, síntesis o secreción de inmunoglobulinas, induce la liberación de la hepcidina de los hepatocitos a través de la unión con el receptor STAT3. La hepcidina es una proteína que participa en el metabolismo férrico dado que regula la expresión del transportador de metales divalente (DMT-1), y de esta forma controla la degradación de la ferroportina, responsable de la transferencia o absorción del hierro en el duodeno. Como resultado, la hepcidina bloquea la absorción intestinal de hierro y el reciclado del hierro en los macrófagos. De la misma manera, induce la transcripción de la ferritina, lo que conlleva a la retención y a la sobrecarga de hierro en el sistema fagocítico mononuclear. Por lo tanto, la ferritina refleja la alteración en la regulación del metabolismo férrico y la inhibición de la eritropoyesis, a pesar de que de hecho haya una sobrecarga férrica sistémica y un aumento en los depósitos de hierro [129-131]. Todos estos mecanismos conllevan una mayor anisocitosis y un aumento del ADE, reflejo de la alteración de la eritropoyesis y el bloqueo de la síntesis de hemoglobina en la médula ósea, cambios particularmente notables en el paciente crítico y en situaciones o estados hiperinflamatorios [132]. Además del estudio de Jian *et al.,* en el que se muestra que los pacientes con sepsis ingresados en la UCI desarrollaban anemia durante la primera semana de ingreso, y que su aparición lo hace en paralelo a la elevación de la hepcidina, ferritina y la IL-6; otros autores han confirmado una asociación entre el ADE y citoquinas inflamatorias o marcadores de la anemia de trastorno crónico como la IL-6, el factor de necrosis tumoral o la hiperferritinemia, demostrando que el ADE es un marcador subrogado del ambiente inflamatorio sistémico [133-135].

Finalmente, a pesar de que se pueda considerar el ADE como un interesante y potencial marcador inflamatorio, este ha sido criticado por su escasa especificidad, ya que es un parámetro

sujeto a otros muchos condicionantes como la anemia, la edad o las enfermedades crónicas, y cuya elevación depende del ambiente pro-inflamatorio y no de una causa concreta [117-119].

3. JUSTIFICACIÓN Y OBJETIVOS

3.1. JUSTIFICACIÓN

El ADE es un parámetro fácilmente disponible y accesible que puede ayudar a determinar la intensidad de la respuesta inflamatoria y tener por lo tanto un papel pronóstico en la sepsis y en la infección grave por COVID-19. Por otra parte, el tratamiento con tocilizumab y la presencia de infecciones bacterianas en pacientes con COVID-19 pueden ser un potencial factor de confusión de la capacidad del ADE en este escenario.

3.2. OBJETIVOS

Objetivo primario: Evaluar la capacidad del ADE como marcador de gravedad y mortalidad tanto en pacientes ingresados en la UCI por sepsis como en pacientes con infección grave por COVID-19.

Objetivos secundarios:

1. Estudiar la dinámica del ADE y las diferencias entre los supervivientes y no supervivientes, en pacientes con sepsis y con infección grave por COVID-19.
2. Determinar la capacidad predictiva de mortalidad del ADE y su asociación con otros potenciales factores de confusión.
3. Evaluar la correlación y comparar las características del ADE con otros biomarcadores inflamatorios.
4. Analizar el valor añadido que supondría incluir el ADE sobre las escalas pronósticas de sepsis existentes y determinar el mejor modelo predictor de mortalidad.
5. Examinar la prevalencia y factores de riesgo de infecciones bacterianas en pacientes ingresados por COVID-19 como posible factor de confusión de la capacidad pronóstica del ADE en este contexto.

4. MATERIALES Y MÉTODOS

4.1. DISEÑO, MARCO Y POBLACIONES DEL ESTUDIO

El estudio se diseñó como un análisis observacional y retrospectivo de tres grupos poblacionales, con tres objetivos distintos. En primer lugar, se analizó la capacidad predictiva de mortalidad del ADE en la sepsis en los pacientes que ingresaron en la UCI por este motivo. En segundo lugar, se analizó el rol pronóstico del ADE y su correlación con otros marcadores inflamatorios en pacientes con neumonía grave por COVID-19. En tercer lugar, se estudiaron la prevalencia, los factores de riesgo de infecciones bacterianas y su impacto en la mortalidad en los pacientes que ingresaron en la planta de hospitalización por neumonía por COVID-19 durante la primera ola, independientemente de su gravedad. Este último análisis se realizó con el objetivo de dilucidar si las infecciones bacterianas, durante el ingreso por infección COVID-19, podían ser un potencial factor de confusión de la capacidad pronóstica del ADE en la cohorte anterior. Las tres poblaciones proceden del Hospital Puerta de Hierro Majadahonda, un hospital terciario dotado de 620 camas de hospitalización y 52 camas de cuidados intensivos, repartidos en un área médica (UCI) y quirúrgica (UCPQ), aunque esta proporción se vio modificada durante la primera ola de la pandemia [80, documento suplementario 5].

4.1.1. Pacientes ingresados en la UCI por sepsis.

Este grupo poblacional consistió en una cohorte retrospectiva de pacientes ingresados en la UCI Médica por sepsis en el Hospital Puerta de Hierro Majadahonda, desde el 1 de enero de 2018 hasta el 31 de diciembre de 2019. Tras el análisis de las historias clínicas de los ingresos en la UCI durante este periodo, se seleccionaron los pacientes mayores de 18 años que ingresaron por sepsis o si cumplían los criterios diagnósticos de sepsis al ingreso de acuerdo con la definición actual de sepsis: pacientes con sospecha o infección confirmada y disfunción orgánica demostrada por una puntuación del *score* SOFA mayor o igual a 2 [1]. Por otra parte, si un paciente ingresaba en varias ocasiones en la UCI por sepsis, se registró y analizó el último episodio para asegurar el criterio de independencia entre las observaciones. Se excluyeron los pacientes que ingresaron en la UCPQ o que desarrollaron la sepsis durante su estancia en la UCI como complicación de otro proceso.

4.1.2. Pacientes ingresados por neumonía grave por COVID-19

Se incluyeron pacientes adultos ingresados por infección por COVID-19 confirmada mediante la reacción en cadena de la polimerasa con transcriptasa inversa (RT-PCR) nasofaríngea desde el 3 de marzo al 20 de abril de 2020 y que presentasen neumonía intersticial con insuficiencia respiratoria grave (puntuación de la escala de gravedad BRESCIA-COVID=2) o que presentasen deterioro

respiratorio o necesidad en aumento de la oxigenoterapia sin la necesidad de ventilación mecánica invasiva (puntuación de la escala de gravedad BRESCIA-COVID =3) [136] y al menos uno de los siguientes parámetros: IL-6 >40 pg/mL, aumento de la lactato dehidrogenasa (LDH) o valores de LDH que duplicasen el límite de la normalidad, PCR en aumento, D-Dímero >1500 ng/mL, linfocitos <1200/uL o ferritina >500 ng/l. El seguimiento se realizó hasta el 30 de junio de 2020. Se excluyeron a los pacientes que cumplieron los criterios durante el ingreso en UCI y a los que fallecieron a las 24 horas del ingreso hospitalario o a las 24 horas de la inclusión en el estudio. Todos estos pacientes se habían incorporado previamente desde nuestro centro a un estudio, denominado TOCICOV. Este estudio multicéntrico, observacional y retrospectivo, se diseñó para comparar la mortalidad y la tasa de ingreso en la UCI en función del tratamiento con corticoides y tocilizumab durante la primera ola de la pandemia [74, documento suplementario 4].

4.1.3. Pacientes ingresados por neumonía por COVID-19

Se analizaron pacientes adultos que ingresaron por neumonía debido a COVID-19 desde el 1 de marzo a 30 de abril de 2020, con seguimiento clínico hasta el 30 de junio de 2020, con o sin confirmación microbiológica. En este grupo también se incluyeron a los pacientes con neumonía grave que se habían analizado en la cohorte anterior.

4.2. RECOGIDA DE DATOS

Los datos epidemiológicos, situación basal, comorbilidades, datos clínicos, analíticos y microbiológicos, además de los diagnósticos y tratamientos realizados durante el ingreso, se recogieron mediante la historia clínica electrónica (SELENE System, Cerner Iberia, S.L.U, Madrid, España) a través de unos formularios diseñados por los investigadores. Para cada cohorte se analizaron unas variables o parámetros en momentos distintos.

En el primer grupo, se registraron los parámetros de laboratorio en el momento del ingreso o en las 24 horas anteriores o posteriores al ingreso en UCI. Además, los valores de los biomarcadores (RDW, PCR y PCT) se recogieron e incluyeron en el momento del ingreso, a las 24, 48 y 72 horas, a los 7 y a los 30 días desde el ingreso, independientemente del alta de la UCI. Por otra parte, las escalas diagnósticas y pronósticas qSOFA, NEWS2, SOFA, LODS, SIRS, APACHE-II o SAPS-II de los pacientes con sepsis se calcularon de acuerdo con los datos recogidos mediante un formulario específico (**tablas 1-7 del Anexo**). Para su estimación, se consideró el valor más patológico o el más relevante acaecido en las 24 horas antes, durante o 24 horas después del ingreso en UCI. Si el paciente padecía enfermedades crónicas o elevaciones basales de algún parámetro, las escalas se calculaban

considerando la diferencia entre el valor basal y el valor del ingreso, de acuerdo con la bibliografía [1,6,7,51,56,58,60]. Además, la ausencia o error de un parámetro se interpretó como valor nulo o no patológico, de forma que no suponía un cambio en el valor de la escala. Por ello, y de acuerdo con su definición, los pacientes cumplían los criterios qSOFA si presentaban 2 de las 3 variables (puntuación de la Escala de Coma de Glasgow menor o igual a 13, tensión arterial sistólica menor de 100 mmHg o frecuencia respiratoria mayor o igual de 22 respiraciones por minuto) [1,8,9]. Igualmente, los pacientes presentaban SIRS si cumplían 2 de cuatro criterios (temperatura mayor de 38°C o menor de 36°C, frecuencia cardíaca mayor de 90 latidos por minuto, frecuencia respiratoria mayor de 20 respiraciones por minuto o presión arterial de dióxido de carbono (PaCO2) menor de 32 mmHg o recuento leucocitario mayor de 12.000/mm³ o menor de 4.000/mm³ o presencia de más de 10% de formas inmaduras [2]. Las variables que incluye cada escala y la fórmula para determinar el valor de cada una de ellas se muestran en las tablas 1-7 del apéndice suplementario.

Por otra parte, en la población de pacientes del estudio TOCICOV se registraron los siguientes parámetros analíticos en el momento de inclusión en el estudio (día 0): linfocitos, neutrófilos, plaquetas, hemoglobina, ADE, actividad de protrombina (AP), D-Dímero (DD), fibrinógeno, aspartato aminotransferasa (AST), PCR, LDH, ferritina e IL-6. Los valores de linfocitos, neutrófilos, AP, DD y LDH se seriaron a las 24 horas (día 1), 72 horas (día 3), 7 días (día 7) y 14 días (día 14) desde el momento de inclusión en el estudio. Por último, también se determinaron las cifras de ferritina, PCR, IL-6 y ADE en el momento del ingreso, además de en los días 0,1, 3,7 y 14.

4.3. DEFINICIONES

Antibioterapia empírica adecuada. Empleo o uso de antibioterapia que ofrezca cobertura adecuada para el germen causal en las 3 primeras horas desde la notificación, sospecha o diagnóstico de sepsis, o desde deterioro del paciente, según los datos analizados en la historia clínica [23,34].

Cardiopatía. Presencia de insuficiencia cardíaca o de enfermedad coronaria.

Control del foco. Abordaje quirúrgico, endoscópico o intervencionista del probable foco causal, en las primeras 6-12 horas desde la notificación, sospecha o diagnóstico de sepsis, o desde deterioro del paciente, según los datos analizados en la historia clínica [23,34].

Código sepsis. Notificación en la historia clínica de sospecha o diagnóstico de sepsis que conllevase un cambio en la actitud diagnóstica o terapéutica dirigida a la sepsis.

Enfermedad cerebrovascular. Antecedente de ictus, accidente isquémico transitorio o lesiones isquémicas antiguas, aunque cursasen de forma asintomática.

Enfermedad renal crónica. Tasa de filtrado glomerular por debajo de 60 ml/min/m2.

Hepatopatía crónica. Antecedente de insuficiencia hepática (Child A, B o C).

Índice de comorbilidad de Charlson (ICC). Sistema de evaluación de esperanza de vida a los 10 años basado en la edad y en las comorbilidades, factores que han demostrado disminuir el pronóstico vital del individuo [137,138]. Las variables que determinan la puntuación del ICC se reflejan en la Tabla 8 del anexo.

Infección bacteriana. Presencia de datos clínicos (fiebre o escalofríos en ausencia de otros diagnósticos, esputo purulento, diarrea inflamatoria, dolor abdominal, eritema peri-catéter, inflamación articular o celulitis…), junto con datos microbiológicos (hemocultivos, urocultivos, cultivos del tracto respiratorio superior e inferior, muestras del líquido cefalorraquídeo, muestras intra-operatorias, antigenurias, toxinas en heces…) o radiológicos (infiltrado, colitis, colecistitis, abcesos…) que lo apoyen.

Infección comunitaria: Adquirida fuera del hospital, que no cumpla criterios de infección nosocomial.

Infección nosocomial: Cuando su sintomatología aparece tras las primeras 48 horas de ingreso o durante el primer mes tras un ingreso hospitalario previo.

Inmunosupresión. Presencia de enfermedad hematológica, trasplante de órgano sólido, neoplasia de órgano sólido activa o diseminada o cualquier patología, incluyendo enfermedad autoinmune, que haya requerido tratamiento inmunosupresor durante al menos 3 meses. A su vez, se considera que un paciente estaba en tratamiento inmunosupresor si recibía tratamiento activo en el momento del ingreso, incluyendo dosis de prednisona mayores 5 mg (o equivalentes), o si había recibido quimioterapia o inmunoterapia en los 6 meses previos.

Foco primario. Localización de la infección que origina la sepsis y que determina el ingreso en la UCI.

Foco secundario. Otras infecciones que puedan aparecer de forma sincrónica con el foco primario o durante el ingreso, pero que no son los responsables de la sepsis.

Organismo multi-resistente (*MDR*). Micro-organismo resistente a uno o más agentes microbianos de al menos 3 grupos o familias antibióticas distintas [139].

Organismo con resistencia de difícil tratamiento (*DTR*). Bacilos gram-negativos que presenten resistencia a todos los agentes de primera línea, incluyendo todos los beta-lactámicos y fluoroquinolonas [140].

Sepsis. Pacientes con infección posible o documentada que presenten variación del *score* SOFA mayor o igual de dos en este contexto [1].

Shock séptico. Pacientes con sepsis que precisen vasopresores para mantener una TAM mayor de 65 mmHg y un lactato mayor de 2 mmol/L, a pesar de un volumen de resucitación adecuado [1].

Síndrome de distrés respiratorio Agudo (SDRA). El SDRA y su gravedad se definieron de acuerdo con la fracción inspirada de oxigeno (FiO2) los criterios de Berlín [141]. La ratio saturación periférica de oxigeno-fracción inspirada de oxigeno (SAFI) se aplicó para definir y estratificar el SDRA en los pacientes en los que la presión parcial de O2 (PaO2) no estaba disponible [142]. De esta forma, la ratio PaO2/FiO2 (PAFI) >200 o SAFI >235 supone un SDRA leve, la PAFI >100 o SAFI >160 un SDRA moderado y la PAFI $\leq$100 un SDRA grave.

Sospecha de neumonía por COVID-19. Se consideró que los pacientes presentaban una neumonía por SARS-CoV-2 a pesar de no disponer de una RT-PCR positiva si el paciente presentaba, en el contexto de la pandemia, una neumonía intersticial en ausencia de otras causas como insuficiencia cardíaca, neumonía bacteriana o viral con otros organismos identificados, sepsis u otras neumopatías intersticiales.

Sueroterapia. Empleo de al menos 500 ml de cristaloides antes del ingreso en UCI.

4.4. ANÁLISIS ESTADÍSTICO

Los datos obtenidos tras la revisión de las historias clínicas se introdujeron en un formulario diseñado con este propósito y en una base de datos de Excel que posteriormente se analizaron mediante el software Stata v16 StataCorp. 2019 (Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC) y SPSS versión 15.0 (IBM). Para todos los análisis, el nivel de significación estadística se definió como 0.05.

En el análisis descriptivo se utilizaron la mediana (percentiles 25 y 75) o media (desviación estándar, DE) para las variables numéricas y frecuencias absolutas (y relativas) para las variables categóricas. El análisis univariante, para comparar las diferencias entre los dos grupos de pacientes (supervivientes vs no supervivientes o pacientes con infecciones bacterianas vs pacientes sin infecciones bacterianas), se realizó mediante el test de Chi-Cuadrado para variables categóricas y los test t de Student o U de Mann-Whitney para las variables numéricas.

Por otra parte, se evaluó y comparó la capacidad discriminatoria de ciertas variables en distintas cohortes. La discriminación es la capacidad que tiene un modelo o parámetro para diferenciar un evento en un paciente. Se evalúa mediante el análisis del área bajo la curva Característica Operativa del Receptor (AUC-ROC por su acrónimo en inglés "Area Under the Curve- Receiver Operating Characteristic"). Oscila entre los valores 0,5 a 1; siendo igual a 0.5 una nula capacidad discriminatoria, mientras que un valor de 1 indica una discriminación perfecta. Un modelo con un AUC-ROC mayor de 0,7 se considera una capacidad discriminatoria suficiente para diferenciar la respuesta o evento en un sujeto [140]. El comando *roccomp* de Stata se utilizó para comparar las áreas bajo la curva.

Además de los análisis descriptivos y univariantes realizados en las tres poblaciones, en cada una se evaluaron aspectos distintos, según se relata a continuación.

4.4.1. Pacientes ingresados en la UCI por sepsis

Inicialmente, se determinaron los valores de las escalas pronósticas y se comparó su capacidad discriminatoria mediante el análisis del AUC-ROC. A continuación, se examinó la dinámica y el rendimiento de los biomarcadores. Puesto que cada paciente es medido varias veces en el tiempo (ingreso en UCI, 24h, 48h, 72h y 7 días posteriores, respectivamente), no se puede asumir que las observaciones son independientes entre sí, y, por tanto, los métodos clásicos de análisis no aplican en este caso. Por ello, se han utilizado los modelos de ecuaciones de estimación generalizados (EEG) para estudiar la asociación del ADE, PCR y PCT con la mortalidad durante la primera semana del ingreso en

UCI. En el modelo se introdujo la mortalidad como variable dependiente y se seleccionó la familia binomial y la función *logit* como función de enlace. Las variables ADE, PCR y PCT, así como el tiempo al que se realizaron cada una de las medidas, se introdujeron como variables independientes.

En tercer lugar, se realizó un análisis multivariante de mortalidad mediante regresión logística binaria para cada una de las medidas del ADE en cada momento, considerando el índice de comorbilidad de Charlson, la inmunosupresión, la infección nosocomial, la cifra de hemoglobina y las escalas NEWS2, SOFA y SAPS-II. Estas variables se eligieron para evaluar su potencial efecto de confusión de acuerdo con lo siguiente: la edad y las comorbilidades se valoraron mediante el índice de comorbilidad de Charlson, la inmunosupresión y la infección nosocomial; mientras que las escalas se utilizaron para evaluar la situación clínica (NEWS2) el daño orgánico (SOFA) y la gravedad (SAPS-II), de acuerdo con los hallazgos del punto anterior. Además, se consideró la hemoglobina puesto que la anemia puede condicionar una elevación del ADE [117,118]. A continuación, se analizó la capacidad discriminatoria de mortalidad de cada uno de los modelos, o para cada momento del periodo de estudio, mediante el AUC-ROC.

Finalmente, se analizó el valor añadido que supone el ADE sobre las escalas diagnósticas y pronósticas mediante la comparación de la capacidad discriminatoria de mortalidad, expresada mediante AUC-ROC. Solo se consideraron los valores del ADE al ingreso y a las 24 horas, ya que las escalas se calculan con los valores analíticos más patológicos en las primeras 24 horas de ingreso. Para ello, se desarrollaron sendos modelos logísticos que incluyeron a las correspondientes escalas, y a esos mismos modelos añadiendo las mediciones de ADE en cada tiempo. Para cada uno de estos modelos logísticos, se estimó el AUC-ROC de su probabilidad predicha y se compararon las áreas.

4.4.2. Pacientes ingresados por neumonía grave por COVID-19

En primer lugar, se estudió la dinámica hasta el día 14 y la asociación de los marcadores inflamatorios (linfocitos, neutrófilos, plaquetas, hemoglobina, ADE, AP, DD, fibrinógeno, AST, PCR, LDH, ferritina e IL-6) con la mortalidad durante la primera semana tras la inclusión en el estudio. La precisión de la predicción de la mortalidad de los parámetros que demostraron asociación estadísticamente significativa se evaluó mediante el análisis del AUC-ROC.

Posteriormente, se consideró el posible efecto del bloqueo del receptor de la IL-6 en estos parámetros, comparando sus valores en función del tratamiento con tocilizumab. Con este mismo

propósito, se realizó un análisis multivariante de mortalidad de estos marcadores inflamatorios, considerando el tratamiento con tocilizumab y las cifras de hemoglobina.

Por último, y para una mejor interpretación del comportamiento y rol de estos parámetros, se estudió la correlación entre los marcadores inflamatorios hasta el día 7 del periodo de estudio.

4.4.3. Pacientes ingresados por neumonía por COVID-19

En la tercera cohorte, y de acuerdo con las variables estudiadas en los análisis univariantes, se realizaron dos análisis multivariantes mediante regresión logística binaria. El primero analizó los factores de riesgo de infecciones bacterianas en pacientes con neumonía por COVID-19 y el segundo analizó los factores relacionados con la mortalidad, incluyendo las infecciones y el tratamiento inmunosupresor.

4.5. ASPECTOS ÉTICOS

El estudio no supuso contacto, intervención o riesgo para los pacientes que participaron en él. Los investigadores involucrados preservaron la confidencialidad y privacidad de los datos mediante el tratamiento agregado de los mismos y la codificación de los datos personales. El análisis de las tres poblaciones del estudio fue aprobado por el Comité Ético de Investigación Clínica del Hospital Universitario Puerta de Hierro Majadahonda.

5. RESULTADOS

5.1. PACIENTES INGRESADOS EN LA UCI POR SEPSIS

5.1.1. Características de la población y del ingreso en UCI

En el periodo comprendido entre el 1 de enero de 2018 y 31 de diciembre de 2019, 203 pacientes adultos ingresaron en la UCI por sepsis. La mortalidad global fue del 31,6%, y en un 81,3% de los casos la causa del fallecimiento fue la sepsis. Las características de la cohorte se muestran en la **tabla 1**. El 63,6% eran varones, con una media de edad de 63 años. En relación con las comorbilidades, el 32% padecía cardiopatía (insuficiencia cardíaca o enfermedad coronaria), el 13,8% enfermedad arterial periférica, el 12,3% enfermedad cerebrovascular, el 32,5% enfermedad pulmonar, el 14,8% hepatopatía crónica, el 21,2% enfermedad renal, el 12,3% enfermedad autoinmune, el 4,5% presentaba antecedentes de enfermedad ulcerosa, el 18,2% patología hematológica, el 31,5% diabetes y el 12,8% alcoholismo. Tres pacientes (1,5%) padecían VIH en el momento del ingreso. Por otra parte, en un 36,5% de los pacientes se identificó cierto grado de inmunosupresión, incluyendo 38 pacientes trasplantados (18,7%): 6,9% de médula ósea, 5,4% de hígado, 3,5% de pulmón, 2,5% de riñón y 4% de corazón. En relación con la patología tumoral, identificada en un el 42,3% del total, se atribuyó en su mayoría a las neoplasias de órgano sólido (28,6%) y a las neoplasias hematológicas (13.3%). Cinco pacientes (2,5%) padecían demencia o deterioro cognitivo, y un 14,3% algún grado de dependencia. La media del índice de comorbilidad de Charlson fue de 6,17 puntos. Por otra parte, un 37% de los pacientes habían sido tratados con antibiótico en los 3 meses previos.

El análisis de comorbilidades mostró una asociación estadísticamente significativa de la mortalidad con la cardiopatía (45,3% vs 25,9%, p=0,001), con la enfermedad pulmonar (42,2% vs 28,1%, p=0,028), con la enfermedad autoinmune (20,3% vs 8,6%, p=0,019), con la inmunosupresión (48,4% vs 30,9%, p=0,018), con cualquier tipo de trasplante (28,1% vs 14,4%, p=0,020), con el trasplante renal (6,3% vs 0,7%, p=0,018), con la leucemia (10,9% vs 3,6%, p=0,039), con el índice de comorbilidad de Charlson (7,03 vs 5,78 puntos, p=0,0048) y con la toma previa de antibioterapia (54,7% vs 28,8%, p=0,002) (**tabla 1**).

Tabla 1. Características basales de los pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p
Sexo masculino (N, %)	129 (63,6)	91 (65,5)	38 (64,9)	0,402
Edad, media, (DE)	63,1 (14,3)	61,81 (14,5)	65,94 (11,6)	0,147
Cardiopatía (%)	65 (32,0)	36 (25,9)	29 (45,3)	0,001
Enfermedad arterial periférica (%)	28 (13,8)	21 (15,1)	7 (10,9)	0,413
Enfermedad cerebrovascular (%)	25 (12.3)	13 (9.4)	12 (18.8)	0.058
Enfermedad pulmonar (%)	66 (32,5)	39 (28,1)	27 (42,2)	0,028
Hepatopatía crónica (%)	30 (14,8)	16 (11,5)	14 (21,9)	0,120
Insuficiencia renal (%)	43 (21,2)	26 (18,7)	17 (26,6)	0,270
Enfermedad autoinmune (%)	25 (12,3)	12 (8,6)	13 (20,3)	0,019
Úlcera péptica (%)	9 (4,5)	4 (2,9)	5 (7,8)	0,115
Enfermedad hematológica (%)	37 (18,2)	21 (15,1)	16 (25)	0,084
Diabetes mellitus (%)	64 (31,5)	39 (28,1)	25 (39,1)	0,116
Infección por VIH (%)	3 (1,5)	2 (1,4)	1 (1,6)	0,214
Inmunosupresión (%)	74 (36,5)	43 (30,9)	31 (48,4)	0,018
Trasplante (%)	38 (18,7)	20 (14,4)	18 (28,1)	0,020
Hígado (%)	11 (5,4)	8 (5,8)	3 (4,7)	0,755
Pulmón (%)	7 (3,5)	3 (2,2)	4 (6,3)	0,138
Riñón (%)	5 (2,5)	1 (0,7)	4 (6,3)	0,018
Corazón (%)	2 (1)	1 (0,7)	1 (1,6)	0,572
Médula ósea (%)	14 (6,9)	7 (5)	7 (10,9)	0,123
Neoplasia	86 (42,5)	55 (39,6)	31 (48,4)	0,109
Leucemia (%)	12 (5,9)	5 (3,6)	7 (10,9)	0,039
Linfoma/MM (%)	15 (7,4)	10 (7,2)	5 (7,8)	0,876
Sólido local (%)	41 (20,2)	27 (19,4)	14 (21,9)	0,686
Sólido MTX (%)	17 (8,4)	14 (10)	3 (4,7)	0,198
Otros (%)	4 (2)	0 (0)	4 (6,3)	0,003

Antibioterapia previa (%)	75 (37)	40 (28,8)	35 (54,7)	0,001
Demencia (%)	5 (2,5)	2 (1,4)	3 (4,7)	0,168
Dependencia (%)	29 (14,3)	17 (12,2)	12 (18,8)	0,261
ICC, media (DE)	6,17 (3,98)	5,78 (4,31)	7,03 (3,01)	0,0048

DE: Desviación estándar, VIH: Virus de la inmunodeficiencia humana, MM: Mieloma múltiple, MTX: metastásico, ICC: Índice de comorbilidad de Charlson.

La **tabla 2** muestra la mortalidad en función del lugar de procedencia, del servicio de origen y en función de los procedimientos realizados durante el ingreso. El 78,3% procedía del domicilio, un 2% de una residencia de ancianos o centro socio-sanitario y un 19,2% había sido trasladado desde otro centro hospitalario. Un 33,8% de los pacientes había estado hospitalizado, durante al menos 24 horas, en los 90 días previos al ingreso actual. El 39,9% de las infecciones tenían origen nosocomial. En relación con el origen del ingreso a la UCI, el 52,7% procedía de Urgencias, mientras que el 7,4% fue un traslado directo. De los pacientes ingresados en la UCI durante su estancia hospitalaria, el 9,4% ingresaron desde cirugía general, el 6.4% desde la planta de trasplante y el 5,9% desde la planta de medicina interna y hematología, respectivamente. En 111 pacientes (54,7%) se llevó a cabo algún procedimiento quirúrgico durante el ingreso en UCI o con anterioridad. Doce pacientes (5,9%) ingresaron por cirugía programada, en un 48,8% se realizó alguna técnica o procedimiento urgente y en un 38,4% la intervención se realizó para el control del foco infeccioso. De ellos, en un 35,1% se realizaron tratamientos intervencionistas, en un 23,7% cirugía abierta o laparoscópica y en un 21,6% procedimientos endoscópicos. Treinta y dos pacientes fueron sometidos a una segunda intervención quirúrgica, endoscópica o mediante radiología intervencionista.

El análisis de mortalidad mostró asociación estadística con la hospitalización previa (48,4% vs 26,6%, p=0.009), con la infección de origen nosocomial (54,7% vs 33,1%, p=0,004) y con los ingresos procedentes de Medicina Interna (10,9% vs 3,6%, p=0,039). Los ingresos procedentes de Urgencias (37,5% vs 59,7%, p=0,003) y la realización de procedimientos endoscópicos (20,3% vs 7,9%, p=0,023) o de la intervención para control del foco (34,4% vs 40,3%, p=0,045) se asociaron a una menor tasa de mortalidad (**Tabla 2**).

Tabla 2. Características de los pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p para mortalidad
Domicilio (%)	159 (78,3)	112 (80,6)	47 (73,4)	0,251
Residencia (%)	4 (12)	3 (2,2)	1 (1,6)	0,777
Traslado desde otro centro (%)	39 (19,2)	24 (17,3)	16 (25)	0,216
Hospitalización previa (%)	68 (33,8)	37 (26,6)	31 (48,4)	0,009
Nosocomial (%)	81 (39,9)	46 (33,1)	35 (54,7)	0,004
Servicio de origen				
Urgencias (%)	107 (52,7)	83 (59,7)	24 (37,5)	0,003
Traslado directo (%)	15 (7,4)	9 (6,5)	6 (9,4)	0,463
Cirugía general (%)	19 (9,4)	12 (8,6)	7 (10,9)	0,600
Trasplante (%)	13 (6,4)	7 (5)	6 (9,4)	0,240
Medicina interna (%)	12 (5,9)	5 (3,6)	7 (10,9)	0,039
Hematología (%)	12 (5,9)	7 (5)	5 (7,8)	0,459
Box vital (%)	4 (2)	2 (1,4)	2 (3,1)	0,422
Nefrología (%)	4 (2)	2 (1,4)	2 (3,1)	0,422
Digestivo (%)	4 (2)	2 (1,4)	2 (3,1)	0,422
Otros (%)	15 (7,39)	11 (7,9)	4 (6,3)	0,664
Intervención quirúrgica (%)	111 (54,7)	73 (52,5)	38 (59,4)	0,362
Programada (%)	12 (5,9)	6 (4,3)	6 (9,4)	0,304
Urgente (%)	99 (48,8)	67 (48,2)	32 (50)	0,812
Para control foco (%)	78 (38,4)	56 (40,3)	22 (34,4)	0,045
Endoscópica (%)	24 (21,6)	11 (7,9)	13 (20,3)	0,023
Abierta/laparoscopia (%)	48 (23,7)	31 (22,3)	17 (26,6)	0,507
Radiología intervencionista (%)	39 (35,1)	31 (22,3)	8 (12,5)	0,099
Segunda intervención (%)	32 (15,8)	26 (18,7)	6 (9,4)	0,090

DE: Desviación estándar, UCI: Unidad cuidados intensivos.

Por otra parte, durante el ingreso en UCI y de acuerdo con los criterios clasificatorios expuestos, 161 pacientes (79,3%) padecieron shock séptico (74,1% en el grupo de supervivientes vs 90,6% en el grupo de no supervivientes, p=0,007) (**tabla 3),** mientras que 29 pacientes (14,3%) fueron diagnosticados de SDRA, sin que ello conllevase mayor tasa de mortalidad. La mediana de la estancia en UCI fue de 6 días y la mediana de la estancia hospitalaria 20 días.

Tabla 3. Diagnósticos y estancias de los pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p
Sepsis (%)	163 (80,3)	113 (69,3)	50 (30,7)	0,598
Shock séptico (%)	161 (79,3)	103 (74,1)	58 (90,6)	0,007
SDRA (%)	29 (14,3)	18 (62,47)	11 (37,93)	0,423
Estancia en UCI, mediana (P25-P75)	6 (2-16)	5 (3-12)	7 (2-23,5)	0,3318
Estancia hospitalaria, mediana (P25-P75)	20 (10-44)	19 (11-42)	19,5 (5-56,5)	0,226

SDRA: Síndrome de distrés respiratorio agudo, UCI: Unidad de Cuidados Intensivos, P25: Percentil 25, P75: Percentil 75.

5.1.2. Foco y etiología de la infección.

En la **tabla 4** se presentan los focos infecciosos de la sepsis (foco primario) u otros focos infecciosos que aparecieron de forma sincrónica o posterior (foco secundario). El foco respiratorio fue el más frecuente (38,9%), seguido del foco abdominal (36%), del urinario (12,3%), del de piel y partes blandas (6,90%), de la bacteriemia asociada a catéter y de la endocarditis (3,9%). Otros focos (7,4%) incluyeron a 4 pacientes con infecciones en el SNC o en el territorio otorrinolaringológico, 1 paciente con infección diseminada tras la instilación del bacilo de Calmette-Guérin (BCG, "BCG-itis") y un paciente con bacteriemia primaria sin foco identificado. 15 pacientes presentaron más de un foco primario y en 5 pacientes no se identificó el foco infeccioso. En el total, un 35,5% de los pacientes presentó bacteriemia asociada al foco primario.

Por otra parte, se analizaron otros focos que pudieron aparecer asociados o de forma independiente al foco primario durante el ingreso en la UCI (focos secundarios), presentes en un 21,7% de los pacientes (**Tabla 4**). Los más frecuentes fueron el foco abdominal (4,9%) y la bacteriemia asociada a catéter (4,4%). En tercer lugar, un 3,9% padeció una infección secundaria de origen

respiratorio y de piel o partes blandas. Un 6,4% presentó bacteriemia en relación con el foco secundario.

En el total, considerando todos los focos, un 40,4% presentó bacteriemia y un 2% candidemia. Entre los fallecidos hubo un mayor número de pacientes con un foco secundario de origen abdominal (9,4% vs 2,9%, p=0,047).

Por otro lado, la causa microbiológica se analizó de forma conjunta para los focos primarios y secundarios, documentándose el microorganismo causal en un 80,8% de los pacientes (**tabla 5**). Se identificaron estafilococos en un 9,4% de los pacientes, estreptococos en un 14,3%, enterococos en un 14,8%, enterobacterias en un 33%, bacterias gram-negativas no fermentadoras en un 9,4%, anaerobios en un 8,4%, hongos en un 7,9% y virus en un 10,3% de los pacientes con sepsis. Sólo las infecciones fúngicas se asociaron significativamente con la mortalidad (4,7% vs 0,7%, p=0,046).

Tabla 4. Origen de la infección en pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p
Foco primario				
Respiratorio (%)	79 (38,9)	54 (38,8)	25 (39,1)	0,977
Urinario (%)	25 (12,3)	20 (14,4)	5 (7,8)	0,185
PyPB (%)	14 (6,9)	10 (7,2)	4 (6,3)	0,805
Abdominal (%)	73 (36)	49 (35,3)	24 (37,5)	0,756
BAC (%)	8 (3,9)	3 (2,2)	5 (7,8)	0,054
Endocarditis (%)	8 (3,9)	3 (2,2)	5 (7,8)	0,054
Otros (%)	6 (3)	5 (3,6)	1 (1,6)	0,463
Sin foco (%)	5 (2,5)	2 (1,4)	3 (4,7)	0,165
Foco secundario/complicaciones infecciosas (%)	44 (21,7)	27 (19,4)	17 (26,6)	0,686
Respiratorio (%)	8 (3,9)	6 (4,3)	2 (3,1)	0,685
Urinario (%)	6 (3)	4 (2,9)	2 (3,1)	0,923
PyPB (%)	8 (3,9)	5 (3,6)	3 (4,7)	0,711
Abdominal (%)	10 (4,9)	4 (2,9)	6 (9,4)	0,047
BAC (%)	9 (4,4)	6 (4,3)	3 (4,7)	0,905
Sin foco (%)	1 (0,5)	1 (0,7)	0 (0)	0,496
Otros (%)	2 (1)	1 (0,7)	1 (1,6)	0,572
Bacteriemia	82 (40,4)	56 (40,3)	26 (40,6)	0,964
Bacteriemia primaria (%)	72 (35,5)	49 (35,3)	23 (35,9)	0,993
Bacteriemia secundaria (%)	13 (6,4)	9 (6,5)	4 (6,3)	0,838
Candidemia (%)	4 (2)	1 (0,7)	3 (4,7)	0,060

PyPB: Piel y partes blandas, BAC: Bacteriemia asociada a catéter.

Tabla 5. Microbiología de la infección en pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p
Estafilococos (%)	19 (9,4)	12 (8,6)	7 (10,9)	0,610
SAMS (%)	12 (5,9)	9 (6,5)	3 (4,7)	0,756
SAMR (%)	3 (1,5)	2 (1,4)	1 (1,6)	1,000
Coagulasa-negativo (%)	4 (2)	1 (0,7)	3 (4,7)	0,094
Estreptococos (%)	29 (14,3)	24 (17,3)	5 (7,8)	0,086
Enterococos (%)	30 (14,8)	20 (14,4)	10 (15,6)	0,833
Enterobacterias (%)	67 (33)	47 (33,8)	20 (31,30)	0,751
Sensibles (%)	48 (23,6)	36 (25,9)	12 (18,8)	0,291
BLEE (%)	11 (5,4)	6 (4,3)	5 (7,8)	0,328
MDR (%)	3 (1,5)	2 (1,4)	1 (1,6)	1,000
BGNNF (%)	19 (9,4)	13 (9,4)	6 (9,4)	1,000
Anaerobios (%)	17 (8,4)	12 (8,6)	5 (7,8)	1,000
Hongos (%)	16 (7,9)	7 (5)	9 (14,1)	0,046
Virus (%)	21 (10,3)	16 (11,5)	5 (7,8%)	0,620
Organismo desconocido (N%)	39 (19,2)	29 (20,9)	10 (15,6%)	0,446

SAMS: *Staphylococcus aureus* meticilin-sensible, SAMR: *Staphylococcus aureus* meticilin-resistente, BLEE: Betalactamasas de espectro extendido, MDR: Multirresistentes, BGNNG: Bacterias gram-negativas no fermentadoras.

5.1.3. Parámetros clínicos y analíticos al ingreso en UCI

En relación con el estudio de mortalidad y cálculo de las distintas escalas pronósticas o de riesgo, se analizaron los valores clínicos o analíticos más patológicos obtenidos en las primeras 24 horas del ingreso en la UCI (**tablas 6 y 7**). Los pacientes fallecidos tuvieron una menor puntuación en la escala de coma de Glasgow (13,3 vs 14,2, p<0,001), menor temperatura (36,5 vs 37,4 ºC, p=0,002), mayor frecuencia respiratoria (30 vs 27,3 respiraciones por minuto, p=0,0258), menor diuresis (p<0,001), mayor necesidad de noradrenalina (92,2% vs 75,5%, p=0,007), mayor uso de ventilación

mecánica invasiva (70,3% vs 33,1%, p<0,001) y mayor necesidad de terapia renal sustitutiva (57,9% vs 42,1%, p<0,001).

Considerando los valores analíticos, la mortalidad se asoció con los valores del pH (7,260 vs 7,319, p<0,001), con la PaO2 (99,9 vs 90,5 mmHg, p=0,047), con la PaCO2 (42,79 vs 39,19 mmHg p=0,034), con la urea sérica (111,4 vs 85 mg/dl, p<0,001), con la creatinina sérica (2,35 vs 2,06 mg/dl, p=0,037), con los leucocitos (16.061 vs 16.914 vs x1000µ/L, p=0,041), con los linfocitos (1129 vs 884 vs x1000µ/L, p=0,013), con las plaquetas (161.000 vs 197.000 x1000µ/L, p<0,001), con la hemoglobina (10,7 vs 12 g/dl, p<0,001), con la AP (49 vs 56,2 segundos, p=0,014), con el tiempo de tromboplastina parcial activada (TTPA) (51,9 vs 43,1 segundos, p<0,001), con el índice internacional normalizado (INR) (2,10 vs 1,80, p=0,046) y con el fibrinógeno (522 vs 661 mg/dl, p=0,002).

Tabla 6. Parámetros clínicos en pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p
GCS (Media, DE)	13,91 (2,47)	14,18 (2,24)	13,33 (2,85)	<0,001
Temperatura (ºC) (Media, DE)	37,12 (1,57)	37,38 (1,47)	36,53 (1,66)	0,002
TAM (mmHG) (Media, DE)	69,48 (20,67)	69,71 (21,81)	68,99 (18,15)	0,829
Defecto relleno capilar (N, %)	63 (31,19%)	38 (27,3%)	25 (39,1%)	0,100
FC (lpm) (Media, DE)	107 (23)	107 (21)	107 (27)	0,862
FR (rpm) (Media, DE)	28 (8)	27 (8)	30 (8)	0,0258
Sat02 (%) (Media, DE)	92,60 (6,41)	92,55 (5,91)	92,70 (7,43)	0,280
Diuresis (ml/24h) (N, %)				
>500 (N, %)	134 (66,34%)	108 (77,8%)	26 (40,6%)	<0,001
200-500 (N, %)	35 (17,33%)	16 (11,5%)	19 (29,7%)	0,001
<200 (N, %)	33 (16,34%)	15 (10,8%)	18 (28,1%)	0,001
Vasopresores (N, %)				
Dopamina (N, %)	23 (11,33%)	18 (12,9%)	5 (7,8%)	0,195
Dobutamina (N, %)	12 (6,06%)	7 (5%)	5 (7,8%)	0,450
Noradrenalina (N, %)	164 (80,79%)	105 (75,5%)	59 (92,2%)	0,007
Soporte respiratorio (N, %)	194 (95,57%)	132 (95%)	62 (97%)	0,539
GN-Ventimask (N, %)	70 (34,5%)	57 (41%)	13 (20,1%)	0,004

GNAF (N, %)	28 (13,79%)	25 (18%)	3 (4,7%)	0,01
VMNI (N, %)	5 (2,46%)	4 (2,9%)	1 (1,6%)	0,574
VMI (N, %)	91 (44,83%)	46 (33,1%)	45 (70,3%)	<0,001
FiO2 (Media, DE)	52,8 (26,5)	51,8 (26,3)	55 (27,1)	0,386
PaO2/FiO2 (Media, DE)	232 (157)	230 (155)	237 (163)	0,793
Terapia renal sustitutiva (N, %)	28,1% (57)	24 (42,1%)	33 (57,9)	<0,001

GCS: Escala de coma de Glasgow, DE: Desviación estándar, TAM: Tensión arterial media, FC: Frecuencia cardíaca, FR: Frecuencia respiratoria, Sat02: Saturación de oxígeno, GN: Gafas nasales, GNAF: Gafas nasales de alto flujo, VMNI: Ventilación mecánica no invasiva, VMI: Ventilación mecánica invasiva, FiO2: Fracción inspirada de oxígeno, PaO2/FiO2: Ratio presión arterial de oxígeno/fracción inspirada de oxígeno.

Tabla 7. Parámetros analíticos en pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p
Bioquímica				
pH (Media, DE)	7,30 (0,11)	7,32 (0,11)	7,26 (0,11)	<0,001
Bicarbonato (mmol/L), media (DE)	19,9 (4,5)	20,2 (4,2)	19,1 (5,1)	0,144
Lactato (mmol/L), media (DE)	3,8 (3,1)	3,5 (2,3)	4,7 (4,4)	0,505
PaO2 (mmHG), media (DE)	93,5 (37,5)	90,5 (38,1)	99,9 (35,6)	0,047
PaCO2 (mmHG), media (DE)	40,3 (12,2)	39,2 (11,6)	42,8 (13,1)	0,033
Urea (mg/dl), media (DE)	93 (61)	85 (60)	111 (60)	<0,001
Creatinina (mg/dl), media (DE)	2,2 (1,7)	2,1 (1,7)	2,4 (1,5)	0,036
Glucosa (mg/dl), media (DE)	173 (90)	175 (81)	169 (107)	0,278
Sodio (mmol/L), media (DE)	138 (6)	137 (5)	139 (8)	0,249
Potasio (mmol/L), media (DE)	4,4 (1)	4,3 (0,9)	4,6 (1)	0,150
Cloro (mmol/L), media (DE)	103 (8)	103 (8)	104 (7)	0,426
Bilirrubina (mg/dl), media (DE)	2,2 (3,8)	1,9 (3,2)	2,81 (4,8)	0,113
PCR (mg/L), media (DE)	166 (90)	167 (86)	162 (98)	0,819
PCT (ng/ml), media (DE)	19 (32)	18 (27)	22 (42)	0,513

Hemograma				
Leucocitos (x1000µ/L), media (DE)	16.645 (14.595)	16.914 (11.525)	16.061 (19.788)	0,041
Neutrófilos (x1000µ/L), media (DE)	13.954 (12.564)	14.228 (10.112)	13.359 (16.784)	0,052
Linfocitos (x1000µ/L), media (DE)	961 (2.109)	884 (896)	1129 (3.530)	0,013
Eosinófilos (x1000µ/L), media (DE)	125 (709)	153(847)	64 (182)	0,791
Plaquetas (x1000µ/L), media (DE)	185(138)	197 (128)	160 (158)	<0,001
Hemoglobina (g/dL), media (DE)	11,60 (2,65)	12,00 (2,53)	10,73 (2,72)	<0,001
Hematocrito (%), media (DE)	35 (8)	36 (7)	33 (9)	<0,001
VCM (fl), media (DE)	90 (8)	89 (7)	91 (10)	0,292
CHCM (g/dl), media (DE)	33 (2)	33 (2)	33 (2)	0,643
ADE (%), media (DE)	15,66 (2,88)	15,06 (2,82)	16,95 (2,58)	<0,001
Coagulación				
AP (%), media (DE)	54 (21)	56 (21)	49 (21)	0,014
TTPA (segundos), media (DE)	45,8 (13,8)	43,1 (9,4)	51,9 (19,3)	<0,001
INR, media (DE)	1,89 (1,51)	1,80 (1,48)	2,10 (1,56)	0,046
Fibrinógeno (mg/dl), media (DE)	618 (237)	661 (210)	522 (265)	0,002

DE: Desviación estándar, PaO2: Presión arterial de oxígeno, PaCO2: Presión arterial de dióxido de carbono. PCR: Proteína C reactiva, PCT: Procalcitonina, VCM: Volumen corpuscular medio, CHCM: Concentración hemoglobina corpuscular media, AP: Actividad de protrombina, TTPA: Tiempo de tromboplastina parcial activada, INR: índice internacional normalizado.

5.1.4. Escalas diagnósticas y pronósticas

Los valores de las escalas diagnósticas y pronósticas se calcularon con los datos del ingreso en la UCI y se muestran en la **tabla 8**. Los valores medios de las escalas fueron de 9.06 puntos para la escala SOFA, 7,92 para la escala LODS, 19,94 para la escala APACHE-II y 51,42 para la escala SAPS-II. El 33,51% cumplía los criterios del qSOFA y un 77,83% los del SIRS. Los pacientes fallecidos presentaron mayores puntuaciones que los supervivientes en las escalas NEWS2 (10,93 vs 9,21, p=0.08), SOFA (11,3 vs 7,99, p<0,001), LODS (9,09 vs 7,37, p<0,001), APACHE-II (22,72 vs 18,65, p<0,001) y SAPS-II (59,53 vs 47,68, p<0,001), mientras que no se encontraron diferencias estadísticamente significativas en relación con los criterios qSOFA o SIRS. De acuerdo con lo anterior, la capacidad discriminatoria de la mortalidad de las escalas se evaluó mediante un análisis del AUC-ROC (**tabla 9)**. Sólo la escala SOFA y SAPS-II mostraron un AUC-ROC por encima de 0,700, mientras que las escalas qSOFA y SIRS estaban por debajo de 0,600.

Tabla 8. Escalas diagnósticas y pronósticas en pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	No supervivientes (n=64)	Valor de p
QSOFA (%)	66 (32,5)	42 (30,2)	24 (37,5)	0,304
NEWS2, media (DE)	9,72 (3,36)	9,21 (2,91)	10,83 (3,98)	0,008
SIRS (%)	158 (77,8)	110 (79,1)	48 (75)	0,510
SOFA, media (DE)	9,06 (3,58)	7,99 (3,24)	11,39 (3,19)	<0,001
LODS, media (DE)	7,92 (2,181)	7,37 (1,69)	9,09 (2,64)	<0,001
APACHE-II, media (DE)	19,94 (5,94)	18,65 (5,14)	22,72 (6,62)	<0,001
SAPS-II, media (DE)	51,42 (13,93)	47,68 (12,13)	59,53 (14,22)	<0,001

qSOFA: quick-SOFA, DE: Desviación estándar, NEWS2: National Early Warning Score 2, SIRS: Síndrome de Respuesta Inflamatoria Sistémica, SOFA: Sequential Organ Failure Assessment, LODS: Logistic Organ Dysfunction System, APACHE-II: Acute Physiology and Chronic Health Evaluation-II, SAPS-II: Simplified Acute Physiology Score-II.

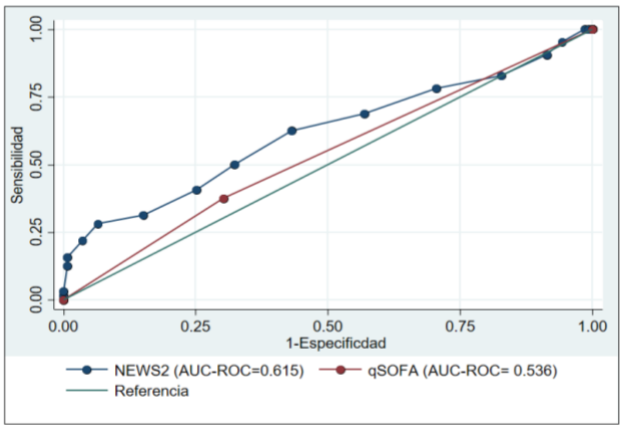
Tabla 9. Capacidad de discriminación de mortalidad de las escalas diagnósticas y pronósticas en pacientes ingresados en la UCI por sepsis

Escala	OR mortalidad (IC 95%)	AUC-ROC (IC 95%)
qSOFA	1,39 (0,74-2,58)	0,536 (0,465-0,607)
NEWS2	1,16 (1,06-1,27)	0,615 (0,526-0,704)
SOFA	1,39 (1,24-1,55)	0,776 (0,705-0,846)
LODS	1,48 (1,26-7,74)	0,693 (0,613-0,772)
SIRS	0,79 (0,39-1,59)	0,521 (0,457-0,584)
APACHE-II	1,13 (1,07-1,20)	0,673 (0,592-0,755)
SAPS-II	1,07 (1,04-1,10)	0,738 (0,665-0,811)

OR: Odds ratio, AUC-ROC: Área Bajo la Curva Característica Operativa del Receptor. IC: Intevarlo de confianza, qSOFA: quick-SOFA, NEWS2: National Early Warning Score 2, SOFA: Sequential Organ Failure Assessment, LODS: Logistic Organ Dysfunction System, SIRS: Síndrome de Respuesta Inflamatoria Sistémica, APACHE-II: Acute Physiology and Chronic Health Evaluation-II, SAPS-II: Simplified Acute Physiology Score-II.

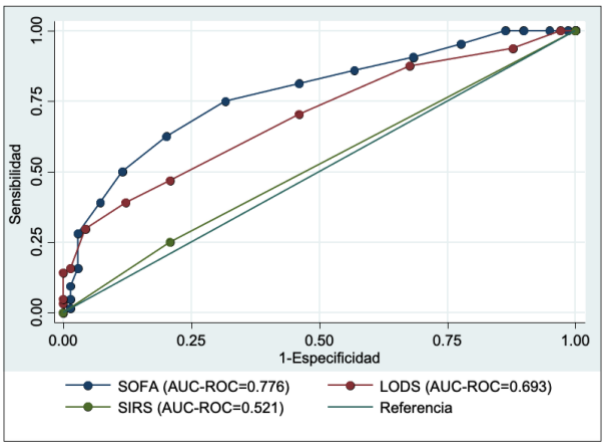
Finalmente, la capacidad de discriminación de las escalas se comparó en función de su aplicación, diseño y las variables analizadas. De esta forma, se evaluaron las escalas utilizadas para la identificación precoz de la sepsis basadas en parámetros clínicos (qSOFA y NEWS) (**figura 1**), las escalas que determinan y cuantifican el daño orgánico (SOFA, LODS, SIRS) (**figura 2**) y las escalas pronósticas de mortalidad y gravedad (APACHE-II y SAPS-II) (**figura 3**). La escala NEWS2 mostró mejor capacidad predictiva que qSOFA (AUC-ROC=0,615 vs 0,536, p=0,039), así como la escala SOFA en comparación con LODS y SIRS (AUC-ROC= 0,776 vs 0,693 vs 0,521, respectivamente, p<0.01). A pesar de que SAPS-II presentó mayor AUC-ROC que APACHE-II (AUROC=0,738 vs AUROC=0,673), esta diferencia no alcanzó la significación estadística (p=0,08).

Figura 1. Capacidad discriminatoria de las escalas qSOFA y NEWS2 en pacientes ingresados en la UCI por sepsis



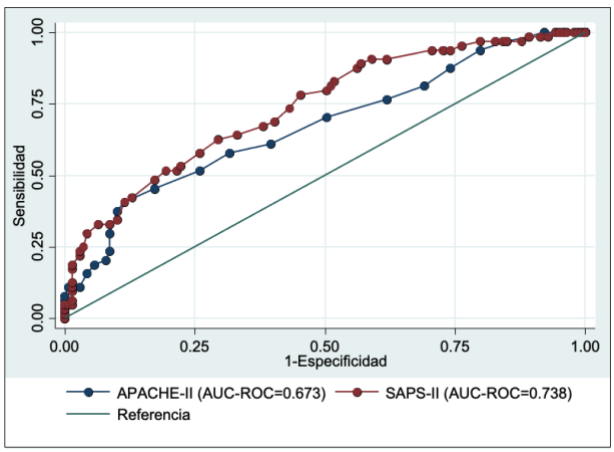
NEWS2: National Early Warning Score 2, AUC-ROC: Área bajo la curva operativa característica del receptor, qSOFA: Quick-SOFA

Figura 2. Capacidad discriminatoria de las escalas SOFA, LODS y SIRS en pacientes ingresados en la UCI por sepsis



SOFA: Sequential Organ Failure Assessment, AUC-ROC: Área bajo la curva operativa característica del receptor, LODS: Logistic Organ Dysfunction System. SIRS: Síndrome de Respuesta Inflamatoria Sistémica.

Figura 3. Capacidad discriminatoria de las escalas APACHE-II y SAPS-II en pacientes ingresados en la UCI por sepsis



APACHE-II: Acute Physiology and Chronic Health Evaluation-II, AUC-ROC: Área bajo la curva operativa característica del receptor, SAPS-II: Simplified Acute Physiology Score-II.

5.1.5. Análisis de los biomarcadores

Durante el ingreso en UCI se analizaron los biomarcadores y sus variaciones durante los 30 días tras el ingreso, aunque el análisis longitudinal se realizó durante la primera semana.

5.1.5.1. Proteína C reactiva (PCR)

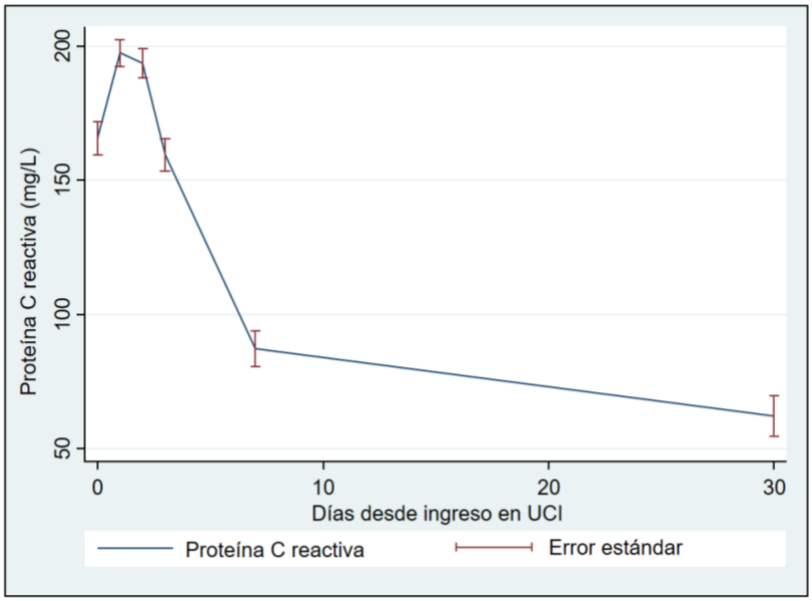
La dinámica de los valores de la PCR durante los 30 días tras el ingreso en UCI se muestra en la **tabla 9 y figura 4**. Sus cifras se elevan durante las primeras 24 horas de ingreso en UCI, momento en el que alcanzan su valor máximo promedio (219 mg/L), con una tendencia descendente posterior. En relación con la mortalidad, los pacientes fallecidos presentaron menores valores de PCR a las 24 horas del ingreso (media de 180 vs 206 mg/L, p=0,038) pero mayores valores a las 72 horas (media de 184 vs 151mg/L, p=0,023) y a los 7 días (media de 130 vs 76 mg/L, p=0,001) (**Figura 5**). No se demostró asociación de la mortalidad con los valores de la PCR al ingreso, a las 48 horas, a los 30 días ni con los valores máximos o momento en el que se identificó el valor máximo. En el análisis longitudinal (EEG) de la PCR durante los primeros 7 días de ingreso no se observaron diferencias estadísticamente significativas entre los pacientes supervivientes y fallecidos (OR=1,00, IC 95% 0,99-1,00, p=0,957).

Tabla 9. Valores de la Proteína C reactiva en pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p
PCR al ingreso (mg/L), media (DE)	166 (90)	167 (86)	162 (98)	0,820
PCR 24h (mg/L), media (DE)	197 (68)	206 (61)	180 (80)	0,038
PCR 48h (mg/L), media (DE)	164 (70)	200 (68)	179 (73)	0,105
PCR 72h (mg/L), media (DE)	159 (77)	151 (77)	184 (73)	0,023
PCR 7d (mg/L), media (DE)	87 (85)	76 (76)	130 (100)	0,001
PCR 30d (mg/L), media (DE)	62 (66)	52 (54)	108 (92)	0,07
PCR máxima (mg/L), media (DE)	219 (67)	226 (60)	204 (79)	0,064
Días desde ingreso hasta PCR máxima, mediana (P25-P75)	0 (0-1)	0 (0-1)	0 (0-1)	0,632

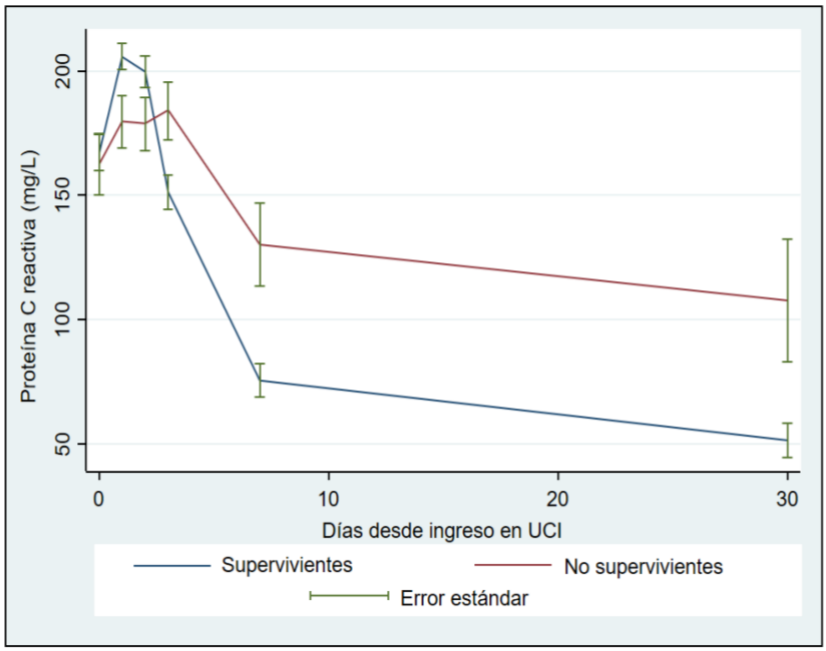
PCR: Proteína C reactiva, DE: Desviación estándar, P25: Percentil 25, P75: Percentil 75.

Figura 4. Dinámica de la PCR en pacientes ingresados en la UCI por sepsis



Los valores se muestran como media y desviación estándar (barras)

Figura 5. Dinámica de la PCR según la mortalidad en pacientes ingresados en la UCI por sepsis



Los valores se muestran como media y desviación estándar (barras)

5.1.5.2. Procalcitonina (PCT)

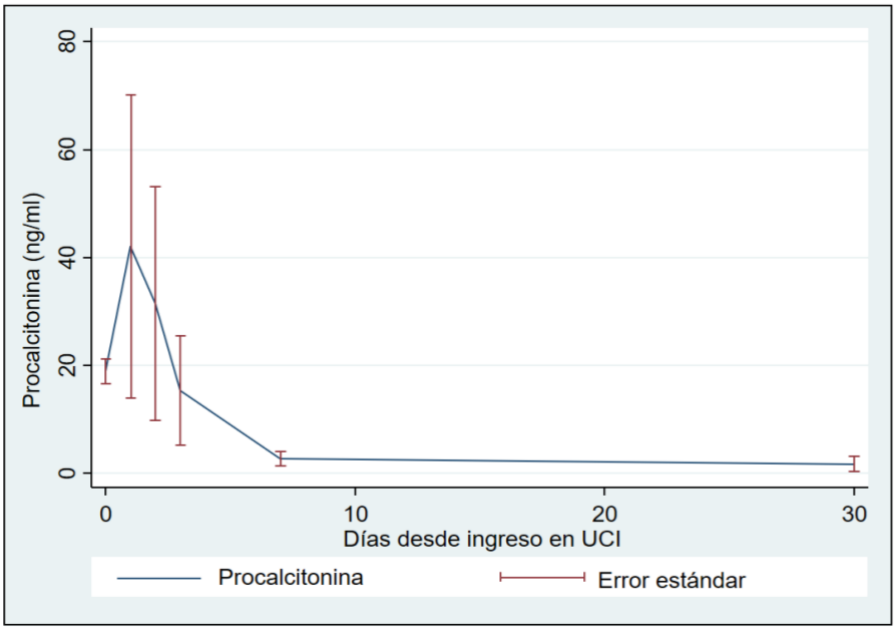
Los mayores valores de la PCT se identificaron durante las primeras 24 horas del ingreso en la UCI y disminuyeron posteriormente, tal y como se muestra en la **tabla 10** y en la **figura 6**. Los pacientes fallecidos presentaron mayores valores de PCT a los 7 días (media de 7,7 vs 0,9 ng/ml, p<0,001) y a los 30 días del ingreso en UCI (media de 5,7 vs 0,2 ng/ml, p=0,003). No se identificaron diferencias para el resto de los valores de PCT o los días que transcurrieron desde el ingreso hasta su mayor valor (**figura 7**). En paralelo, los valores de la PCT durante la primera semana de ingreso en UCI no se asociaron con un mayor riesgo de mortalidad (OR=1,01, IC 95% 0,99-1,02, p=0,130).

Tabla 10. Valores de la Procalcitonina en pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p
PCT al ingreso (ng/ml), media (DE)	18,9 (32,3)	17,8 (27,2)	21,8 (42)	0,513
PCT 24h (ng/ml), media (DE)	45,1 (188,6)	11,7 (17,2)	97,2 (314,2)	0,393
PCT 48h (ng/ml), media (DE)	31,5 (154,6)	9,6 (11,1)	75,3 (267,1)	0,596
PCT 72h (ng/ml), media (DE)	15,4 (91,6)	4,4 (9,7)	39 (161,6)	0,557
PCT 7d (ng/ml), media (DE)	2,8 (14,1)	0,9 (3)	7,7 (26,4)	<0,001
PCT 30d (ng/ml), media (DE)	1,7 (9,5)	0,2 (0,2)	5,7 (17,8)	0,003
PCT máxima (ng/ml), media (DE)	40,1 (202,3)	19,7 (28,4)	88,1 (365,2)	0,921
Días desde ingreso hasta PCT máxima, mediana (P25-P75)	1 (0-1)	1 (0-2)	1 (0-3)	0,547

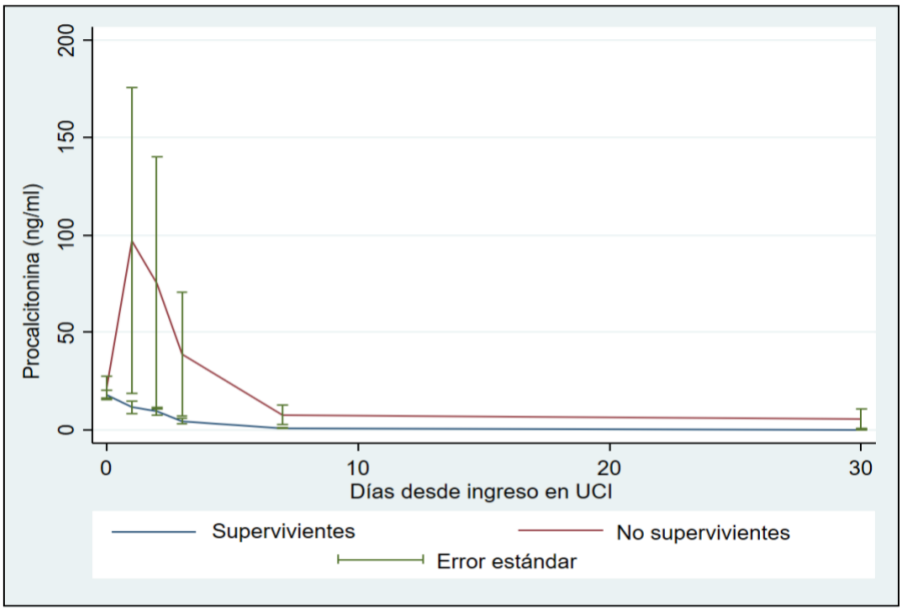
PCT: Procalcitonina, DE: Desviación estándar, P25: Percentil 25, P75: Percentil 75.

Figura 6. Dinámica de la PCT en pacientes ingresados en la UCI por sepsis



Los valores se muestran como media y desviación estándar (barras)

Figura 7. Dinámica de la PCT según la mortalidad en pacientes ingresados en la UCI por sepsis



Los valores se muestran como media y desviación estándar (barras)

5.1.5.3. Ancho de distribución eritrocitaria (ADE)

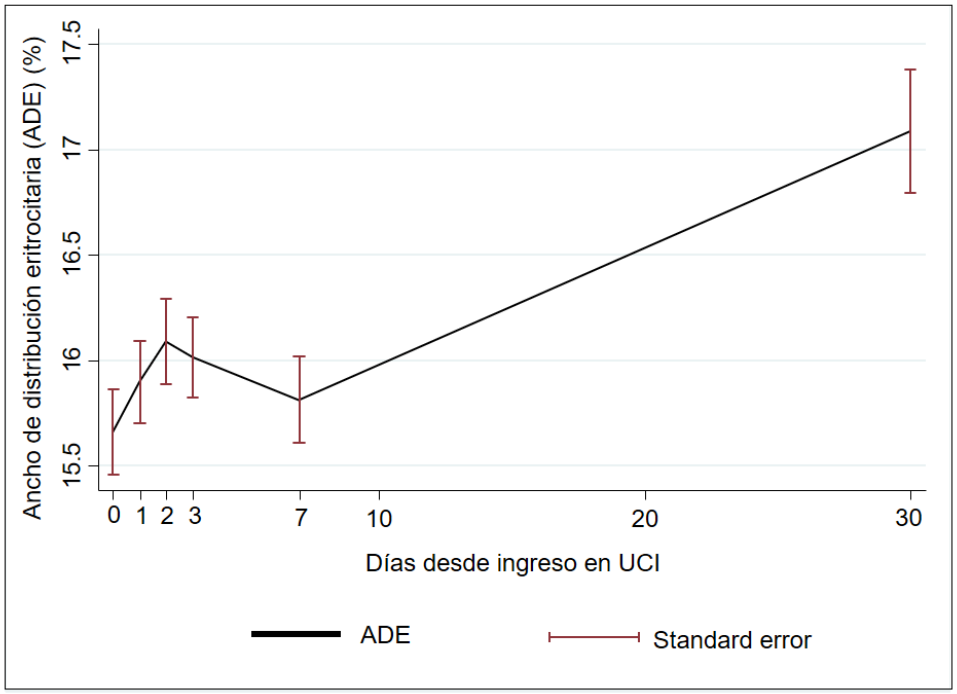
Las variaciones y la dinámica del ADE se muestran en la **tabla 11 y figura 8 y 9**. Los mayores valores medios de ADE durante la primera semana se dieron a las 48 horas (media 16,09%) y 72 horas (media 16,02%) del ingreso, aunque los valores máximos del ADE durante el periodo de hospitalización (media 17,70%) se identificaron a los 16,1 días de media desde el ingreso en UCI. El análisis univariante del ADE con la mortalidad demostró que los pacientes fallecidos presentaban mayores valores de ADE al ingreso (16,95% vs 15,06%, p<0,001, AUC-ROC=0,737), a las 24 horas (17,15% vs 15,35%, p<0,001, AUC-ROC=0,737), a las 48 horas (17,54% vs 15,49%, p<0,001, AUC-ROC=0,750), a las 72 horas (17,55% vs 15,48%, p<0,001, AUC-ROC=0,747) y a los 7 días del ingreso en UCI (17,33% vs 15,40%, p<0,001, AUC-ROC=0,740) (**Tabla 11 y figuras 9-11**). Además, los pacientes que fallecieron presentaron mayores valores máximos de ADE (19,27% vs 16,99%, p<0,001, AUC-ROC=0,733) y este apareció antes (10,5 vs 13,4 días tras el ingreso, p=0,004) con respecto a los supervivientes. El análisis longitudinal (EEG) confirmó que, durante la primera semana de ingreso, el ADE se asociaba con un mayor riesgo de mortalidad (**OR=1,05, 95% IC 1,01-1,10, p=0,048**), (**Figura 12)**.

Tabla 11. Valores del ADE en pacientes ingresados en la UCI por sepsis

	Global (n=203)	Supervivientes (n=139)	Fallecidos (n=64)	Valor de p	AUC-ROC
ADE al ingreso (%), media (DE)	15,66 (2,46)	15,06 (2,82)	16,95 (2,58)	<0,001	0,737
ADE 24h (%), media (DE)	15,90 (2,76)	15,35 (2,73)	17,15 (2,41)	<0,001	0,737
ADE 48h (%), media (DE)	16,09 (2,80)	15,49 (2,66)	17,54 (2,61)	<0,001	0,750
ADE 72h (%), media (DE)	16,02 (2,50)	15,48 (2,18)	17,55 (2,74)	<0,001	0,747
ADE 7d (%), media (DE)	15,81 (2,66)	15,40 (2,45)	17,33 (2,86)	<0,001	0, 740
ADE 30d (%), media (DE)	17,09 (2,71)	16,86 (2,67)	18,22 (2,74)	0,09	-
ADE máximo (%), media (DE)	17,70 (3,39)	16,99 (3,36)	19,27 (2,93)	<0,001	0,733
Días desde ingreso hasta ADE máximo, media (DE)	16,1 (41,1)	13,4 (14,3)	10,5 (16,3)	0,004	-

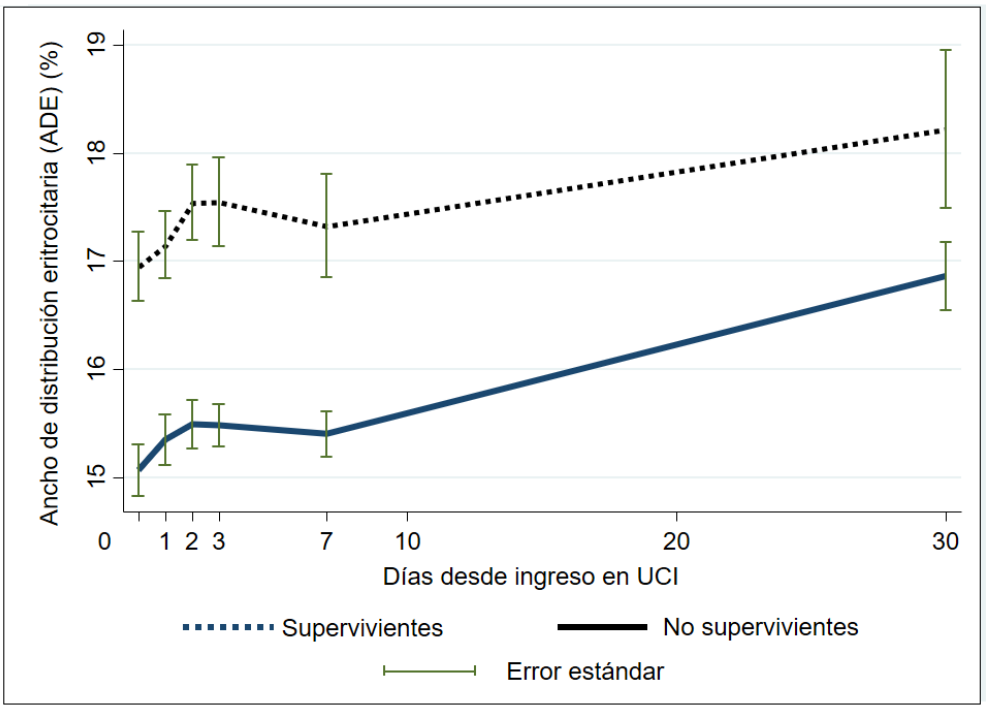
ADE: Ancho de distribución eritrocitaria, DE: Desviación estándar, P25: Percentil 25, P75: Percentil 75.

Figura 8. Dinámica del ADE en pacientes ingresados en la UCI por sepsis



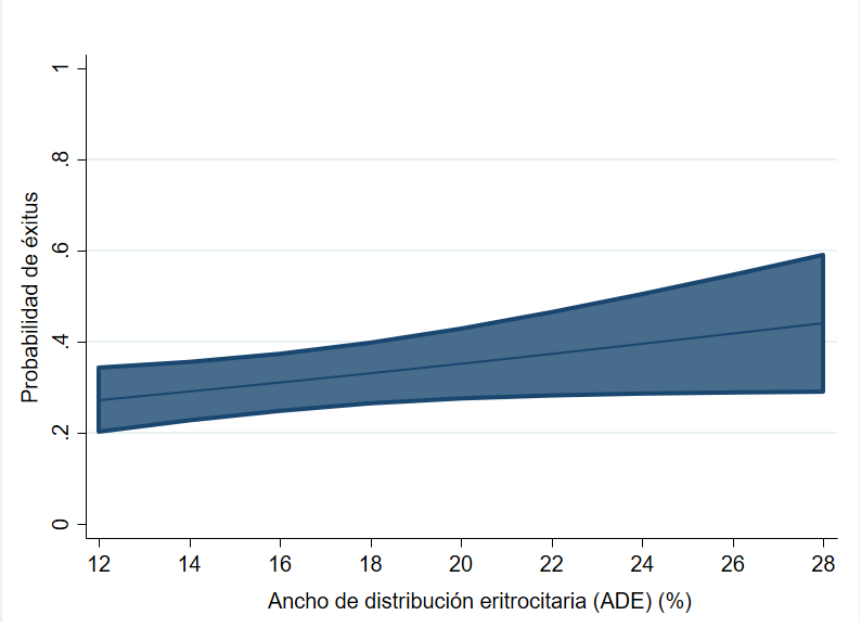
ADE: Ancho de distribución eritrocitaria. Los valores se muestran como media y desviación estándar (barras)

Figura 9. Dinámica del ADE según éxitus en pacientes ingresados en la UCI por sepsis



ADE: Ancho de distribución eritrocitaria. Los valores se muestran como media y desviación estándar (barras)

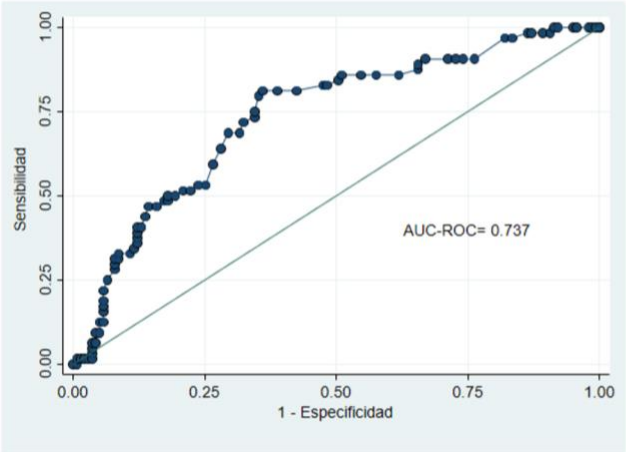
Figura 10. Probabilidad de fallecimiento del paciente en función del ADE en pacientes ingresados en la UCI por sepsis



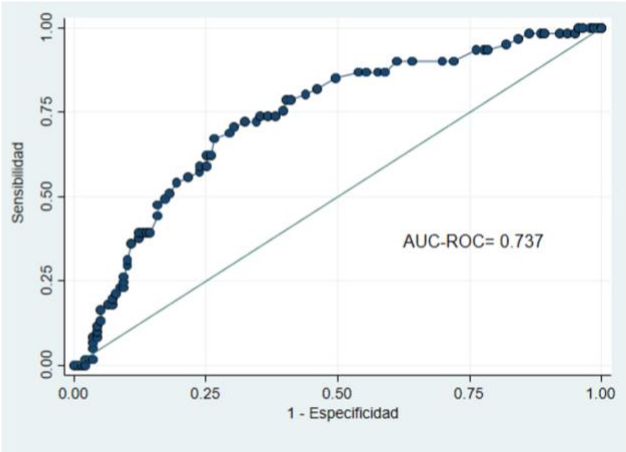
La figura representa la probabilidad predicha por el modelo de la ecuación de estimación generalizada (EEG) de fallecer para cada uno de los valores de ADE a lo largo del tiempo, expresado con un intervalo de confianza al 95% (zona sombreada). ADE: Ancho de distribución eritrocitaria.

Figura 11. Capacidad discriminatoria de mortalidad del ancho de distribución eritrocitaria en pacientes ingresados en la UCI por sepsis

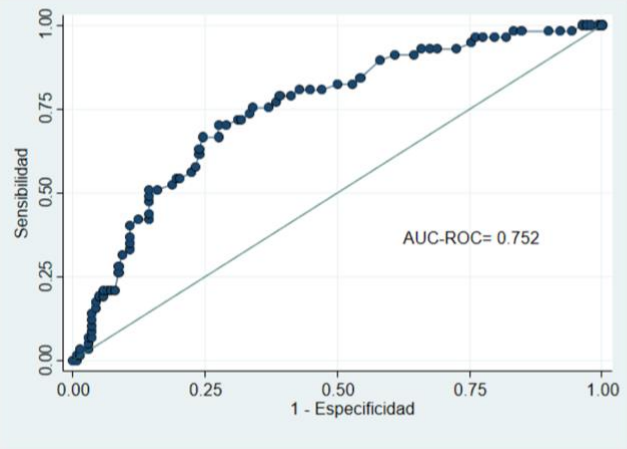
A. ADE al ingreso



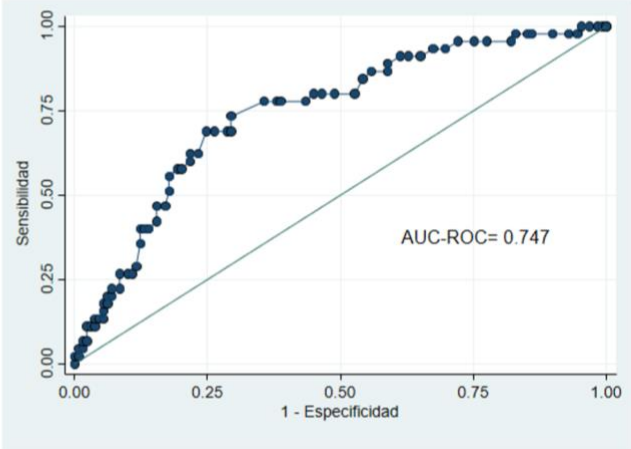
B. ADE a las 24 horas



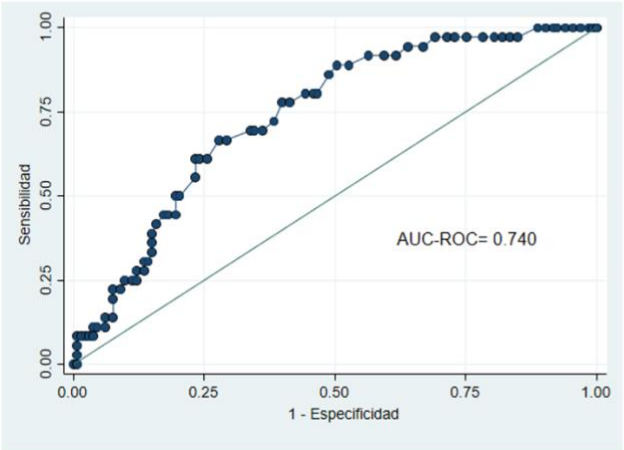
C. ADE a las 48 horas



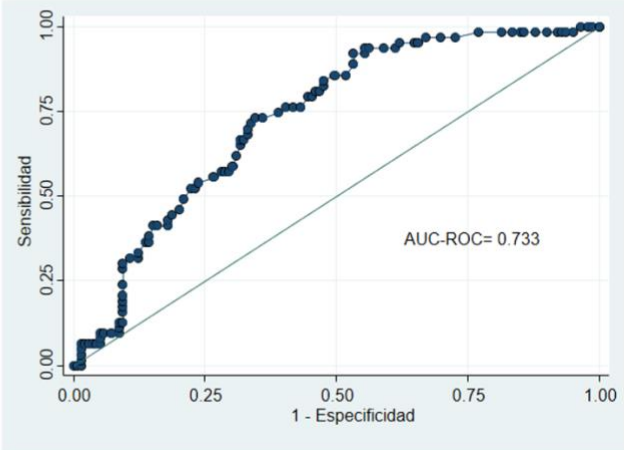
D. ADE a las 72horas



E. ADE a los 7 días



F. Valores máximos de ADE



ADE: Ancho de distribución eritrocitaria, AUC-ROC: Área bajo la curva operativa característica del receptor.

5.1.5.3.1. Análisis multivariante de mortalidad

Con el objetivo de determinar la asociación independiente del ADE con la mortalidad, se realizó un análisis multivariante de mortalidad para cada valor del ADE (ingreso, 24h, 48h, 72h, 7 días y valor máximo), considerando como variables de ajuste: el índice de comorbilidad de Charlson, la inmunosupresión y la infección nosocomial para evaluar la edad, las comorbilidades y la situación basal, la escala NEWS2 para cuantificar las variables clínicas, la escala SOFA para determinar el daño orgánico y la escala SAPS-II para estratificar la gravedad de cada paciente, de acuerdo con los resultados del análisis previo de la capacidad discriminatoria de estas escalas. Además, se incluyó el valor de la hemoglobina al ingreso puesto que podría potencialmente influir en el valor del ADE [117,118]. En las **Tablas 12-17** se muestran los distintos análisis multivariantes, para cada determinación del ADE. Sólo la escala SOFA y el ADE al ingreso, 24 horas, 48 horas, 72 horas, y sus valores máximos, fueron factores estadísticamente asociados con la mortalidad.

Tabla 12. Análisis multivariante de mortalidad considerando ADE al ingreso

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Charlson	1,01	0,91-1,12	0,826
Inmunosupresión	1,02	0,46-2,28	0,959
Infección nosocomial	1,15	0,49-2,74	0,748
NEWS2	1,06	0,94-1,20	0,340
SOFA	**1,29**	**1,11-1,49**	**0,001**
SAPS-II	1,01	0,97-1,05	0,508
ADE al ingreso	**1,17**	**1,03-1,34**	**0,018**
Hemoglobina	0,90	0,76-1,05	0,180

NEWS2: National Early Warning Score 2, SOFA: Sequential Organ Failure Assessment, SAPS-II: Simplified Acute Physiology Score-II, ADE: Ancho de distribución eritrocitaria.

Tabla 13. Análisis multivariante de mortalidad considerando ADE a las 24 horas

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Charlson	1,02	0,92-1,13	0,691
Inmunosupresión	1,05	0,47-2,35	0,910
Infección nosocomial	1,03	0,42-2,49	0,956
NEWS2	1,06	0,94-1,20	0,333
SOFA	**1,28**	**1,11-1,49**	**0,001**
SAPS-II	1,01	0,97-1,05	0,618
ADE a las 24 horas	**1,16**	**1,01-1,34**	**0,034**
Hemoglobina	0,89	0,75-1,05	0,166

NEWS2: National Early Warning Score 2, SOFA: Sequential Organ Failure Assessment, SAPS-II: Simplified Acute Physiology Score-II, ADE: Ancho de distribución eritrocitaria.

Tabla 14. Análisis multivariante de mortalidad considerando ADE a las 48 horas

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Charlson	1,01	0,91-1,13	0,796
Inmunosupresión	1,05	0,46-2,37	0,912
Infección nosocomial	1,10	0,45-2,70	0,839
NEWS2	1,07	0,94-1,21	0,319
SOFA	**1,30**	**1,11-1,51**	**0,001**
SAPS-II	1	0,96-1,04	0,975
ADE a las 48 horas	**1,20**	**1,04-1,39**	**0,011**
Hemoglobina	0,87	0,73-1,04	0,118

NEWS2: National Early Warning Score 2, SOFA: Sequential Organ Failure Assessment, SAPS-II: Simplified Acute Physiology Score-II, ADE: Ancho de distribución eritrocitaria.

Tabla 15. Análisis multivariante de mortalidad considerando ADE a las 72 horas

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Charlson	0,98	0,86-1,11	0,726
Inmunosupresión	0,90	0,36-2,23	0,816
Infección nosocomial	1,80	0,65-4,94	0,257
NEWS2	1,09	0,95-1,26	0,223
SOFA	**1,30**	**1,09-1,55**	**0,003**
SAPS-II	0,98	0,94-1,03	0,467
ADE a las 72 horas	**1,30**	**1,09-1,55**	**0,003**
Hemoglobina	0,84	0,69-1,03	0,103

NEWS2: National Early Warning Score 2, SOFA: Sequential Organ Failure Assessment, SAPS-II: Simplified Acute Physiology Score-II, ADE: Ancho de distribución eritrocitaria.

Tabla 16. Análisis multivariante de mortalidad considerando ADE a los 7 días

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Charlson	1,02	0,89-1,16	0,795
Inmunosupresión	0,95	0,37-2,43	0,908
Infección nosocomial	2,33	0,82-6,61	0,111
NEWS2	1,10	0,94-1,28	0,221
SOFA	**1,30**	**1,08-1,57**	**0,006**
SAPS-II	0,96	0,91-1,01	0,136
ADE a los 7 días	1,16	0,99-1,35	0,065
Hemoglobina	0,82	0,66-1,02	0,081

NEWS2: National Early Warning Score 2, SOFA: Sequential Organ Failure Assessment, SAPS-II: Simplified Acute Physiology Score-II, ADE: Ancho de distribución eritrocitaria.

Tabla 17. Análisis multivariante de mortalidad considerando mayor valor de ADE

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Charlson	1	0,89-1,11	0,974
Inmunosupresión	1,06	0,47-2,38	0,887
Infección nosocomial	1,40	0,58-3,63	0,457
NEWS2	1,07	0,95-1,22	0,276
SOFA	**1,29**	**1,11-1,50**	**0,001**
SAPS-II	1,01	0,97-1,05	0,523
Mayor valor de ADE	**1,18**	**1,05-1,32**	**0,004**
Hemoglobina	0,92	0,78-1,09	0,341

NEWS2: National Early Warning Score 2, SOFA: Sequential Organ Failure Assessment, SAPS-II: Simplified Acute Physiology Score-II, ADE: Ancho de distribución eritrocitaria.

La capacidad discriminatoria del modelo resultante, considerando las variables previas, se analizó mediante el área bajo la curva Característica Operativa del Receptor (AUC-ROC). Todos los modelos presentaron un AUC-ROC superior a 0,700, siendo el mayor para el que consideró el ADE a las 72 horas de ingreso (AUC-ROC=0,831), (**tabla 18, figura 12**).

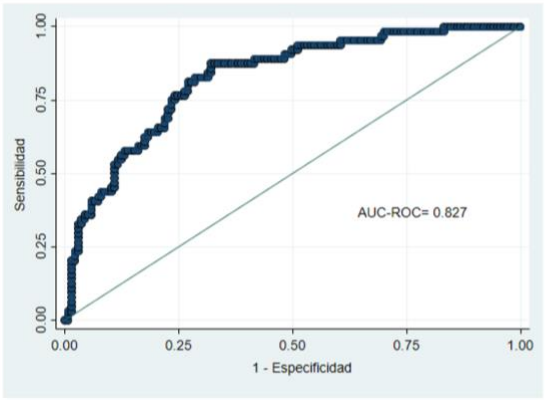
Tabla 18. Capacidad discriminatoria de mortalidad de cada uno de los modelos, considerando el ADE durante la primera semana de ingreso en la UCI, en pacientes con sepsis

	AUC-ROC	Intervalo de confianza al 95%
ADE al ingreso	0,827	0,767-0,886
ADE 24 horas	0,821	0,760-0,882
ADE 48 horas	0,826	0,764-0,888
ADE 72 horas	0,831	0,763-0,898
ADE 7 días	0,812	0,738-0,885
ADE máximo	0,812	0,778-0,892

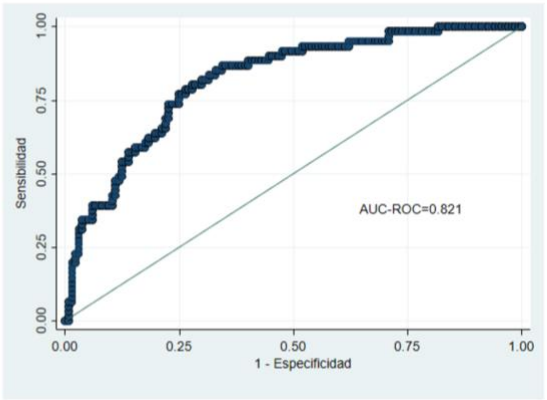
Los análisis se ajustaron por el índice de comorbilidad de Charlson, la inmunosupresión, la infección nosocomial, la escala National Early Warning Score 2, Simplified Acute Physiology Score-II y las cifras de hemoglobina, AUC-ROC= Área bajo la curva operativa característica del receptor, ADE= Ancho de distribución eritrocitaria.

Figura 12. Capacidad discriminatoria de mortalidad de cada uno de los modelos, considerando el ADE durante la primera semana de ingreso en la UCI, en pacientes con sepsis.

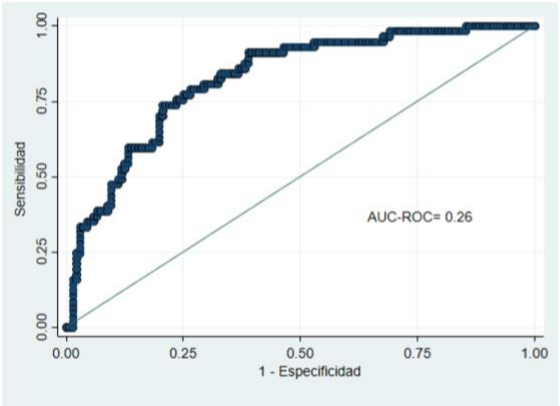
A. ADE al ingreso



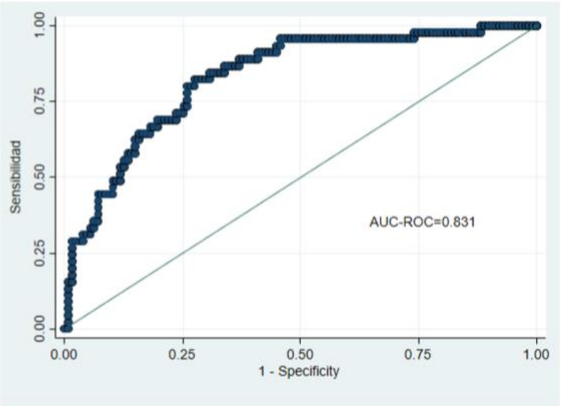
B. ADE a las 24 horas



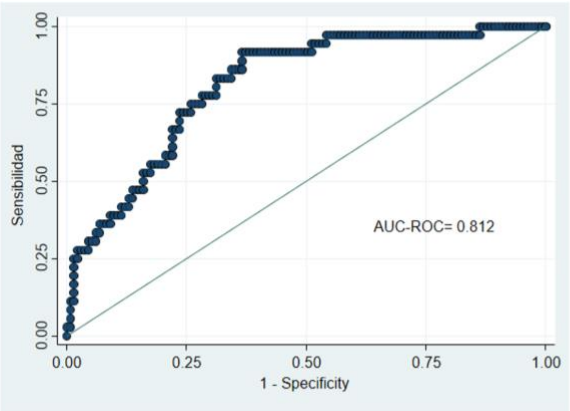
C. ADE a las 48 horas



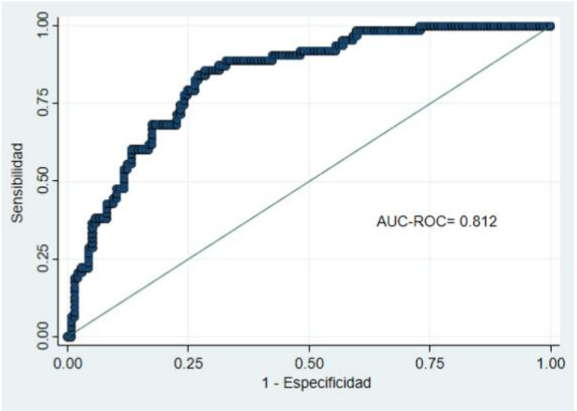
D. ADE a las 72horas



E. ADE a los 7 días horas



F. Valores máximos de ADE



Los análisis se ajustaron por el índice de comorbilidad de Charlson, la inmunosupresión, la infección nosocomial, la escala National Early Warning Score 2, Sequential Organ Failure Assessment y Simplified Acute Physiology Score-II y las cifras de hemoglobina. AUC-ROC= Área bajo la curva operativa característica del receptor, ADE= Ancho de distribución eritrocitaria.

5.1.5.3.2. Valor añadido del ADE a las escalas pronósticas

Finalmente, planteamos analizar el valor añadido que supone el ADE sobre las escalas pronósticas. Puesto que estas escalas se calculan con el valor más patológico de los parámetros obtenidos durante las primeras 24 horas del ingreso en UCI, solo se analizó el ADE en el ingreso y a las 24 horas (**Tabla 19, figura 13**). Todas las escalas (SOFA, LODS, SAPS-II y APACHE-II) presentaron una mejor capacidad discriminatoria de mortalidad cuando incorporaron al ADE como variable pronóstica ($p<0,05$), aunque los valores del AUC-ROC fueron superiores cuando se consideró el ADE al ingreso en UCI. Por consiguiente, el modelo resultante de la combinación de la escala SOFA con el ADE al ingreso mostró la mejor capacidad discriminatoria de mortalidad (AUC-ROC=0,812).

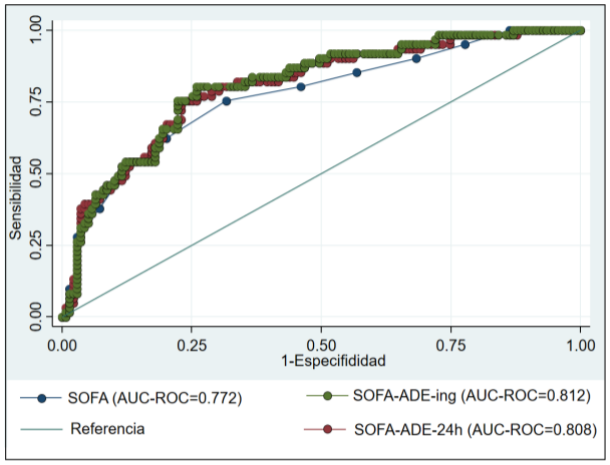
Tabla 19. Capacidad discriminatoria de las escalas pronósticas tras añadir el ADE, en pacientes ingresados en la UCI por sepsis

	AUC-ROC	Intervalo de confianza al 95%	Valor de p para la comparación entre AUC-ROCs
SOFA	0,772	0,700-0,844	
+ ADE al ingreso	0,812	0,748-0,876	0,041
+ ADE 24 horas	0,808	0,744-0,873	0,042
LODS	0,687	0,605-0,768	
+ ADE al ingreso	0,771	0,702-0,839	0,002
+ ADE 24 horas	0,770	0,701-0,839	0,002
SAPS-II	0,734	0,660-0,809	
+ ADE al ingreso	0,785	0,721-0,849	0,021
+ ADE 24 horas	0,781	0,716-0,846	0,030
APACHE-II	0,672	0,588-0,756	
+ ADE al ingreso	0,755	0,682-0,827	0,003
+ ADE 24 horas	0,752	0,678-0,826	0,004

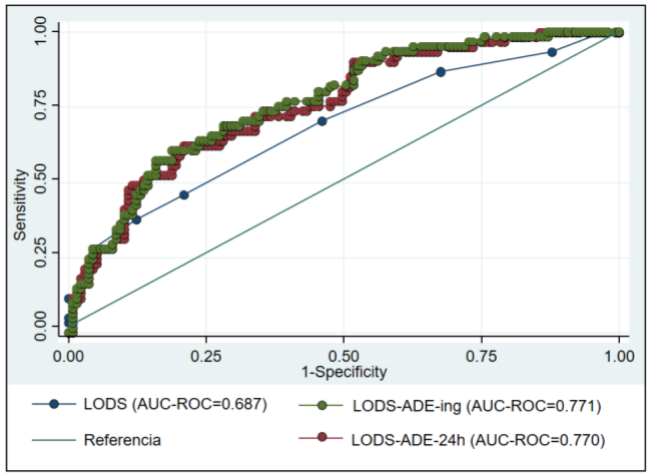
SOFA: Sequential Organ Failure Assessment, ADE: Ancho de distribución eritrocitaria, LODS: Logistic Organ Dysfunction System, SAPS-II: Simplified Acute Physiology Score-II, APACHE-II: Acute Physiology and Chronic Health Evaluation-II, AUC-ROC: Área Bajo la Curva Característica Operativa del Receptor

Figura 13. Capacidad discriminatoria de mortalidad de las escalas pronósticas tras añadir el ADE, en pacientes ingresados en la UCI por sepsis

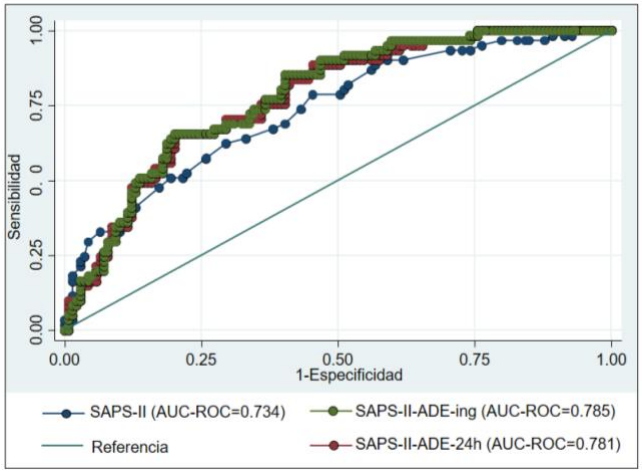
A. Capacidad discriminatoria de SOFA tras añadir el ADE



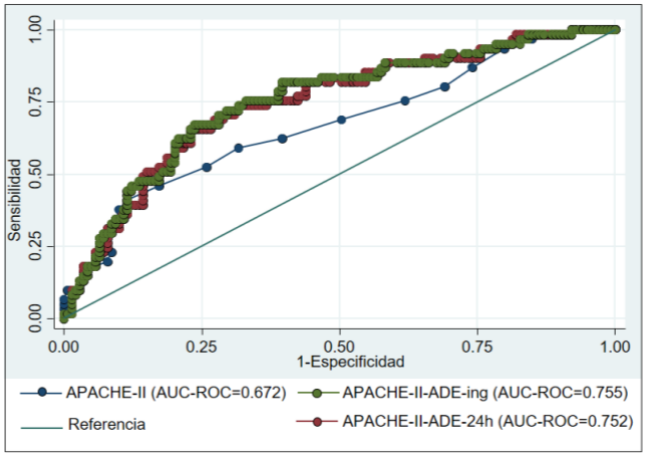
B. Capacidad discriminatoria de LODS tras añadir el ADE



C. Capacidad discriminatoria de SAPS-II tras añadir el ADE



D. Capacidad discriminatoria de APACHE-II tras añadir el ADE



5.2. PACIENTES INGRESADOS POR NEUMONÍA GRAVE POR COVID-19

5.2.1. Características de la población y del ingreso hospitalario

En nuestro centro se incluyeron en el estudio TOCICOV 173 pacientes con neumonía por COVID-19 con insuficiencia respiratoria o con aumento de los parámetros inflamatorios, de acuerdo con los criterios descritos. Este grupo de pacientes se consideró adecuado para evaluar la utilidad pronóstica del ADE y otros marcadores analíticos. Las características basales de la cohorte se muestran en la **tabla 20.** Un 67,1% de los pacientes eran varones con una edad media de 66,6 años. La media del índice de comorbilidad de Charlson fue de 3.5 puntos y un 54.3% padecía hipertensión, un 28.3% diabetes mellitus, un 25.4% enfermedad cardiovascular, un 12.1% enfermedad neurológica, un 17.9% enfermedad pulmonar, un 7.5% hepatopatía crónica y un 8.7% insuficiencia renal. Un 1.7% eran pacientes trasplantados y un 4.6% pacientes onco-hematológicos.

Las características clínicas durante el ingreso se muestran en la **tabla 21**. En el momento de la inclusión en el estudio, la media del SAFI era de 202. En ese momento, un 66,7% estaba recibiendo oxigenoterapia mediante gafas nasales o mascarilla tipo Venturi y un 31.4% mediante reservorio. Durante el ingreso, un 98,3% recibió hidroxicloroquina, un 82,7% lopinavir-ritonavir, un 29% interferón, un 43,4% azitromicina, un 48,6% ceftriaxona, un 13,9% levofloxacino, un 90,2% esteroides y un 62,4% tocilizumab. El 87,2% de los pacientes que recibieron tocilizumab lo hicieron en el día de inclusión de estudio (día 0), coincidiendo con el deterioro respiratorio y/o analítico. En un 8,7% se identificaron complicaciones infecciosas y un 85,5% de los pacientes precisó ingreso en UCI.

La mortalidad global fue del 20,8%. El análisis univariante de la mortalidad demostró asociación significativa con la edad (78 vs 63,6 años, p<0,001) , con todas las comorbilidades excepto con el trasplante y con la enfermedad onco-hematológica, así como con la tensión arterial sistólica (TAS) en el momento de la inclusión (138 vs 115 mmHg, p=0,038), el tratamiento con lopinavir-ritonavir (63,9 vs 83,9%, p=0,002), con interferón (72,2% vs 67,2%, p<0,001), con levofloxacino (2,8% vs 16,8%, p=0,03) y con tocilizumab (41,7 vs 67,9%, p=0,006); pero no con los parámetros respiratorios en el momento de la inclusión ni con la presencia de complicaciones infecciosas (**tablas 20 y 21**).

Tabla 20. Características basales de los pacientes ingresados por neumonía grave por COVID-19

	Global (n=173)	Supervivientes (n=137)	Fallecidos (n=36)	Valor de p
Sexo masculino (%)	116 (67,1)	91 (66,4)	25 (69,4)	0,843
Edad, media (DE)	66,6 (13,6)	63,6 (12,5)	78 (1,5)	<0,001
Índice de comorbilidad de Charlson, media (DE)	3,5 (2,5)	2.9 (2,1)	5,4 (2,8)	<0,001
Hipertensión (%)	94 (54,3)	54 (40,9)	23 (63,9)	0,015
Diabetes (%)	49 (28,3)	31 (22,6)	18 (50)	0,003
Enfermedad cardiovascular (%)	44 (25,4)	25 (18,2)	19 (52,8)	<0,001
Enfermedad neurológica (%)	21 (12,1)	10 (7,3)	11 (30,6)	<0,001
Enfermedad pulmonar (%)	31 (17,9)	20 (14,6)	11 (30,6)	0,048
Hepatopatía crónica (%)	13 (7,5)	5 (3,6)	8 (22,2)	<0,001
Insuficiencia renal (%)	15 (8,7)	6 (4,4)	9 (25)	<0,001
Trasplante (%)	3 (1,7)	1 (0,7)	2 (5,6)	0,110
Enfermedad onco-hematológica (N, %)	8 (4,6)	5 (3,6)	3 (8,3)	0,365

DE: Desviación estándar.

Tabla 21. Características clínicas de los pacientes ingresados por neumonía grave por COVID-19

	Global (n=173)	Supervivientes (n=137)	Fallecidos (n=36)	Valor de p
Parámetros clínicos				
TAS (mmHg), media (DE)	117 (21)	115 (19)	138 (37)	0,038
TAD (mmHg), media (DE)	68 (11)	68 (11)	66 (13)	0,654
Temperatura (`C), media (DE)	37 (1)	37 (1)	37 (1)	0,111
FR (rpm), media (DE)	26 (7)	25 (7)	28 (1)	0,112
Sat02 (%), media (DE)	93 (4)	93 (3)	92 (6)	0,097
FiO2 (%), media (DE)	53 (28)	53 (28)	55 (27)	0,769
SAFI, media (DE)	202 (84)	206 (85)	188 (78)	0,266
Tratamiento recibido				
Hidroxicloroquina (%)	170 (98,3)	135 (98,5)	35 (93,2)	0,506
Lopinavir-Ritonavir (%)	143 (82,7)	120 (83,9)	23 (63,9)	0,002
Interferon (%)	102 (59)	92 (67,2)	10 (72,2)	<0,001
Azitromicina (%)	75 (43,4)	54 (39,4)	21 (58,3)	0.058
Ceftriaxona (%)	84 (48,6)	68 (49,6)	16 (44,4)	0.708
Levofloxacino (%)	24 (13,9)	23 (16,8)	1 (2,8)	0.03
Esteroides (%)	156 (90,2)	124 (90,5)	32 (88,9)	0,757
Tocilizumab (%)	108 (62,4)	93 (67,9)	15 (41,7)	0,006
Evolución clínica				
Complicaciones infecciosas (%)	15 (8,7)	12 (8,8)	3 (8,3)	0,990
Ingreso en UCI (%)	148 (85,5)	116 (84,7)	32 (88,9)	0.485

TAS: Tensión arterial sistólica, DE: Desviación estándar, TAD: Tensión arterial media, FR: Frecuencia respiratoria, RPM: Respiraciones por minuto, Sat02: Saturación periférica de oxígeno, FiO2: Fracción inspirada de oxígeno, SAFI: Ratio saturación periférica de oxígeno-Fracción inspirada de oxígeno, UCI: Unidad de cuidados intensivos.

5.2.2. Dinámica de los parámetros inflamatorios y relación con la mortalidad

Los parámetros inflamatorios y analíticos (linfocitos, neutrófilos, plaquetas, ADE, AP, dímero-D, PCR, LDH, IL-6 y ferritina) se registraron y analizaron el día del ingreso y los días 0, 1,3, 7 y 14 tras la inclusión en el estudio. En la **tabla 22** se muestran los valores de estos parámetros el día de inclusión (día 0), junto con los de las plaquetas, hemoglobina, fibrinógeno y AST. La dinámica en función de la supervivencia se muestra en las **figuras 14-18.**

Ninguno de los recuentos celulares (linfocitos, neutrófilos o plaquetas), ni los valores de la coagulación (AP, fibrinógeno o DD) o las enzimas LDH o AST mostraron diferencias estadísticamente significativas entre supervivientes y fallecidos (p>0,05, **tabla 23, figuras 14-16**). Sí se demostró asociación de la mortalidad con los valores de la PCR en el ingreso (136 vs 105 mg/L, p=0,002), en el día 1 (176 vs 128 mg/L, p<0,001) y en el día 7 (55 vs 23 mg/L, p=0,01), así como los valores de la IL-6 en el día 1 (454 vs 243 pg/ml, p=0,046) y los valores de la ferritina en el día 3 (1934 vs 1444 ng/ml, p=0,029), (**figuras 16 y 17)**.

Además, los pacientes fallecidos presentaron mayor ADE en el momento del ingreso (14,35% vs 13,52%, p=0,002, **tabla 23**), en el día 0 (14,42% vs 13,60%, p=0,026), en el día 3 (14,35% vs 13,43%, p<0,001) y en el día 7 tras la inclusión en el estudio (14,31% vs 13,41%, p=0,046), tal y como se representa en la **figura 18**.

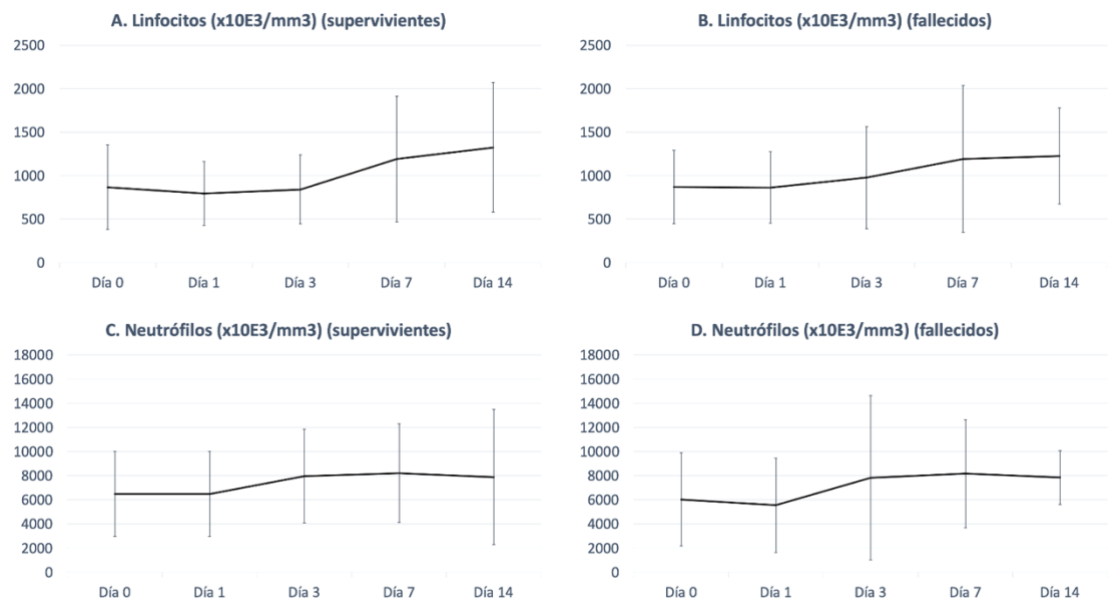
Por otra parte, y de cara a comparar la precisión en la predicción de la mortalidad de estos parámetros, se realizó un análisis del AUC-ROC (**tabla 24).** Los valores del AUC-ROC del ADE en el ingreso, en el día 0, en el día 3 y en el día 7 superaron el 0,650, así como los valores de la PCR y de la IL-6 en el día 1. El ADE del día 3 presentó la mejor capacidad predictiva de mortalidad de todos los parámetros analizados (AUC-ROC= 0,696, IC al 95% 0,587-0,803).

Tabla 23. Parámetros analíticos, en el día 0, de los pacientes ingresados por neumonía grave por COVID-19

	Global (n=173)	Supervivientes (n=137)	Fallecidos (n=36)	p para mortalidad
Linfocitos/uL, media (DE)	869 (473)	868 (488)	869 (421)	0.990
Neutrófilos/uL, media (DE)	6381 (3588)	6477 (3520)	6028 (3864)	0.520
Plaquetas (10^3/uL), media (DE)	228 (85)	235 (85)	204 (82)	0.06
Hemoglobina (g/dl), media (DE)	13.58 (1.96)	13.55 (1.99)	13.72 (1.84)	0.308
ADE (%), media (DE)	13.76 (1.84)	13.60 (1.81)	14.42 (1.87)	0.026
AP (%), media (DE)	87.01 (16.50)	86.74 (18.20)	86.53 (13.40)	0.954
Fibrinógeno (mg/dl), media (DE)	676 (143)	682 (148)	653 (113)	0.399
DD (ng/ml), media (DE)	1395 (1700)	1330 (1658)	1646 (1858)	0.336
AST (U/L), media (DE)	71 (112)	69 (106)	79 (134)	0.659
PCR (mg/L), media (DE)	144 (82)	142 (86)	154 (67)	0.462
LDH (UI/L), media (DE)	404 (174)	396 (178)	437 (155)	0.213
IL-6 (pg/ml), media (DE)	199 (228)	186 (221)	293 (274)	0,286
Ferritina (ng/ml), media (DE)	1478 (1638)	1394 (834)	1933 (3793)	0,344

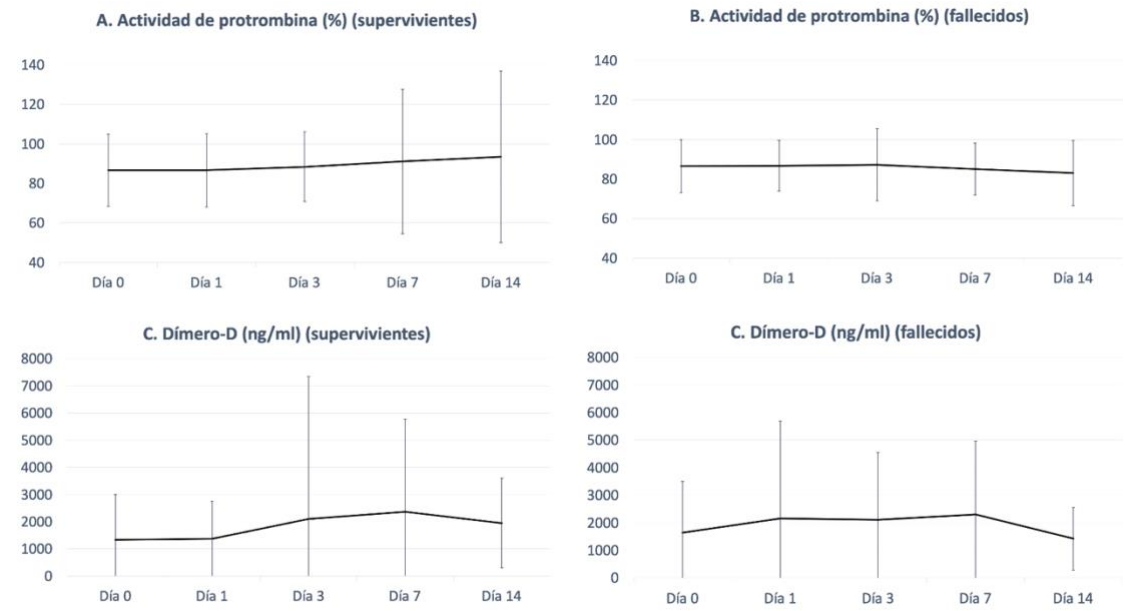
DE: Desviación estándar, ADE: Ancho de distribución eritrocitaria, AP: Actividad de protrombina, DD: Dímero-D, PCR: Proteína C reactiva, AST: Aspartato-aminotrasferasa, LDH: Lactato dehidrogenasa, IL-6: Interleukina 6.

Figura 14. Valores de linfocitos y neutrófilos en pacientes ingresados por neumonía grave por COVID-19



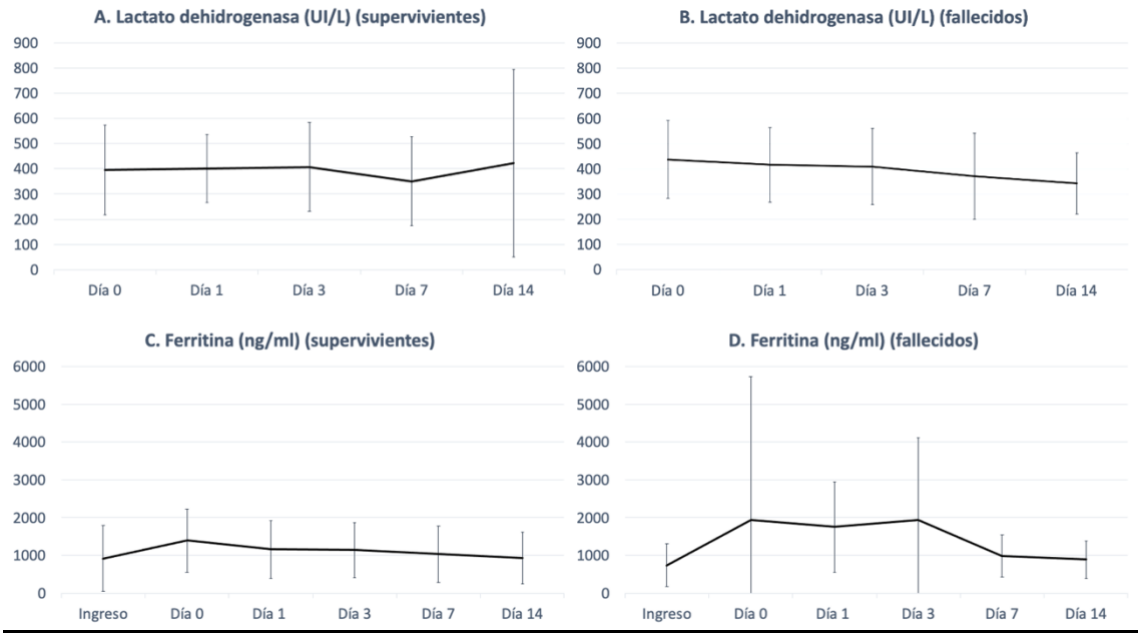
Los parámetros se muestran como media y desviación estándar (barras)

Figura 15. Valores de la AP y DD en pacientes ingresados por neumonía grave por COVID-19



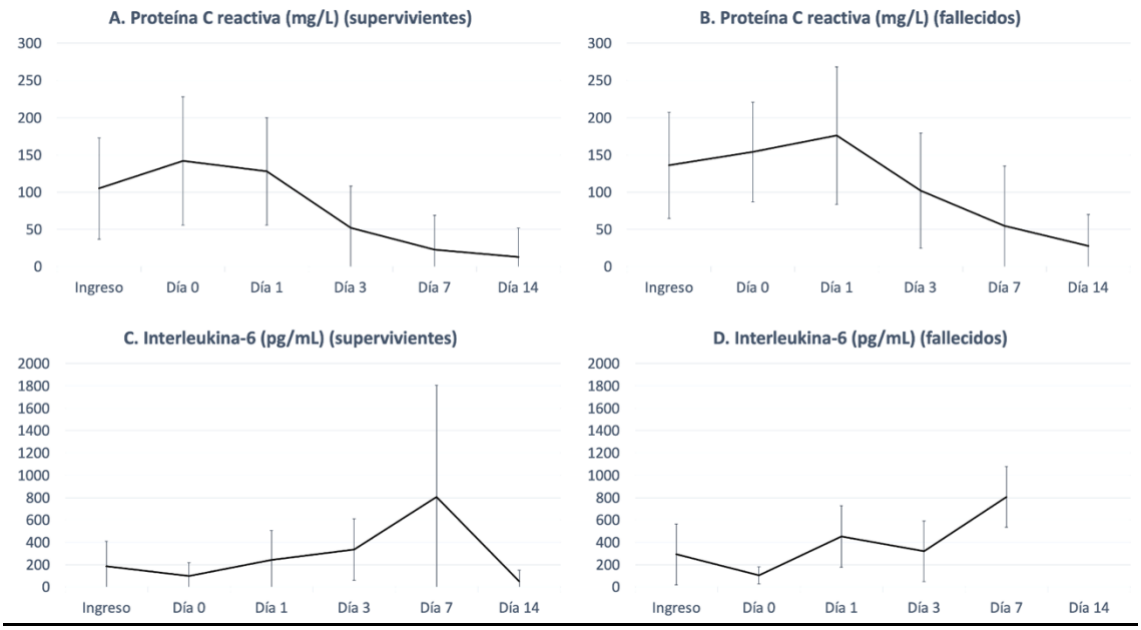
Los parámetros se muestran como media y desviación estándar (barras)

Figura 16. Valores de la LDH y ferritina en pacientes ingresados por neumonía grave por COVID-19



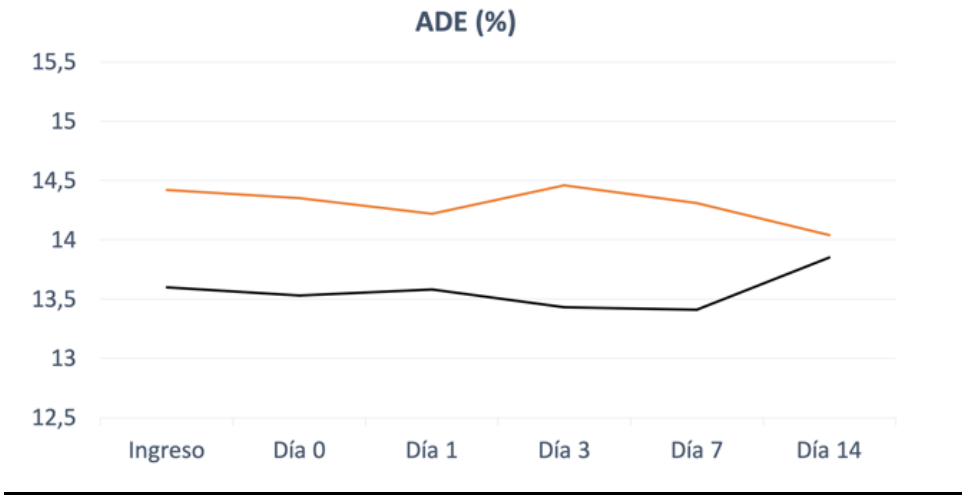
Los parámetros se muestran como media y desviación estándar (barras)

Figura 17. Valores de la PCR e IL-6 en pacientes ingresados por neumonía grave por COVID-19



Los parámetros se muestran como media y desviación estándar (barras)

Figura 18. Valores del ADE en pacientes ingresados por neumonía grave por COVID-19



Los parámetros se muestran como media, en naranja (fallecidos) y negro (supervivientes). ADE: Ancho de distribución eritrocitaria.

Tabla 24. Capacidad discriminatoria de mortalidad de los parámetros inflamatorios en pacientes ingresados por neumonía grave por COVID-19

	AUC-ROC	IC al 95%
ADE al ingreso	0,668	0,567-0,769
ADE día 0	0,680	0,574-0,785
ADE día 3	0,695	0,587-0,803
ADE día 7	0,666	0,548-0,783
PCR al ingreso	0,625	0,523-0,727
PCR día 1	0,662	0,549-0,775
PCR día 7	0,633	0,494-0,773
IL-6 día 1	0,681	0,471-0,892
Ferritina día 3	0,540	0,348-0,731

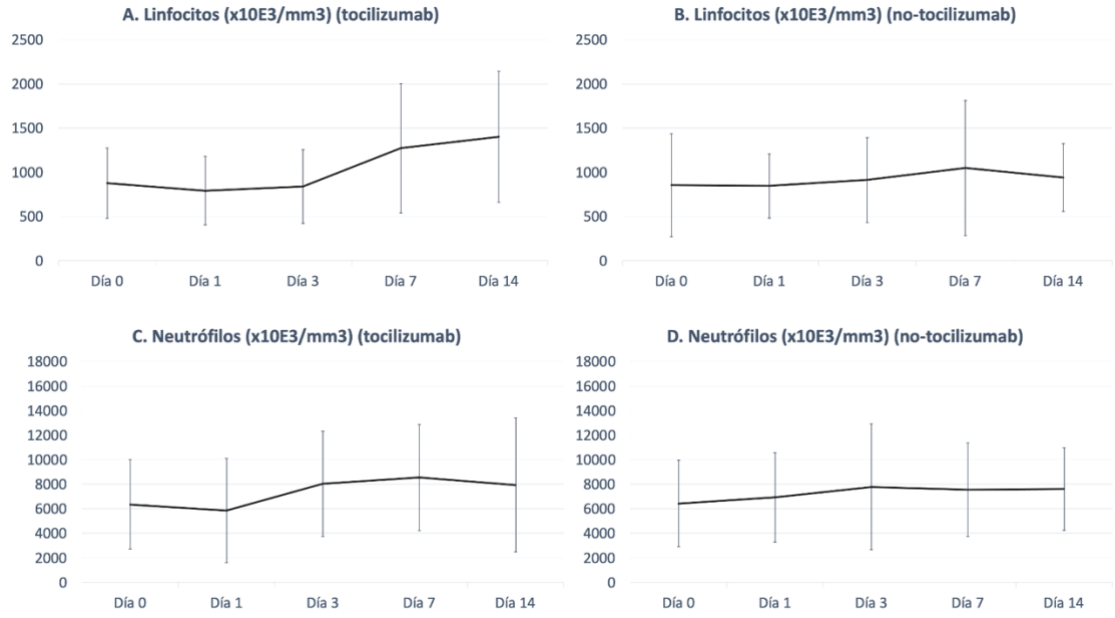
AUC-ROC: Área Bajo la Curva Característica Operativa del Receptor, ADE: Ancho de distribución eritrocitaria, PCR: Proteína C reactiva, IL-6: Interleukina 6.

5.2.3. Dinámica de los parámetros según el tratamiento con tocilizumab

Con el objetivo de entender la influencia del bloqueo de la IL-6, se estudiaron los valores y la dinámica de los parámetros inflamatorios en función del tratamiento con tocilizumab (**figuras 19-23**). Los pacientes que recibieron tocilizumab presentaron mayores valores de PCR en el día 0 (155 vs 127 mg/L, p=0,0038) pero menores cifras de PCR en el día 3 (43 vs 92 mg/L, p<0,001) y en el día 7 (12,5 vs 55 mg/L, p<0,001), (**figura 22**). Por otra parte, los niveles de IL-6 fueron mayores en los pacientes que recibieron tocilizumab en el ingreso (270 vs 98 pg/ml, p=0,008), en el día 1 (346 vs 95 pg/ml, p=0,004) y en el día 3 (379 vs 141 pg/ml, p=0,016), (**figura 23**). Sin embargo, no se identificaron variaciones en los valores de linfocitos, neutrófilos, DD, fibrinógeno, LDH, ferritina o el ADE según el tratamiento con tocilizumab (**figuras 19-21 y 24**).

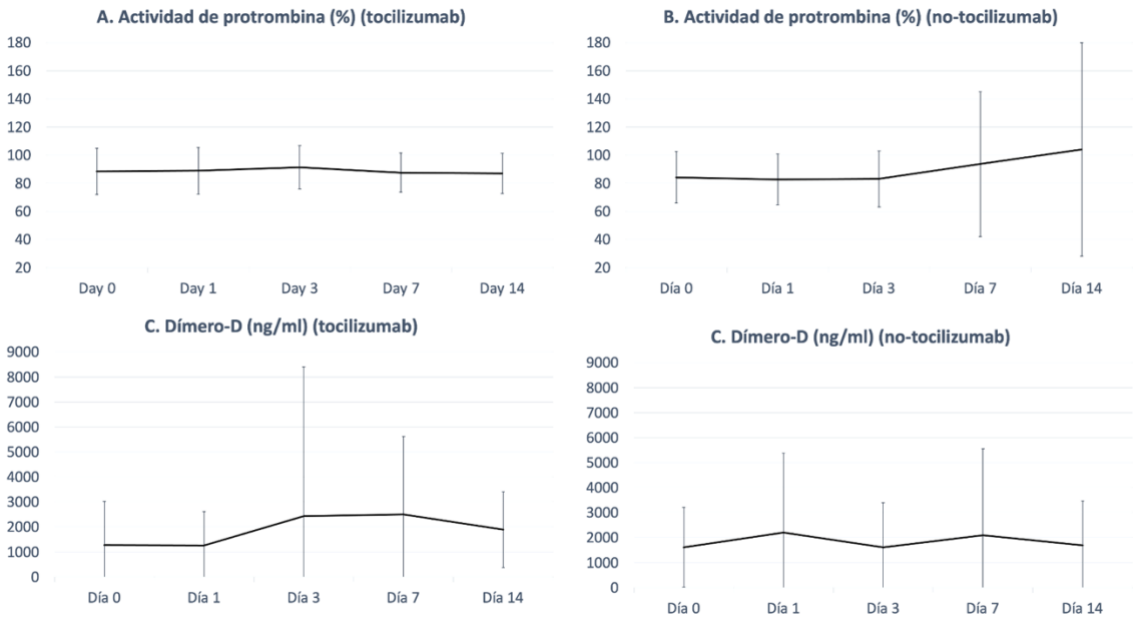
Además, la capacidad pronóstica de los parámetros que habían demostrado asociación con la mortalidad se evaluó mediante un análisis multivariante, considerando las cifras de hemoglobina y el tratamiento con tocilizumab (**tablas 25-32)**. Tanto el ADE al ingreso (OR=1,23, IC 95% 1,01-1,49, p=0,041), en el día 0 (OR=1,22, 95% CI 1-1,49, p=0,05), y día 3 (OR=1,25, 95% CI 1,01-1,56, p=0,047), como la PCR en el ingreso (OR=1,01, 95% CI 1-1,01, p=0,043) y en el día 1 (OR=1,01 95% CI 1,01-1,02, p=0,001) fueron factores que mostraron capacidad pronóstica de mortalidad; mientras que los valores de la PCR en el día 7, de IL-6 el día 1 y de ferritina el día 3 no.

Figura 19. Valores de linfocitos y neutrófilos en pacientes ingresados por neumonía grave por COVID-19 en función del tratamiento con tocilizumab



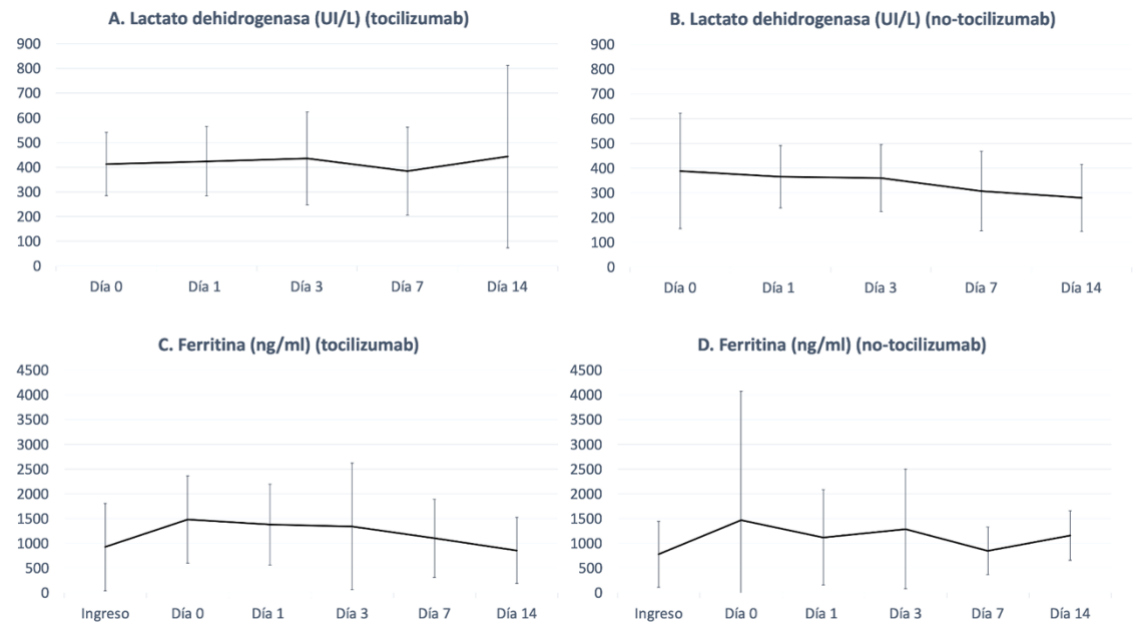
Los parámetros se muestran como media y desviación estándar (barras).

Figura 20. Valores de la AP y DD en pacientes ingresados por neumonía grave por COVID-19 en función del tratamiento con tocilizumab



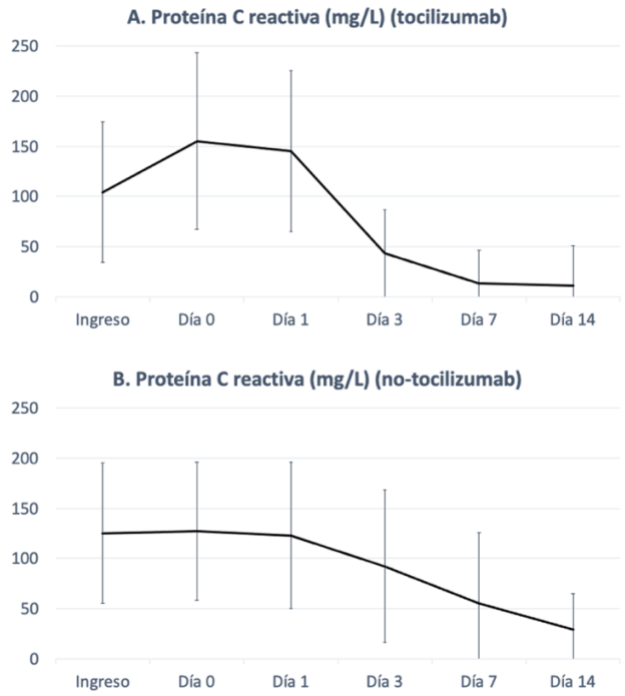
Los parámetros se muestran como media y desviación estándar (barras)

Figura 21. Valores de la LDH y ferritina en pacientes ingresados por neumonía grave por COVID-19 en función del tratamiento con tocilizumab



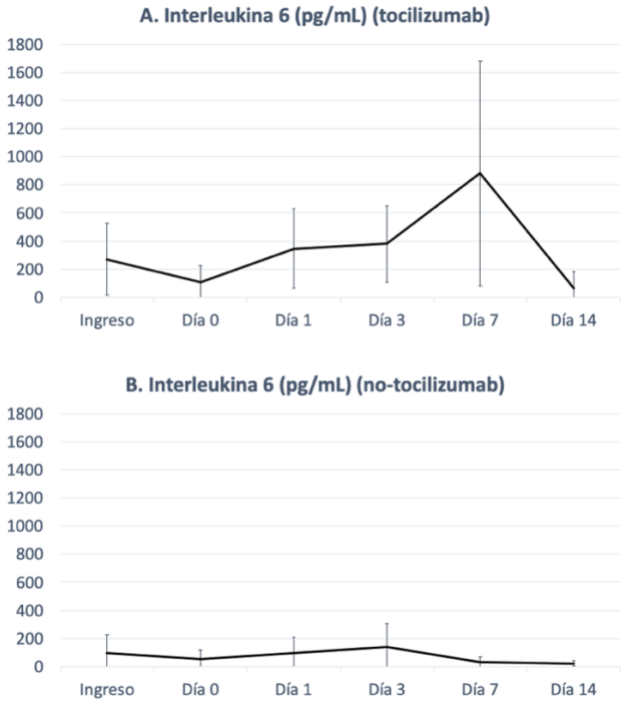
Los parámetros se muestran como media y desviación estándar (barras)

Figura 22. Valores de la proteína C reactiva en pacientes ingresados por neumonía grave por COVID-19 en función del tratamiento con tocilizumab



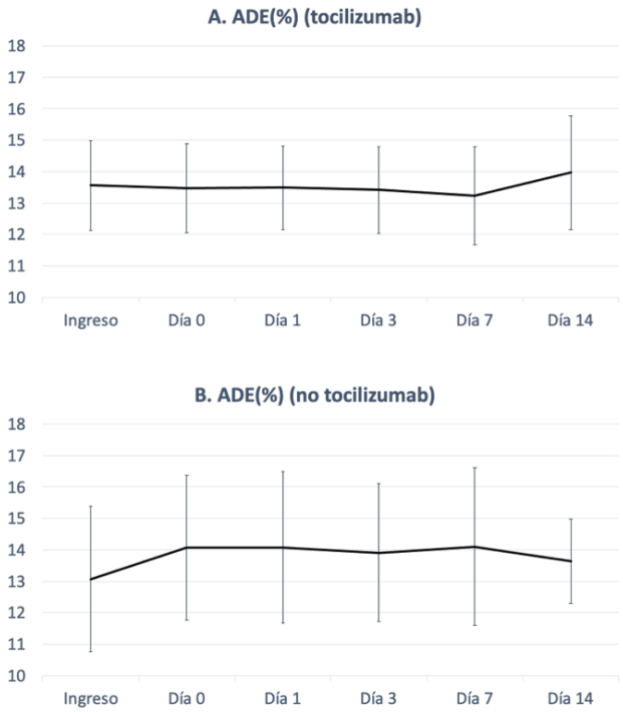
Los parámetros se muestran como media y desviación estándar (barras)

Figura 23. Valores de la IL-6 en pacientes ingresados por neumonía grave por COVID-19 en función del tratamiento con tocilizumab



Los parámetros se muestran como media y desviación estándar (barras)

Figura 24. Valores del ADE en pacientes ingresados por neumonía grave por COVID-19 en función del tratamiento con tocilizumab



Los parámetros se muestran como media y desviación estándar (barras)

Tabla 25. Análisis multivariante de mortalidad considerando el tratamiento con tocilizumab y el ADE al ingreso

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Tocilizumab	**0,33**	**0,15-0,72**	**0,005**
ADE al ingreso	**1,23**	**1,01-1,49**	**0,041**
Hemoglobina	0,79	0,54-1,16	0,226

ADE: Ancho de distribución eritrocitaria.

Tabla 26. Análisis multivariante de mortalidad considerando el tratamiento con tocilizumab y el ADE en el día 0

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Tocilizumab	**0,29**	**0,13-0,68**	**0,004**
ADE día 0	**1,22**	**1-1,49**	**0,05**
Hemoglobina	0,82	0,54-1,23	0,337

ADE: Ancho de distribución eritrocitaria.

Tabla 27. Análisis multivariante de mortalidad considerando el tratamiento con tocilizumab y el ADE en el día 3

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Tocilizumab	**0,28**	**0,12-0,67**	**0,004**
ADE día 3	**1,25**	**1,01-1,56**	**0,047**
Hemoglobina	0,79	0,51-1,22	0,292

ADE: Ancho de distribución eritrocitaria.

Tabla 28. Análisis multivariante de mortalidad considerando el tratamiento con tocilizumab y la PCR al ingreso

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Tocilizumab	**0,42**	**0,19-0,95**	**0,037**
PCR al ingreso	**1,01**	**1-1,01**	**0,043**
Hemoglobina	0,70	0,47-1,05	0,083

PCR: Proteína C reactiva.

Tabla 29. Análisis multivariante de mortalidad considerando el tratamiento con tocilizumab y la PCR en el día 1

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Tocilizumab	**0,16**	**0,06-0,43**	**<0,001**
PCR en el día 1	**1,01**	**1,01-1,02**	**0,001**
Hemoglobina	0,71	0,46-1,10	0,128

PCR: Proteína C reactiva.

Tabla 30. Análisis multivariante de mortalidad considerando el tratamiento con tocilizumab y la PCR en el día 7

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Tocilizumab	**0,25**	**0,10-0,65**	**0,004**
PCR en el día 7	1,00	0,99-1,01	0,180
Hemoglobina	0,71	0,44-1,16	0,170

PCR: Proteína C reactiva.

Tabla 31. Análisis multivariante de mortalidad considerando el tratamiento con tocilizumab y la IL-6 en el día 1

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Tocilizumab	0,09	0,01-2,91	0,173
IL-6 en el día 1	1,01	0,99-1,01	0,67
Hemoglobina	**0,34**	**0,12-0,94**	**0,037**

IL-6: Interleukina 6.

Tabla 32. Análisis multivariante de mortalidad considerando el tratamiento con tocilizumab y la ferritina en el día 3

	Odds ratio	Intervalo de confianza al 95%	Valor de p
Tocilizumab	0,44	0,120-1,62	0,218
Ferritina en el día 3	1,00	0,99-1,01	0,056
Hemoglobina	**0,54**	**0,30-0,99**	**0,046**

5.2.4. Correlación entre los marcadores inflamatorios

Por último, se analizaron las correlaciones de los distintos parámetros inflamatorios para comprender la fisiopatología de las alteraciones inflamatorias asociadas a la infección por el SARS-CoV-2. La **tabla 33** muestra las correlaciones estadísticamente significativas. La IL-6 del ingreso presentó correlación con la PCR del día 0 o de la inclusión (r=0.332, p=0,007) y del día 1(r=0,303, p=0,015), mientras que la IL-6 el día 0 se correlacionó con la PCR del día 0 (r=0,297, p=0,003) y con la ferritina del día 0 (r=0,539, p=0,008). En paralelo, la PCR del día 1 se correlacionó con el ADE del ingreso (r=0,186, p=0,026), con el ADE del día 0 (r=0,275, p=0,002), con el del día 1 (r=0,255, p=0,04) y con el del día 3 (r=0,277, p=0,001). Además, la PCR del día 3 también mostró correlación con el ADE el día 0 (r=0,188, p=0,03), con el del día 1 (r=0,245, p=0,001) y con el del día 3 (r=0.266, p=0.001). Por último, la PCR del día 7 se correlacionó con el ADE del día 7 (r=0,218, p=0,017).

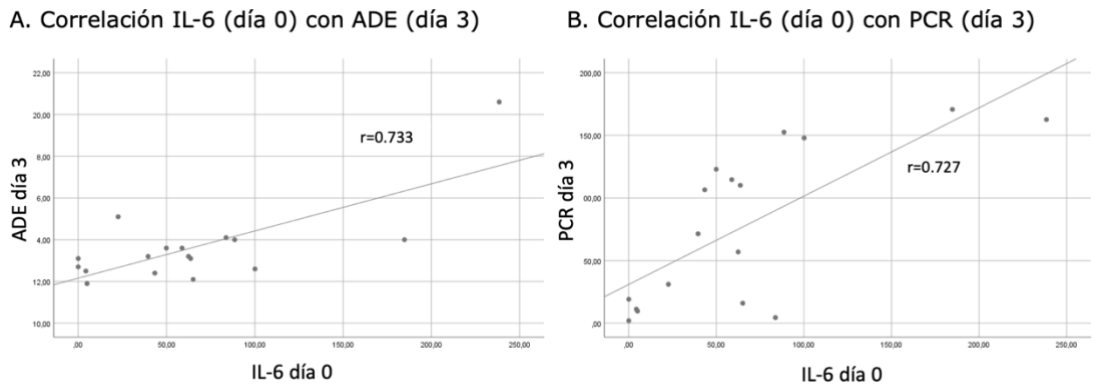
A pesar de lo previo, no se demostró significación entre los valores de IL-6 y ADE en la cohorte global. Sin embargo, en los pacientes que no recibieron tocilizumab, los valores de IL-6 en el momento de la inclusión (día 0) mostraron una fuerte correlación con el ADE del día 3 (r=0,733, p=0.004) y con la PCR del día 3 (r=0,727, p=0,022) (**figura 25).** En este grupo de pacientes, las cifras de ADE y PCR el día 3 también mostraron una correlación significativa (r=0,358, p=0,005) (**figura 26**).

Tabla 33. Correlaciones estadísticamente significativas de los marcadores inflamatorios en pacientes ingresados por neumonía grave por COVID-19

Parámetros	Coeficiente de correlación	Valor de p
IL-6 en el ingreso		
PCR día 0	0,332	0,007
PCR día 1	0,303	0,015
IL-6 día 0		
PCR día 0	0,297	0,003
Ferritina día 0	0,539	0,008
PCR día 1		
ADE en el ingreso	0,186	0,026
ADE día 0	0,275	0,002
ADE día 1	0,255	0,04
ADE día 3	0,277	0,001
PCR día 3		
ADE día 0	0,188	0,03
ADE día 1	0,245	0,001
ADE día 3	0,266	0,001
PCR día 7		
ADE día 7	0,218	0,017

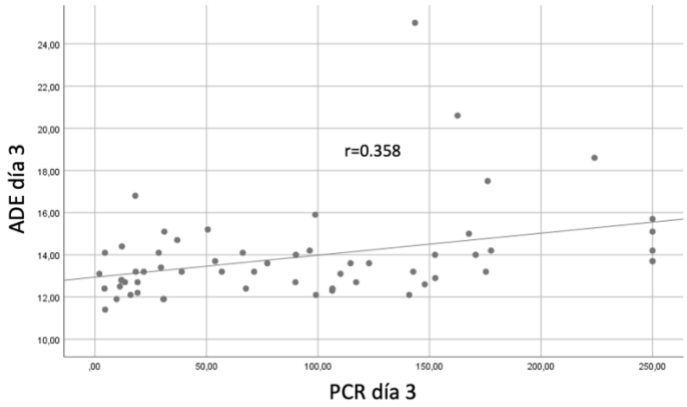
PCR: Proteína c reactiva, ADE= Ancho de distribución eritrocitaria.

Figura 25. Correlación de Il-6 en el día 0 con el ADE y PCR en el día 3 en pacientes no tratados con tocilizumab



ADE: Ancho de distribución eritrocitaria. PCR: Proteína c reactiva.

Figura 26. Correlación del ADE y PCR en el día 3 en pacientes no tratados con tocilizumab



ADE: Ancho de distribución eritrocitaria. PCR: Proteína c reactiva.

5.3. PACIENTES INGRESADOS POR NEUMONÍA POR COVID-19

5.3.1. Características de la población y del ingreso hospitalario

Durante la primera ola de la pandemia, entre marzo y abril de 2020, ingresaron un total de 1.594 pacientes por neumonía por COVID-19. Un 87,2% presentaba RT-PCR positiva. En los demás pacientes la infección se asumió de acuerdo con las manifestaciones clínicas, radiológicas y analíticas, en el ambiente epidemiológico de pandemia y en ausencia de otros diagnósticos alternativos. Las características de esta población se muestran en la **tabla 34**. En el global, un 62,1% eran varones y la edad media fue de 64,8 años. En relación con las comorbilidades previas al ingreso, un 43,9% eran hipertensos, un 17,6% diabéticos, un 35,2% obesos, un 16,9% presentaban cardiopatía, un 14,1% enfermedad neurológica, un 15,6% enfermedad pulmonar, un 3% hepatopatía crónica, un 7% insuficiencia renal, un 10,4% algún grado de inmunosupresión: un 1,9% eran pacientes trasplantados, un 3,8% tenía una neoplasia de órgano solido, un 2,3% enfermedad hematológica y un 6,3% alguna enfermedad autoinmune.

Por otra parte, la **tabla 35** muestra los datos clínicos de los pacientes durante el ingreso. Un 76,1 % presentó un SDRA (en un 41,4% leve, en un 26,3% moderado y en un 8,1% grave). La media de la SAFI fue de 273. En el total, un 58,1% recibió esteroides durante el ingreso, un 18,8% tocilizumab y un 2,6% anakinra. La estancia media fue de 9,3 días y 110 pacientes (6,9%) ingresaron en la UCI. La tasa de mortalidad global fue del 15,1%.

El análisis univariante demostró asociación estadísticamente significativa de la mortalidad con: la edad (79,9 vs 62,1 años, p<0,001), la hipertensión (73% vs 8,7%, p<0,001, la diabetes (34,9% vs 14,6%, p<0,001), la cardiopatía previa (39,8% vs 12,9%, p<0,001), la enfermedad neurológica (39,4% vs 9,6%, p<0,001), la hepatopatía crónica (5,4% vs 2,6%, p=0,021), la insuficiencia renal (21,2% vs 4,5%, p<0,001), la inmunosupresión (8,7% vs 19,9%, p<0,001), la neoplasia de órgano solido (9,1% vs 2,8%, p<0,001) y el antecedente de enfermedad hematológica (5,8% vs 1,7%, p<0,001). Además, los pacientes fallecidos desarrollaron con mayor frecuencia SDRA (98,3% vs 72,1%, p <0,001), presentaron menor SAFI (140 vs 297, p<0,001) y con mayor frecuencia SDRA grave (19,5% vs 6,1%, p <0,001). Por lo tanto, recibieron más esteroides (70,5% vs 55,9%, p <0,001) y presentaron una mayor tasa de ingreso en UCI (11,6% vs 6,1%, p <0,001).

Tabla 34. Características basales de los pacientes ingresados por neumonía por COVID-19

	Global (n=1594)	Supervivientes (n=1353)	Fallecidos (n=241)	Valor de p
Sexo masculino (%)	990 (62,1)	845 (62,5)	145 (60,2)	0,273
Edad, media (DE)	64,8 (14,8)	62,1 (13,8)	79,9 (10,8)	<0,001
Hipertensión (%)	699 (43,9)	523 (38,7)	176 (73)	<0,001
Diabetes (%)	281 (17,6)	197 (14,6)	84 (34,9)	<0,001
Obesidad (%)	424 (35,2)	355 (35,2)	69 (35)	0,518
Cardiopatía (%)	270 (16,9)	174 (12,9)	96 (39,8)	<0,001
Enfermedad neurológica (%)	225 (14,1)	130 (9,6)	95 (39,4)	<0,001
Enfermedad pulmonar (%)	248 (15,6)	206 (15,2)	42 (17,4)	0,218
Hepatopatía crónica (%)	48 (3)	35 (2,6)	13 (5,4)	0,021
Insuficiencia renal (%)	112 (7)	61 (4,5)	51 (21,2)	<0,001
Inmunosupresión (%)	166 (10,4)	48 (19,9)	118 (8,7)	<0,001
Trasplante (%)	30 (1,9)	22 (1,6)	8 (3,3)	0,071
Neoplasia de órgano solido (%)	60 (3,8)	38 (2,8)	22 (9,1)	<0,001
Enfermedad hematológica (%)	37 (2,3)	23 (1,7)	14 (5,8)	<0,001
Enfermedad autoinmune (%)	100 (6,3)	80 (5,9)	20 (8,3)	0,106

DE: Desviación estándar.

Tabla 35. Datos clínicos de los pacientes ingresados por neumonía por COVID-19

	Global (n=1594)	Supervivientes (n=1353)	Fallecidos (n=241)	Valor de p
SDRA (N, %)	1212 (76,1)	975 (72,1)	237 (98,3)	<0,001
SAFI, media (DE)	273 (124)	297 (116)	140 (74)	<0,001
Leve (%)	660 (41,4)	627 (46,3)	33 (13,7)	<0,001
Moderado (%)	420 (26,3)	263 (19,4)	157 (65,1)	<0,001
Grave (%)	129 (8,1)	82 (6,1)	47 (19,5)	<0,001
Tratamiento recibido				
Esteroides (%)	926 (58,1)	756 (55,9)	170 (70,5)	<0,001
Tocilizumab (%)	300 (18,8)	246 (18,2)	54 (22,4)	0,075
Anakinra (%)	42 (2,6)	33 (2,4)	9 (3,7)	0,172
Estancia (días), media (DE)	9,3 (10,1)	9,3 (9,7)	9,2 (12,1)	0,908
Ingreso en UCI (%)	110 (6,9)	82 (6,1)	28 (11,6)	0,002
Estancia en UCI (días), media (DE)	17,9 (15,4)	18,3 (15,7)	16,6 (14,9)	0,646

SDRA: Síndrome de distrés respiratorio agudo, SAFI: Ratio saturación periférica de oxígeno-Fracción inspirada de oxígeno, DE: Desviación estándar, UCI: Unidad de cuidados intensivos.

5.3.2. Prevalencia y características de las infecciones bacterianas

En el total, se identificaron 156 infecciones bacterianas en 135 pacientes (8,5% de la población total), con aislamiento microbiológico en un 91,9% (**tabla 36**). El foco más frecuente fue el urinario (31,6%), seguido de la bacteriemia (31,9%; un 67,4% relacionada con catéter y un 32.6% de origen primario) y de las infecciones pulmonares (31,8%; un 51,2% sobreinfección bacteriana de origen comunitario y un 48,8% de origen nosocomial). Un 6,7% presentó infecciones intraabdominales, o de piel y partes blandas, respectivamente. Otros focos de infección bacteriana (5,9%) incluyeron meningitis (2 pacientes), endocarditis (2 pacientes), foco otorrinolaringológico (2 pacientes), tuberculosis (1 paciente) y un shock séptico de origen indeterminado en un paciente.

Tabla 36. Origen de las infecciones bacterianas en pacientes ingresados por neumonía por COVID-19

Foco de la infección	N (%)
Pulmonar	43 (31.8)
Adquirido en la comunidad/sobreinfección	22/43 (51.2)
Nosocomial	21/43 (48.8)
Bacteriemia:	43 (31.9)
Relacionada con catéter	29/43 (67.4)
Origen desconocido	14/43 (32.6)
Tracto urinario	44 (32.6)
Intraabdominal	9 (6.7)
Piel y partes blandas	9 (6.7)
Otros *	8 (5.9)

*Entre los que se incluyen: meningitis (2 pacientes), endocarditis (2 pacientes), foco otorrinolaringológico (2 pacientes), tuberculosis (1 paciente) y shock séptico de origen indeterminado (1 paciente).

El microorganismo causal de la infección se muestra en la **tabla 37**. En el 54,1% de los pacientes se aislaron cocos gram-positivos (en un 25,2% enterococos, en un 23,7% estafilococos coagulasa-negativos, en un 11,9% estreptococos, en un 8,2% estafilococos aureus meticilin-resistentes, en un 1,5% estafilococos aureus meticilin-sensibles) y en un 29,6% enterobacterias (en un 22,2% *E. coli*, en un 11,9% *Klebsiella* spp y en un 2,2% *Enterobacter* spp). En un 9,6% se identificaron infecciones por gram-negativos no fermentadores (*Pseudomonas aeruginosa* en un 8,9%) y en un 6,7% infecciones por anaerobios (*Clostridium difficile* en un 3%).

La distribución de los patógenos resistentes según los criterios descritos se refleja asimismo en la **tabla 38.** En un 19,3% se identificaron microorganismos *MDR* a expensas de *E. coli* (30,8%), estafilococos (15,4%), *Stenotrophomonas* (15,4%), *Enterobacter* (7,7%), *Klebsilella* (11,1%), *Achromobacter* (5,5%) y *Acinetobacter* (5,5%). En relación con los bacilos gram-negativos *DTR*, documentados en un 13.3%, se debieron a la presencia de especies de *E.Coli* (27,8%), *Stenotrophomonas* (22,2%), *Pseudomonas aeruginosa* (16,7%), *Enterobacter* (11,1%), *Klebsiella* (11.1%), *Achromobacter* (5,5%) y *Acinetobacter* (5,5%). Estas infecciones ocurrieron en su mayoría en pacientes ingresados en la UCI (63,3% de las infecciones por organismos *MDR* y 77,8% de las infecciones por gram-negativos *DTR*).

Finalmente, el foco de la infección en los 11 pacientes en los que no se pudo determinar el organismo responsable fue: neumonía nosocomial (4 pacientes), piel y partes blandas (3 pacientes), tracto urinario (2 pacientes), diverticulitis (1 paciente) y un shock séptico de origen desconocido.

Tabla 37. Microbiología de las infecciones bacterianas en pacientes ingresados por neumonía por COVID-19

Microorganismo	N (%)
Cocos gram-positivos	73 (54.1)
SARM	11 (8.2)
SAMS	2 (1.5)
Estafilococos coagulasa-negativo	32 (23.7)
Enterococcos	34 (25.2)
Estreptococos	16 (11.9)
Enterobacterias	40 (29.6)
E. coli	30 (22.2)
Klebsiella spp.	16 (11.9)
*Enterobacter s*pp.	3 (2.2)
Otros	2 (1.5)
Gram-negativos no fermentadores	13 (9.6)
P. aeruginosa	12 (8.9)
Stenotrophomonas maltophilia	4 (3)
Acinetobacter spp.	1 (1)
Achromobacter spp.	1 (1)
Anaerobios	9 (6.7)
Clostridioides difficile	4 (3)

SARM: Estafilococo aureus resistente a meticilina, SASM: Estafilococo aureus sensible a meticilina.

Tabla 38. Prevalencia de gérmenes *MDR* y bacilos gram-negativos *DTR* en pacientes ingresados por neumonía por COVID-19

Microorganismo	N (%)
MDR	26 (19,3)
E. coli	8 (30,8)
Pseudomonas aeruginosa	4 (15,4)
Estafilococos resistentes	4 (15,4)
Stenotrophomonas maltophilia	4 (15,4)
*Enterobacter s*pp.	2 (7,7)
Klebsiella spp.	2 (7,7)
Achromobacter spp.	1 (3,8)
Acinetobacter spp.	1 (3,8)
Bacilos gram-negativos *DTR*	18 (13,3)
E. coli	5 (27,8)
Stenotrophomonas maltophilia	4 (22,2)
Pseudomonas aeruginosa	3 (16,7)
*Enterobacter s*pp.	2 (11,1)
Klebsiella spp.	2 (11,1)
Achromobacter spp.	1 (5,5)
Acinetobacter spp.	1 (5,5)

MDR: *Multi-drug resistant*, *DTR*: *Difficult to-treat resistance*

5.3.3. Características de los pacientes con infecciones bacterianas

Las características basales y clínicas durante el ingreso de los pacientes con infecciones bacterianas se compararon con el resto de la población (**tablas 39 y 40,** respectivamente**)**. Los pacientes que presentaron complicaciones infecciosas eran significativamente mayores (68 vs 64,5, p=0,007) y presentaban con mayor frecuencia hipertensión arterial (48,1% vs 43,5%, p=0,002), enfermedad neurológica (20,7% vs 13,5%, p=0,0021), enfermedad renal (13,3 % vs 6,4%, p=0,0003), inmunosupresión previa (28,9% vs 8,7%, p <0,001), enfermedad hematológica (7,4% vs 1,9%, p <0,001) y enfermedades autoinmunes (10,4% vs 5,9%, p=0,037) que en los no que no se documentaron infecciones (**tabla 39**).

Tabla 39. Características basales de los pacientes ingresados por neumonía por COVID-19, según la presencia de complicaciones infecciosas bacterianas

	Complicaciones infecciosas bacterianas		
	No (n=1459)	Sí (n=135)	Valor de p
Sexo masculino (%)	903 (61,9)	87 (64,4)	0,559
Edad, media (DE)	64,5 (14,9)	68 (14,3)	<0,001
Hipertensión (%)	634 (43,5)	65 (48,1)	0,570
Diabetes (%)	244 (16,7)	37 (27,4)	<0,001
Obesidad (%)	388 (35,7)	36 (30)	0,547
Cardiopatía (%)	242 (16,6)	28 (20,7)	0,116
Enfermedad neurológica (%)	197 (13,5)	28 (20,7)	0,0021
Enfermedad pulmonar (%)	222 (15,2)	26 (19,3)	0,295
Hepatopatía crónica (%)	42 (6,4)	6 (4,4)	0,308
Insuficiencia renal (%)	94 (6,4)	18 (13,3)	0,003
Inmunosupresión (%)	127 (8,9)	39 (28,9)	<0,001
Trasplante (%)	24 (1,6)	6 (4,4)	0,036
Neoplasia de órgano solido (%)	51 (3,5)	9 (6,7)	0,061
Enfermedad hematológica (%)	27 (1,9)	10 (7,4)	<0,001
Enfermedad autoinmune (%)	86 (5,9)	14 (10,4)	0,037

DE: Desviación estándar.

Tabla 40. Datos clínicos de los pacientes ingresados por neumonía por COVID-19, según la presencia de complicaciones infecciosas bacterianas

	Complicaciones infecciosas		
	No (n=1459)	Sí (n=135)	Valor de p
SDRA (N, %)	1090 (74,8)	122 (90,4)	<0,001
SAFI, media (DE)	280 (123)	198 (114)	<0,001
Leve (%)	627 (43)	33 (24,4)	<0,001
Moderado (%)	386 (26,5)	34 (25,2)	0,748
Grave (%)	74 (5,1)	55 (40,7)	<0,001
Tratamiento recibido			
Esteroides (%)	824 (56,5)	102 (76,1)	<0,001
Tocilizumab (%)	246 (16,9)	54 (40)	<0,001
Anakinra (%)	35 (2,4)	7 (5,2)	0,053
Estancia, media (DE)	8,4 (8,1)	19,5 (19,6)	<0,001
Ingreso en UCI (%)	56 (3,8)	54 (40)	<0,001
Estancia en UCI, media (DE)	10,4 (11)	27,2 (15,2)	<0,001
Reingreso (%)	72 (4,9)	119 (14,2)	<0,001
Éxitus (%)	207 (14,2)	34 (25,2)	<0,001

SDRA: Síndrome de distrés respiratorio agudo, SAFI: Ratio saturación periférica de oxígeno-Fracción inspirada de oxígeno, DE: Desviación estándar, UCI: Unidad de cuidados intensivos.

Por otra parte, se confirmó la asociación estadísticamente significativa del desarrollo de infecciones bacterianas durante el ingreso con el SDRA (90,4% vs 74,8%, p <0,001) y la SAFI (180 vs 280, p<0,001) (**tabla 40**). Por consiguiente, los pacientes que padecieron infecciones bacterianas presentaron menos SDRA leve (24,4% vs 43%, p <0,001), más SDRA grave (40,7% vs 5,1%, p<0,001), mayor uso de esteroides (76,1% vs 56,5%, p<0,001) y tocilizumab (40% vs 16,9%, p<0,001), mayor estancia media (19,5 vs 8,4 días, p <0,001), mayor tasa de ingreso en UCI (40% vs 3,8%, p<0,001) y mayor estancia en UCI (15,2 vs 10,4 días, p<0,001). Asimismo, las tasas de reingresos (14,2% vs 4,9%, p<0,001) y de mortalidad (25,2% vs 14,2%, p<0,001) fueron mayores en los pacientes con complicaciones infecciosas.

5.3.4. Factores de riesgo de desarrollo de infecciones bacterianas

Para la identificación de los factores de riesgo relacionados con el desarrollo de infecciones bacterianas en pacientes ingresados por COVID-19, se realizó un análisis multivariante considerando las características basales de los pacientes, la gravedad de la infección por COVID-19 o sus complicaciones y el tratamiento inmunosupresor recibido para el tratamiento del SDRA (**tabla 41**). Así pues, los factores relacionados de forma independiente con las complicaciones infecciosas fueron la edad (OR=1,69, IC 95% 1,01-1,04), la enfermedad neurológica (OR= 1,69, IC 95% 1,01-2,82), la inmunosupresión anterior al ingreso (OR=4,41, IC 95% 2,76-7,06) y el propio ingreso en UCI (OR=21,36, IC 95% 13,21-34,55). El tratamiento esteroideo, el tratamiento con tocilizumab, o la combinación de ambos no se asoció con un mayor riesgo de infecciones bacterianas tras el ajuste.

Tabla 41. Factores de riesgo desarrollo de infección bacteriana en pacientes con COVID-19

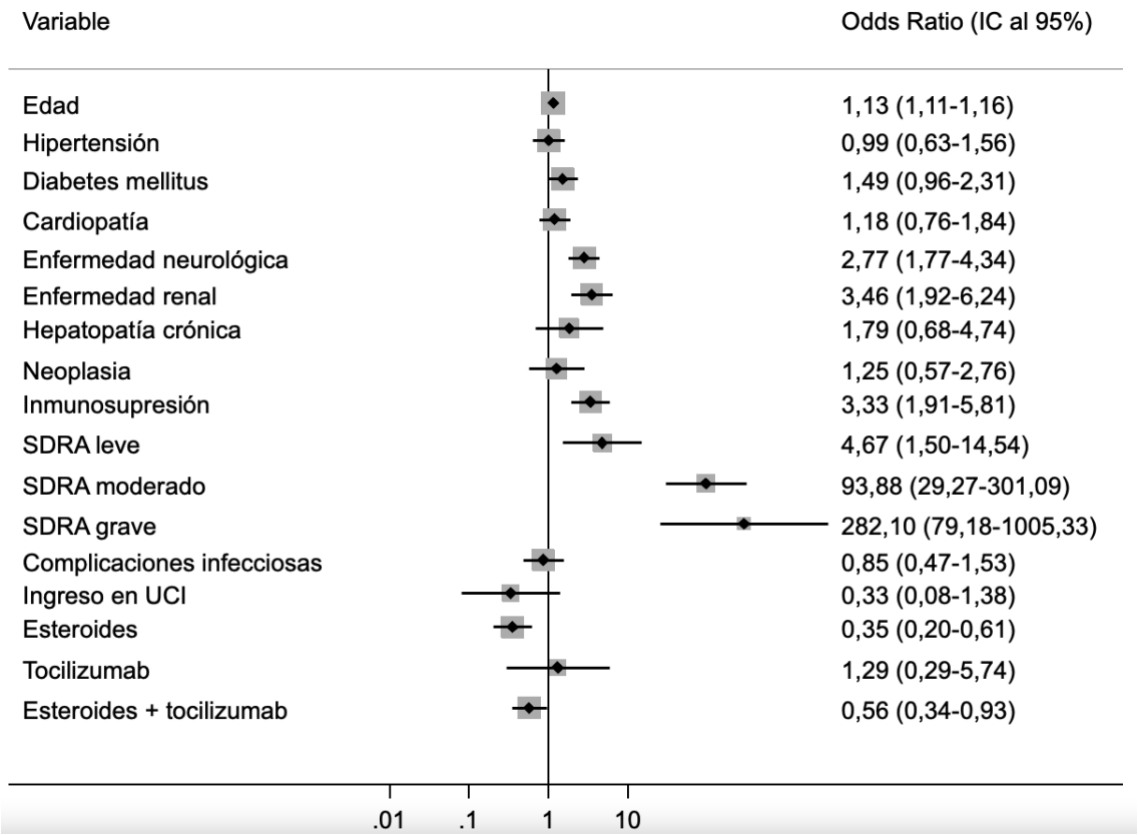
		Análisis univariante*		**Análisis multivariante****	
		OR	**IC al 95%**	**OR**	**IC al 95%**
Características basales	Edad	1,02	1,01-1,03	**1,02**	**1,01-1,04**
	Diabetes mellitus	1,88	1,25-2,81	1,60	0,99-2,49
	Enfermedad neurológica	1,68	1,08-2,60	**1,69**	**1,01-2,82**
	Enfermedad renal	2,23	1,30-3,82	1,22	0,64-2,29
	Neoplasia activa	2,66	1,52-4,65	1,05	0,50-2,20
	Inmunosupresión	4,26	2,82-6,45	**4,41**	**2,76-7,06**
Eventos clínicos	SDRA	3,17	3,17-5,68	1,34	0,70-2,55
	Ingreso en UCI	16,69	10,80-25,81	**21,36**	**13,21-34,55**
Tratamiento	Esteroides	2,46	1,63-3,70	0,94	0,55-1,61
	Tocilizumab	3,29	2,27-4,76	0,66	0,12-3,75
	Esteroides + Tocilizumab^{T}	2,43	1,66-3,58	1,31	0,80-2,16

En negrita aparecen señalados los OR e IC con significación estadística.
OR: Odds ratio, IC: Intervalo de confianza, SDRA: Síndrome de distrés respiratorio agudo, UCI: Unidad de cuidados intensivos.
^{T}: Interacción estadística entre el tratamiento esteroideo y tocilizumab.

5.3.5. Análisis multivariante de mortalidad

En último lugar, y dado que la tasa de mortalidad de los pacientes que desarrollaron infecciones bacterianas fue significativamente mayor en el análisis univariante, se realizó una regresión logística para identificar los factores asociados a la mortalidad (**figura 26**). En los pacientes ingresados por neumonía por COVID-19, la mortalidad vino determinada por las comorbilidades, incluyendo la edad (OR=1,13, IC 95% 1,10-1,16), la enfermedad neurológica (OR=2,77, IC 95% 1,77-4,34), la enfermedad renal (OR=3,46, IC 95% 1,92-6,24) o la inmunosupresión previa (OR= 3,33, IC 95% 1,91-5,82); además de la presencia y la gravedad del SDRA: SDRA leve (OR=4,67, IC 95% 1,50-14,54), SDRA moderado (OR=93,88, IC 95% 29,27-301,08) y el SDRA grave (OR=282,10, IC 95% 79,18-1005,33). Sin embargo, las infecciones bacterianas no se asociaron con la mortalidad tras el ajuste (OR=0,85, IC 95% 0,47-1,53). El tratamiento con esteroides (OR=0,35, IC 95% 0,20-0,60) y el tratamiento combinado de esteroides y tocilizumab (OR=0,56, IC 95% 0,34-0,93) mostraron un efecto protector.

Figura 25. Factores predictores de mortalidad en pacientes ingresados por neumonía por COVID-19



Los Odds ratio se muestran como cuadrados y las líneas corresponden al intervalo de confianza al 95%
IC: Intervalo de confianza. SDRA: Síndrome de distrés respiratorio agudo. UCI: Unidad de cuidados intensivos.

6. DISCUSIÓN

Nuestro estudio demuestra que el ADE es un biomarcador pronóstico de mortalidad en la sepsis y en la infección por COVID-19. Estas dos patologías de elevada prevalencia y mortalidad comparten además mecanismos fisiopatológicos puesto que, tras el estímulo inicial de la infección bacteriana o viral, se produce una respuesta inmunológica desproporcionada y descontrolada. Siendo la IL-6 una de sus principales protagonistas, esta hiperactivación inmune es la responsable del daño sistémico que las caracteriza. Además, en este trabajo se constata que en el contexto de la infección por COVID-19, las infecciones bacterianas *per se* no suponen un mayor riesgo de mortalidad, sino que traducen, por un lado, el ingreso en la UCI, y por otro, la gravedad del SDRA o de la neumonía por COVID-19. Por lo tanto, consideramos que la elevación del ADE en la infección por COVID-19 no se atribuye a la infección bacteriana, sino que refleja la tormenta de citoquinas, en la que la IL-6 es la principal mediadora. En su conjunto, estos resultados posicionan al ADE como un marcador de la respuesta inmune sistémica y como una herramienta pronóstica útil en el manejo de estos pacientes.

6.1. ADE COMO BIOMARCADOR EN LA SEPSIS EN PACIENTES INGRESADOS EN LA UCI

En este grupo poblacional, los resultados muestran que el ADE, a diferencia de la PCR y la PCT, tiene una capacidad predictiva de mortalidad de forma independiente en los pacientes que ingresan en la UCI por sepsis. Además, su incorporación a las principales escalas utilizadas (SOFA, LODS, SAPS-II y APACHE-II) supone una mejora de su capacidad pronóstica. El modelo resultante de la combinación del SOFA con el ADE al ingreso en UCI, a su vez las dos únicas variables asociadas con la mortalidad de forma independiente, presentó la mejor capacidad predictiva de mortalidad.

6.1.1. Comparación de las escalas pronósticas

En primer lugar, y en relación con la selección de variables a incluir en el ajuste o análisis multivariante, y a fin de determinar el mejor modelo predictor de mortalidad, se realizó la comparación de la capacidad predictiva de mortalidad de las principales escalas pronósticas según su diseño o aplicación y según las variables que se utilicen para su cálculo.

Por un lado, las escalas qSOFA y NEWS se diseñaron y validaron para identificar y reconocer de forma precoz al paciente potencialmente grave 1,8,9,47-51]. Estos *scores* se basan únicamente en parámetros clínicos que se pueden determinar a pie de cama, con el objetivo de

ser accesibles y aplicables en cualquier escenario. Como resultado, y tras las recomendaciones del documento actual de definición de sepsis (Sepsis-3), la escala qSOFA se ha convertido en una útil herramienta que se utiliza en los servicios de urgencias y plantas de hospitalización como parte de las estrategias de reconocimiento precoz de la sepsis [3]. Además, Canet *et al.* confirmaron que la escala qSOFA es capaz de predecir la mortalidad y la estancia en UCI en pacientes con sospecha de infección en el ámbito de urgencias [144]. Sin embargo, y aunque la escala presenta un valor predictivo negativo del 98% para la mortalidad hospitalaria, el qSOFA ha sido criticado por su bajo valor predictivo positivo. A su vez, la escala NEWS, diseñada en 2012 por el NHS para su aplicación en el sistema sanitario británico, y actualizada posteriormente en la versión NEWS2, ha demostrado ser más precisa que la escala qSOFA en la predicción de la mortalidad hospitalaria y de la mortalidad relacionada con la sepsis [145,146]. Tanto es así que Mellhamar *et al.* demostraron que el *score* NEWS2 fue superior al qSOFA en la detección de una variable compuesta por sepsis con disfunción orgánica, mortalidad relacionada con la infección e ingreso en la unidad de cuidados intensivos por la infección [147]. Asimismo, nuestro análisis confirma que la escala NEWS2 tiene mejor capacidad predictiva que la escala qSOFA, por lo que se podrá plantear como una alternativa más válida en la identificación del paciente grave o potencialmente grave. Por este motivo, en nuestro estudio se eligió para valorar la situación clínica de los pacientes en el análisis multivariante pero no se incluyó en el diseño del mejor modelo predictor de mortalidad, combinado con el ADE, puesto que no incluye parámetros de laboratorio.

Por otra parte, las escalas SOFA, LODS y SIRS cuantifican el daño orgánico en el paciente con sepsis [1-9, 52-57]. Los criterios SIRS se acuñaron en la primera conferencia de consenso para definir la sepsis y el fracaso orgánico, pero han demostrado ser unos criterios inespecíficos y poco precisos en este contexto [1,2,3,8,9]. Posteriormente, y como ya se indicó, el documento Sepsis-3 posicionó a la escala SOFA como criterio definitorio de sepsis, en atención a que las escalas SOFA y LODS presentan mejor capacidad predictiva que los criterios SIRS y a que la escala SOFA es más sencilla y conocida que la escala LODS. Sin embargo, entre estas dos últimas no hay datos confirmatorios de cuál tiene mejor capacidad predictiva o cuál puede cuantificar con más exactitud y precisión el daño orgánico en pacientes con sepsis; y sólo Wu *et al.* han demostrado, en pacientes con trauma grave, que el LODS tiene mayor capacidad pronóstica que el SOFA [148-154]. En nuestra cohorte, los *scores* SOFA y LODS fueron superiores a los criterios SIRS; a su vez, la escala SOFA, factor independiente de mortalidad, mostró mayor potencia pronóstica que LODS. Por un lado, estos resultados confirman que la escala SIRS es un término inexacto en el ámbito de la sepsis, sin un rol predictivo adecuado, en la línea de la bibliografía reciente que lo

reconoce como un término prácticamente en desuso [1,8,9]. Sin embargo, consideramos que el concepto SIRS no es inútil puesto que permite detectar al paciente con una situación inflamatoria en otros contextos clínicos, lo cual puede ayudar a identificar y contextualizar ciertas patologías [4]. Por ello, siendo un término fisiopatológicamente congruente y bien conocido, no consideramos que los criterios o el concepto SIRS estén obsoletos, aun cuando en el contexto de la sepsis no sean válidos. Por otro lado, nuestros hallazgos confirman que la escala SOFA es la herramienta adecuada para evaluar el daño orgánico de la sepsis. Aunque las escalas SOFA y LODS cuantifiquen y evalúen variables similares, las diferencias encontradas pueden atribuirse a que la escala SOFA incluye el uso y las dosis de vasopresores, dato sólido de gravedad y daño orgánico, mientras que la escala LODS incluye los parámetros de coagulación y la cifra de leucocitos, menos específicos y no necesariamente sinónimos de daño orgánico. De acuerdo con todo lo anterior, en el análisis multivariante se consideró al SOFA como el criterio de daño orgánico. A su vez, y como se discutirá posteriormente, en nuestra población fue el mejor modelo predictor de mortalidad al añadir el ADE.

Finalmente, las escalas APACHE-II y SAPS-II son muy utilizadas en el paciente crítico y determinan la gravedad de este, independientemente de la causa [58-62]. A pesar de que existen versiones más recientes de las dos, su uso se ha mantenido gracias a que son muy accesibles y de fácil aplicación en la práctica clínica. Algunos estudios han analizado la capacidad discriminatoria y la calibración entre estas dos escalas, demostrando que, aunque ambas predicen la mortalidad con precisión, SAPS-II puede tener mejor capacidad predictiva de mortalidad [155-158]. A pesar de que no se logró la significación estadística en la comparación de las AUC-ROC, nuestros hallazgos van en la misma línea, razón por la que se utilizó como criterio o sinónimo de gravedad en el posterior análisis.

6.1.2. Dinámica y capacidad pronóstica del ADE

Estudios previos han demostrado que el ADE es un factor predictor independiente de mortalidad en los pacientes con sepsis [121, 122, 159-163], hallazgos que se confirman en nuestra cohorte. Asimismo, otros autores han analizado la dinámica y los cambios en las cifras del ADE, demostrando que sus valores y variaciones, durante la primera semana del ingreso o tras la presentación de la sepsis, reflejan la gravedad y predicen la muerte. De esta forma, Xu-Feng mostró que, a pesar de la ausencia de diferencias en los valores iniciales del ADE, los pacientes fallecidos con sepsis presentaban un mayor ADE los días 4 y 7 del debut. Además, la mayoría de los pacientes no supervivientes presentaban un ascenso continuado del ADE durante la primera semana del ingreso, mientras que los pacientes supervivientes no [164]. En paralelo,

Ozdogan *et al.* confirmaron que los pacientes fallecidos por sepsis de origen abdominal presentaban mayores valores del ADE los días 1, 3 y 7 del ingreso [165]; así como Lorente *et al.* quienes demostraron, en una cohorte de pacientes ingresados en UCI por sepsis, que los pacientes fallecidos tenían mayores valores de ADE los días 1, 4 y 9 del ingreso, y que estos valores se correlacionaban con la puntuación de la escala SOFA [166]. De forma similar, Kim *et al.* identificaron un mayor riesgo de mortalidad en los pacientes sépticos que presentaban un ascenso del ADE sobre el basal a las 72 horas de ingreso hospitalario, tras el ajuste por otras variables como el SOFA, el Charlson, la hemoglobina y otros parámetros inflamatorios [167]. Ku *et al.* también observaron que el ADE a las 72 horas de la bacteriemia por gram-negativos era mayor en los pacientes que fallecían, y que este valor tenía mejor AUC-ROC que el propio SOFA o que el ADE en el momento de la bacteriemia [168]. Todos los datos anteriores apoyan nuestros resultados, que confirman mediante el análisis longitudinal que el ADE, durante la primera semana de ingreso en UCI por sepsis, se asocia a un mayor riesgo de mortalidad. Además, nuestro estudio pone de manifiesto igualmente que los valores del ADE a las 48 y 72 horas del ingreso en UCI, a su vez los mayores niveles identificados durante la primera semana de ingreso, presentaron la mejor capacidad predictiva de mortalidad, resultados muy similares a los que presentaron Kim *et al.* y Ku *et al.* [167,168]. Por otra parte, Jian *et al.* identificaron en su interesante estudio que los pacientes con sepsis ingresados en la UCI desarrollaban anemia durante la primera semana de ingreso, y que su aparición lo hace en paralelo a la elevación de la hepcidina, la ferritina y la IL-6, parámetros característicos de la anemia de la enfermedad crónica o del trastorno inflamatorio [133]. A su vez, estos parámetros se relacionaban con un aumento del ADE y con el SOFA, únicos marcadores independientes de mortalidad. Los anteriores datos son equivalentes a los nuestros, y probablemente se deban a que las elevaciones del ADE son un reflejo de la acción de la IL-6: respuesta atenuada a la eritropoyetina, inhibición de la eritropoyesis y de la síntesis de hemoglobina [127-132]. Esta hipótesis ha sido tratada previamente por otros autores en patologías como la enfermedad cardiovascular o la artritis reumatoide, donde también se ha visto que el aumento de la anisocitosis discurre en paralelo a otros marcadores de la anemia del trastorno crónico, debido al aumento de las citoquinas inflamatorias, como la IL-6 o el factor de necrosis tumoral. Además del estudio de Jian *et al.*, otros autores han demostrado una asociación entre el ADE y citoquinas inflamatorias responsables de la anemia de trastorno crónico como la IL-6 o el factor de necrosis tumoral, así como con los marcadores de la anemia de trastorno crónico, demostrando que el ADE es un marcador subrogado del ambiente inflamatorio sistémico [134,135,169,170]. Por lo tanto, la dinámica del ADE en nuestro estudio confirma que este se eleva a las 48-72 horas del debut de la sepsis, reflejo del ambiente pro-inflamatorio y de las citoquinas que conllevan a la disfunción

orgánica que define la sepsis. De esta forma, los valores del ADE en el debut, y a las 48 y 72 horas del ingreso, presentaron la mejor capacidad pronóstica de mortalidad.

6.1.3. Comparación del ADE con la PCR y PCT

Como se mencionó previamente, actualmente no se dispone de un biomarcador adecuado para la sepsis. Por lo tanto, el lactato, la PCR y la PCT son los parámetros más estudiados y aplicados en la práctica clínica puesto que, a pesar de la identificación reciente de múltiples moléculas con este fin, ninguna ha conseguido superar a los anteriores y consolidarse [98]. En este sentido, el lactato se utiliza como sinónimo de disfunción hemodinámica y orgánica, la PCR como un marcador sensible pero inespecífico de inflamación y la PCT como un parámetro indicador de infección bacteriana que puede ayudar a monitorizar o guiar el tratamiento antibiótico [94,107,110]. Así pues, las guías clínicas de sepsis contemplan el uso de la PCT y del lactato en la sepsis, pero no de la PCR [21].

A su vez, algunos autores han analizado la capacidad diagnóstica y predictiva del ADE en comparación con estos marcadores. Zhang *et al.* y Laukemann *et al.* demostraron que la PCT identifica con mejor precisión los pacientes que tienen hemocultivos positivos en el seno de la sepsis o SIRS, mientras que Chen *et al.* y Park *et al.* confirmaron que el ADE presentaba mejor capacidad pronóstica en términos de mortalidad que la PCR, la PCT o el lactato [171-174]. De igual forma, otros autores han elaborado escalas pronósticas o diagnósticas como el *score* CHARM, que, en combinación con otras variables, presentaba mejor capacidad predictiva que estos tres marcadores de forma aislada [172,175].

En el presente estudio se analizó no sólo la asociación de estos biomarcadores con la mortalidad sino su dinámica para comprender mejor su rol. En primer lugar, la PCR alcanzó sus valores máximos en el ingreso y a las 24 horas del mismo. De hecho, este último valor fue mayor en los pacientes supervivientes. Por ello, y aunque los pacientes fallecidos presentaron mayores valores de PCR a las 72 horas y 7 días del ingreso, el análisis longitudinal confirmó que la PCR no presentó una asociación estadísticamente significativa con la mortalidad durante la primera semana de ingreso. En segundo lugar, los mayores valores de PCT se identificaron a las 24 horas del ingreso en la UCI, y solo la PCT a los 7 días se asoció con la mortalidad. A su vez, todas las determinaciones del valor del ADE mostraron una asociación independiente con la mortalidad, que se confirmó mediante el análisis longitudinal. A nuestro juicio, estos resultados coinciden con lo comentado previamente y refuerzan la capacidad y utilidad del ADE en este escenario.

Por un lado, y de acuerdo con lo expuesto, el ADE refleja la intensidad y la repercusión de la actividad inflamatoria sistémica y el daño orgánico, factores claramente determinantes de la gravedad y por tanto de la mortalidad. Por otro lado, la PCT es un marcador de infección bacteriana y de la carga de esta, pero no necesariamente refleja la respuesta inflamatoria o el daño orgánico secundario a la infección [95,109,110]. Por este motivo, en los estudios previos la PCT presentaba mayor exactitud en la identificación de bacteriemia sin que ello conllevase una mejor capacidad pronóstica de mortalidad [171,172]. Finalmente, y aunque la PCR sea un marcador muy sensible de inflamación, en la sepsis no refleja con la misma exactitud o precisión que el ADE la repercusión sistémica y la disfunción de los distintos órganos que la inflamación no controlada conlleva. Por estos motivos, nuestros resultados posicionan al ADE como un marcador más fiel y exacto del daño sistémico que conlleva a la muerte en la sepsis, con la ventaja añadida de que es un parámetro fácilmente accesible puesto que forma parte del hemograma rutinario.

6.1.4. Valor añadido del ADE sobre las escalas pronósticas existentes y determinación del mejor modelo predictor de mortalidad

Por otra parte, otros autores han estudiado y comparado el rol del ADE con las escalas pronósticas como el SOFA, SIRS y el APACHE, demostrando, por un lado, que el ADE se correlaciona con el SOFA o el APACHE-II [9,164,166,176,177], y por otro, que incluso podría tener mejor capacidad pronóstica que el SOFA, APACHE-II o los criterios SIRS [162,173,175,178]. De hecho, Sadaka *et al.* demostraron que al añadir el ADE al APACHE-II, el AUC-ROC de la mortalidad hospitalaria en el shock séptico mejoraba [179]. En el presente estudio se evaluó la utilidad del ADE con el SOFA y LODS, escalas pronósticas que definen y cuantifican el daño orgánico, y con las escalas APACHE-II y SAPS-II, predictores de mortalidad hospitalaria y en cuidados intensivos.

Nuestros datos apoyan los resultados de los estudios previos y demuestran que el ADE mejora la capacidad discriminatoria del SOFA, LODS y del SAPS-II. Creemos que estos nuevos hallazgos son prometedores y relevantes puesto que confirman que el ADE es un reflejo sólido de la respuesta inflamatoria alterada que se traduce en la disfunción sistémica de la sepsis. Como consecuencia, el ADE podría ser un marcador de disfunción orgánica, similar a la creatinina, a la bilirrubina o a las plaquetas, a su vez marcadores inespecíficos de sepsis pero que definen la disfunción orgánica y la gravedad del paciente crítico, y que por lo tanto se incluyen en las escalas SOFA y LODS o en las escalas APACHE-II y SAPS-II, respectivamente.

En este sentido, el ADE, añadido al SOFA, a su vez las dos únicas variables asociadas de forma independiente con el mayor riesgo de mortalidad, presentaron la mejor capacidad discriminatoria de mortalidad en comparación con el resto de modelos, probablemente porque reflejan la disfunción sistémica y el daño orgánico de la sepsis con mayor precisión y exactitud. Además, y dado que el SOFA es la herramienta propuesta por el *Third Consensus Definition for Sepsis and Septic shock* (Sepsis-3) para evaluar el daño orgánico y definir la sepsis, la inclusión del ADE podría mejorar el rendimiento de esta escala, además de añadir el concepto de disfunción inflamatoria sistémica en ella, punto clave en el concepto y fisiopatología de la sepsis [1].

6.2. ADE COMO BIOMARCADOR EN LA INFECCIÓN GRAVE POR COVID-19

Como parte del estudio TOCICOV se analizó la capacidad pronóstica de mortalidad del ADE en la infección grave por COVID-19, demostrando que también presenta una mejor capacidad predictiva que otros parámetros y que refleja el ambiente inflamatorio y los efectos de la IL-6, a pesar del bloqueo de su receptor tras el tratamiento con tocilizumab.

6.2.1. Dinámica y capacidad pronóstica del ADE

Actualmente también hay datos claros que confirman que el ADE es mayor en pacientes con COVID-19 grave y en quienes presentan una mayor mortalidad [125,126,180-187]. En este sentido, Hornick *et al.* demostraron que el ADE se asocia con la mortalidad tras el ajuste por la edad, el sexo, la raza, la enfermedad cardiovascular y la hemoglobina [187]. Además, este estudio mostró que el ADE se asociaba con ciertas citoquinas pro-inflamatorias como el factor de necrosis tumoral alfa o la propia IL-6. De igual forma, el trabajo de Martínez-Urbistondo confirmó que los pacientes que presentaban mayores niveles de IL-6 tenían mayores valores de los parámetros inflamatorios, incluyendo el ADE [188]. Sin embargo, hay importantes dudas acerca de los mecanismos, dinámica, significado y utilidad del ADE en la infección por COVID-19 y en la tormenta de citoquinas.

En esta cohorte, la dinámica del ADE, aunque similar, fue menos abrupta que la que se objetivó en los pacientes con sepsis y que la que han descrito otros autores [165-168]. En este caso, el tratamiento con tocilizumab pudo justificar esta diferencia, ya que el bloqueo de la IL-6 puede suponer una menor inhibición de la eritropoyesis y atenuar los mecanismos de la anemia del trastorno crónico [127,128,189]. Por este motivo se consideró evaluar específicamente el efecto del tratamiento con tocilizumab, ya que no sólo podría suponer cambios en la dinámica

del ADE y otros marcadores, sino que su indicación durante el ingreso pudo deberse a diferencias en el perfil del paciente. De esta forma, la gravedad y/o indicación de medidas invasivas o ingreso en UCI de los pacientes que recibieron tocilizumab pudo ser distinta de los que no lo recibieron. Con todo ello, los pacientes fallecidos presentaron mayores cifras de ADE en el momento del ingreso, en el día 0, 3 y 7. Además, el análisis multivariante confirmó que el valor del ADE en el ingreso y en el día 3, a su vez los parámetros que de nuevo presentaron mayor AUC-ROC, eran factores predictores de mortalidad, de forma independiente al tratamiento con tocilizumab y a las cifras de hemoglobina. Por último, el análisis de las correlaciones entre los marcadores inflamatorios demostró que la IL-6 en el día 0, y por lo tanto coincidiendo con el deterioro respiratorio, determina el valor del ADE el día 3. Pero esto no sucede en la población tratada con tocilizumab, en la que los valores en suero de la IL-6 libre se elevan por el bloqueo de su receptor y no necesariamente reflejan inflamación [189]. Estos datos coinciden con lo previamente descrito, y además apoyan que el ADE es un dato indirecto de la actividad sistémica de la IL-6 a las 72 horas, remedando nuestros hallazgos en la cohorte de pacientes sépticos. Además, en este grupo de pacientes tiene especial interés el hecho de que el ADE es capaz de reflejar el valor biológicamente eficaz de la IL-6 y no sus valores en suero, lo que resulta especialmente útil para cuantificar el estado hiperinflamatorio en la tormenta de citoquinas y los efectos de la IL-6 activa, incluso cuando sus niveles se han elevado tras el tratamiento por tocilizumab.

6.1.2. Comparación del ADE con otros marcadores de la tormenta de citoquinas

Además del ADE, en el presente trabajo evaluamos la dinámica y el rol pronóstico de otros marcadores inflamatorios de la tormenta de citoquinas en la infección por COVID-19, haciendo especial énfasis en la ferritina, la PCR y la IL-6 y su relación con el ADE.

Por un lado, la ferritina es una molécula clave en la homeostasis del metabolismo férrico [129,130]. Su principal función es el almacenaje y secuestro del hierro libre, no sólo como molécula de depósito, sino para proteger al resto de tejidos de las especies reactivas del hierro. Sin embargo, la ferritina en suero también se comporta como un reactante de fase aguda y como marcador de inflamación aguda y crónica, pudiendo elevarse bajo el efecto de la IL-1, el factor de necrosis tumoral y la IL-6, en situaciones tan amplias como la enfermedad renal crónica, la artritis reumatoide, las infecciones y las neoplasias malignas. En estos contextos, la elevación de los niveles de ferritina refleja un aumento en los depósitos de hierro que, paradójicamente, no están biológicamente disponibles para la hematopoyesis. Este mecanismo, como ya se ha indicado, es la causa de la anemia de trastorno crónico, inflamatoria o de secuestro [127,128].

De forma similar, en la infección por COVID-19 la ferritina también ha demostrado ser un marcador de inflamación y gravedad [112,189]. Tanto es así que la tormenta de citoquinas inicialmente se describió y comparó fisiopatológicamente con la enfermedad de Still y con el síndrome hemofagocítico, entidades clínicas en las que la elevación de la ferritina es especialmente acusada y en los que también se emplean el tocilizumab y el anakinra, fármacos que bloquean la acción de la IL-6 e IL-1, respectivamente [69, 127,128,191-192].

A pesar de lo anterior, en nuestra cohorte de pacientes con infección grave por COVID-19, los valores de la ferritina mostraron una pobre capacidad predictiva de mortalidad, que además no se confirmó tras el ajuste según el tratamiento con tocilizumab. Según nuestros resultados, y tal y como muestra la correlación de la IL-6 y la ferritina en el día 0, probablemente la elevación de la ferritina en este contexto se produzca por la acción de la tormenta de citoquinas. Sin embargo, es posible que la hiperferritininemia no sólo se deba a la IL-6, sino que intervienen otras moléculas y mecanismos como la IL-1, el factor de necrosis tumoral y el estado previo del almacenaje o deposito férrico previo. Por tanto, la ferritina no refleja con tanta exactitud la actividad de la IL-6, que es la que parece que determina en mayor medida la inflamación sistémica, la elevación del ADE y el daño orgánico. En consecuencia, la ferritina no se comportó como un marcador pronóstico de gravedad y de mortalidad en nuestro análisis.

Por otra parte, la PCR es una proteína que se sintetiza en el hígado tras el estímulo de la IL-6 y que también se comporta como un reactante de fase aguda, en cualquier contexto inflamatorio e independientemente de la causa, incluyendo la infección por COVID-19 [67,93-96, 193]. En nuestro trabajo en pacientes con infección grave por COVID-19, la PCR presentó una capacidad predictiva de mortalidad tras el ajuste multivariante. Además, mostró una correlación significativa con la IL-6 y con las cifras del ADE en diversos momentos de la enfermedad y de forma independiente al tratamiento con tocilizumab, confirmando que ambos parámetros representan en paralelo la actividad biológica de la IL-6 y su efecto sistémico, a diferencia de la ferritina. Aunque plausibles y lógicos desde el punto de vista biológico, estos hallazgos pueden contrastar con los que se han presentado en los pacientes con sepsis, en los que la PCR no se comportó como un marcador pronóstico de mortalidad y gravedad. Sin embargo, nuestros resultados no son necesariamente contradictorios, sino que en nuestra opinión probablemente pongan de manifiesto que, a pesar de que fisiopatológicamente sean muy similares, la sepsis y la infección grave por COVID-19 no son del todo equivalentes u homólogas. Este concepto fue puesto de manifiesto por un interesante artículo de Leisman *et al.*, quienes compararon los valores de diferentes citoquinas y marcadores inflamatorios, incluyendo la PCR y la ferritina, en

la sepsis, el SDRA por otras causas distintas al COVID-19 y otros síndromes de liberación masiva de citoquinas equivalentes a la enfermedad de Still o al síndrome hemofagocítico [194]. En este estudio se demuestra que no todas estas entidades se comportan de igual manera, sino que hay diferencias claras en el perfil bioquímico y analítico, probablemente justificadas por las distintas cascadas inmunológicas y citoquinas implicadas.

A propósito del trabajo anterior, que además mostró que los valores de las citoquinas inflamatorias son significativamente menores en el COVID-19 que en la sepsis o el SDRA por otras causas, algunos trabajos han cuestionado el papel protagonista o incluso la existencia de la tormenta de citoquinas como causa de la disfunción orgánica en la infección por COVID-19 [194,195]. En nuestra cohorte también resultó llamativo que la IL-6 no se asoció de forma significativa con la mortalidad, y que sólo los valores de la IL-6 el día 1 tras la inclusión, probablemente influidos por el tratamiento con tocilizumab como demuestra el análisis multivariante, pudieron asociarse con la mortalidad. Por el contrario, la IL-6 sí que mostró una clara correlación con la ferritina, pero sobre todo con el ADE y la PCR en pacientes no expuestos al tocilizumab, parámetros que en nuestro análisis mostraron un valor pronóstico de mortalidad. Probablemente la fisiopatología de la tormenta de citoquinas no se pueda simplificar en la acción de la IL-6 y pueda seguir el planteamiento que propuso Zizzo *et al.*, según el cual la Interleukina 33 (IL-33) es la principal responsable del daño en la infección por COVID-19 [196]. De acuerdo con esta aproximación, la IL-6, entre otras citoquinas como la IL-1b, la Interleukina 7 (IL-7) o la Interleukina 2 (IL-2), es un mediador de la cascada inflamatoria tras la acción de la IL-33. Por ello, es probable que la IL-6 pueda ser una molécula responsable de la tormenta de citoquinas, pero no la única. A pesar de lo anterior, nuestros hallazgos sí que demuestran que la PCR, pero sobre todo el ADE, son parámetros que reflejan el daño sistémico y el efecto biológico de la IL-6 en los pacientes con infección grave por COVID-19. De nuevo, los valores del ADE a las 72 horas del deterioro clínico, precisamente los que se relacionaron con la IL-6 en día 0 y presentaron buena correlación con la PCR del mismo día en pacientes no tratados con tocilizumab, son el parámetro que presentó la mejor capacidad predictora de mortalidad.

6. 3. INFECCIONES BACTERIANAS EN PACIENTES INGRESADOS POR COVID-19

La relación entre el ADE y el pronóstico de infecciones graves ha quedado evidenciada en estudios previos y se ha confirmado en nuestro trabajo. Además, nuestro análisis ha demostrado que este marcador puede también elevarse en la fase inflamatoria de la infección por SARS-CoV-2. En esta segunda cohorte, sin embargo, se identificó una proporción significativa

de complicaciones infecciosas. Efectivamente, una alta prevalencia de infecciones bacterianas en este grupo de pacientes podría haber supuesto un factor de confusión de la capacidad pronóstica del ADE en la infección por COVID-19. Por ello, en un tercer análisis, se evaluaron la prevalencia, los factores de riesgo y el impacto en términos de mortalidad de las infecciones bacterianas en una cohorte de pacientes más amplia. Por otra parte, el estudio del desarrollo de infecciones bacterianas en este contexto tiene especial interés puesto que, por el momento, las mejores herramientas de las que se dispone para el tratamiento de la infección grave y el SDRA por COVID-19 son los esteroides y el tocilizumab, fármacos inmunosupresores que presentan el reconocido riesgo de desarrollo de infecciones [71-74].

6.3.1. Prevalencia, factores de riesgo e impacto de las infecciones bacterianas

En la cohorte de pacientes ingresados por COVID-19, la prevalencia de las infecciones bacterianas fue del 8,5%. Estas cifras son discretamente superiores a las tasas de infecciones nosocomiales o asociadas a cuidados sanitarios descritas en otras cohortes previas a la pandemia [197,198]. Sin embargo, estos resultados son similares a los que se describen en otras cohortes de pacientes ingresados por COVID-19 [199,200].

Además, en el presente estudio las complicaciones infecciosas no sólo se debieron a infecciones del aparato respiratorio puesto que se identificó una importante tasa de bacteriemias primarias o relacionadas con catéter, infecciones del tracto urinario o infecciones abdominales, con la consiguiente importante participación de cocos gram-positivos, enterobacterias no fermentadoras y patógenos *MDR/DTR*, similar a lo descrito previamente [198,201,202]. Estos datos no son del todo sorprendentes puesto que la infección por COVID-19 puede conllevar estancias de ingreso muy prolongadas, ingreso en UCI, uso de dispositivos vasculares o respiratorios, malnutrición y el uso de antibioterapia empírica, todos ellos conocidos factores de riesgo de infección nosocomial y aparición de gérmenes resistentes [203,204]. Además, este hallazgo no deja de ser otro dato que apoya que la infección grave por COVID-19 supone un estado hiperinflamatorio sistémico más allá del daño local (pulmonar), que a su vez conlleva una situación de inmunosupresión; todo lo cual es muy similar a lo que ocurre en la sepsis [24-27,33,67,68,205,206].

En esta población, el principal factor de riesgo de desarrollo de complicaciones infecciosas bacterianas durante el ingreso fue, además de la edad y las comorbilidades, el ingreso en la UCI, a su vez determinada por la gravedad del SDRA y la situación respiratoria.

Estudios anteriores han descrito altas tasas de infecciones en pacientes críticos con SDRA grave por COVID-19; y de hecho Bardi *et al.* confirmaron que la gravedad de la infección por el SARS-CoV-2 fue el único factor asociado al desarrollo de infecciones en la UCI [207-210]. Lógicamente, la situación epidemiológica en la primera ola supuso una falta de materiales, tiempo y personal, así como cambios infraestructurales, en ocasiones improvisados, y una evidente sobrecarga de trabajo que se tradujo en un funcionamiento y organización subóptima de la UCI [80, documento suplementario 4]. Como consecuencia, probablemente las normas de asepsia y medidas profilácticas habituales no se cumplieron con la rigurosidad habitual, justificando una mayor tasa de complicaciones infecciosas, sobre todo en el caso de las bacteriemias primarias o relacionadas con el catéter.

En paralelo, los principales factores de riesgo de mortalidad en esta cohorte fueron la edad, las comorbilidades y el SDRA. De nuevo, las infecciones bacterianas no fueron un factor de riesgo independiente, fortaleciendo la hipótesis de que las infecciones bacterianas son un marcador subrogado de la fragilidad o de la gravedad de los pacientes afectos por el COVID-19.

6.3.2. Papel del tratamiento inmunosupresor en el desarrollo de infecciones

Este estudio también ha permitido analizar el papel del tratamiento inmunosupresor en el desarrollo de las infecciones bacterianas durante el ingreso por COVID-19. El tratamiento esteroideo fue inicialmente criticado durante los primeros meses de la pandemia puesto que no había datos sólidos que justificasen su uso. De hecho, varios estudios informaron de que el uso precoz de esteroides podría suponer una mayor replicación del RNA y de la carga viral del SARS-CoV-2 [211,212]. Además, las infecciones oportunistas y bacterianas son un efecto secundario claramente reconocido y frecuente de los esteroides, incluso con dosis bajas y esquemas cortos, lo cual podría ser un factor limitante para su uso en pacientes con infección por COVID-19 [213-215]. Tanto es así que Obata *et al.* demostraron que los esteroides se asociaron con mayores tasas de infecciones bacterianas y fúngicas en pacientes ingresados por COVID-19 [216]. De igual forma, el riesgo de complicaciones infecciosas tras el tratamiento con tocilizumab en enfermedades autoinmunes también ha sido ampliamente estudiado y se ha constatado que puede conllevar incluso más riesgo de infecciones bacterianas que los inhibidores del factor de necrosis tumoral [217-219].

Sin embargo, en nuestra cohorte, ni el tratamiento con esteroide ni con tocilizumab conllevaron un mayor riesgo de infección bacteriana tras el ajuste en el análisis multivariante,

confirmando que su uso fue mayor en los pacientes más graves, quienes a su vez presentaban una mayor tasa de complicaciones infecciosas por la peor situación respiratoria e ingreso en UCI. De hecho, el tratamiento con esteroides y la combinación del tocilizumab con el esteroide supuso una mejora en la tasa de mortalidad, hallazgos superponibles a los del estudio TOCICOV [74, documento suplementario 4].

Por ello, este estudio consolida el beneficio del tratamiento inmunosupresor con esteroides y tocilizumab en los pacientes con infección grave por COVID-19 y demuestra que el potencial desarrollo de infecciones no debe ser un limitante para su uso. De forma paralela, el ensayo de Veiga *et al.* no demostró una mayor tasa de sobreinfecciones en los pacientes tratados con tocilizumab, y hasta el estudio de Stone *et al.* identificó una menor proporción de infecciones en los pacientes que se trataron con tocilizumab [73, 220]. En nuestro estudio estos hallazgos podrían justificarse, por un lado, por el tratamiento acortado con corticoides, y, por otro, por el uso puntual del tocilizumab (una o dos dosis), de forma que no se mantuvo el bloqueo del receptor de la IL-6 y por lo tanto el efecto inmunosupresor mantenido que puede traducir un mayor riesgo de infecciones. En cualquier caso, se podría asumir que el efecto antiinflamatorio parece superar al potencial riesgo de infecciones en este contexto, como ya han planteado otros autores [221-223].

6.4. ADE COMO MARCADOR DE INFLAMACION

En su conjunto, todos estos datos y anotaciones refuerzan que el ADE es un marcador potente del ambiente inflamatorio, principalmente protagonizado por la IL-6. Tanto en la sepsis como en la infección por COVID-19, el ADE ha mostrado ser un parámetro pronóstico de forma independiente, lo cual con convierte en una interesante herramienta para monitorizar a estos pacientes, predecir el deterioro clínico y dirigir la intensidad del tratamiento. Además, el ADE es un parámetro disponible en un hemograma rutinario, por lo que su obtención no conlleva un coste adicional o una demora alguna, lo cual supone una ventaja adicional.

Sin embargo, el ADE ha sido criticado por su poca especificidad, ya que su elevación no se debe a una causa o noxa en concreto. Es decir, es incapaz de discernir la etiología del proceso inflamatorio subyacente (enfermedad autoinmune, cardiopatía, infección por COVID-19, sepsis…) [118,119,224]. Este hecho ha quedado confirmado en nuestro tercer análisis, en el que se ha documentado que las infecciones bacterianas en pacientes con infección por COVID-19 presentan, además de una relativa baja prevalencia, una relación estrecha con la gravedad del

SDRA, siendo por lo tanto un marcador subrogado del mismo. Por ello, la elevación del ADE en los pacientes con COVID-19 se debe a la inflamación sistémica o tormenta de citoquinas, y no a la infección bacteriana o a la sepsis. Por consiguiente, y en nuestra opinión, la principal fortaleza del ADE es que efectivamente determina y permite cuantificar la disfunción sistémica relacionada con el ambiente inflamatorio, que en este caso protagoniza la fisiopatología de la sepsis y del COVID-19, aunque no distinga la causa.

En cualquier caso, es necesario que otras cohortes corroboren nuestros hallazgos para poder plantear una generalización de este parámetro y aclarar su utilidad clínica, idealmente en estudios prospectivos. Además, y a la luz de nuestros hallazgos, sería preciso analizar el comportamiento del ADE en función de distintas comorbilidades, tratamientos (hierro, vitamina B12, quimioterapia, inmunoterapia…), tipo de microorganismo (bacterias gram-positivas, gram-negativas, virus, hongos…) y en diversos escenarios clínicos. Por último, creemos que futuros estudios podrían definir puntos de corte que permitan cuantificar los valores del ADE en las puntuaciones del SOFA, además de validar nuestros resultados.

6.5. LIMITACIONES

A pesar de la potencia y congruencia de los hallazgos expuestos, el presente estudio y su diseño presentan algunas limitaciones.

En primer lugar, se trata de un estudio realizado en un único centro, de carácter observacional y retrospectivo. Por ello, y a pesar de que el diseño del estudio y los criterios de selección de las cohortes se realizaron para minimizar la posibilidad de un sesgo de selección, este no se puede reducir de forma absoluta.

En segundo lugar, el ADE y el resto de parámetros inflamatorios se analizaron como variables continuas y no como variables categóricas, agrupadas en cuartiles y en deciles, o en función de un punto de corte. Este tipo de análisis permite evaluar de una forma más exacta, precisa y con mayor rigor desde el punto de vista biológico una variable como el ADE, pero impide la determinación de un umbral patológico o cifra que categorice a un paciente o situación como patológica [225]. Estas limitaciones lógicamente obligan a que los hallazgos del estudio deban ser contrastados en una cohorte prospectiva, idealmente en un registro multicéntrico y considerando unos puntos de corte que permitan clasificar o estratificar a un paciente en

función de los valores del ADE; de forma similar a como se determina el daño orgánico en el SOFA mediante la bilirrubina, la creatinina o la cifra de plaquetas [52,55].

Por otra parte, el análisis de la dinámica del ADE y de la PCR fue distinta en las dos cohortes dado que la recogida de datos y análisis se realizó en circunstancias y momentos distintos. Por ello, la menor disponibilidad de los parámetros inflamatorios durante la primera ola de la pandemia del COVID-19 no permitió la realización de un análisis longitudinal. Por consiguiente, no se puede descartar que un análisis con la misma metodología pudiese conllevar distintos resultados.

Además, y de acuerdo con los argumentos fisiopatológicos expuestos y los hallazgos de Jian *et al.*, entre otros, el valor y dinámica del ADE están íntimamente relacionados con parámetros de la ferrocinética como la ferritina, la transferrina o el índice de saturación de transferrina, habitualmente determinados en el estudio de las anemias y de los trastornos inflamatorios [117, 131, 133]. En este sentido, en el presente estudio, el valor de estos parámetros en las dos cohortes, además de otras moléculas como la hepcidina, la IL-1, el factor de necrosis tumoral y otras citoquinas, o la propia dinámica de la hemoglobina, podrían haber ayudado a esclarecer el patrón inflamatorio que condiciona la anemia del trastorno crónico y la consiguiente elevación del ADE, máxime si tenemos en cuenta que el estado de depósito férrico puede producir variaciones del ADE y que podría haber justificado que la ferritina no se asociara con la mortalidad en los pacientes con infección grave por COVID-19.

Finalmente, en cada una de las cohortes analizadas se pudieron presentar algunas limitaciones, que se especifican por separado.

6.5.1. Pacientes ingresados en la UCI por sepsis

En esta cohorte solo se incluyeron pacientes con sepsis que ingresan en la UCI, lo cual no es una limitación de forma estricta en la práctica clínica dado que el ingreso en UCI es prácticamente una constante en situaciones graves de sepsis o en el shock séptico.

En segundo lugar, ciertos resultados parecen contrastar con la bibliografía y los datos previos. En este grupo de pacientes, los niveles de lactato no se asociaron con la mortalidad, a pesar de que es un reconocido y sólido marcador de gravedad y mortalidad [23]. Como posible explicación, el valor de lactato que se analizó fue el valor más alto de los determinados en las primeras 24 horas de ingreso en UCI y no el lactato inicial o los valores de lactato a las 6 horas

tras el uso de vasopresores, que son los que han demostrado una asociación clara con la mortalidad [226,227]. Por este motivo, pudo haber otros factores de confusión que hayan condicionado los valores de lactato en las primeras 24 horas de ingreso, de forma que la asociación estadística con la mortalidad no pudo ser contrastada. Por otra parte, los pacientes fallecidos presentaron mayores cifras de presión arterial de oxígeno en comparación con los pacientes supervivientes. En este caso, los peores valores de PaO2 se analizaron en el momento de las mayores cifras en la fracción de oxígeno inspirado (FiO2), aportado con el soporte ventilatorio. Por lo tanto y teniendo en cuenta que los pacientes fallecidos recibieron con más frecuencia ventilación mecánica y que la ratio PaO2/FiO2 fue menor, la mayor PaO2 no necesariamente indicaría mejor oxigenación o situación respiratoria, sino probablemente los mayores requisitos respiratorios de los pacientes fallecidos.

6.5.2. Pacientes ingresados por neumonía grave por COVID-19

Este grupo de pacientes se seleccionó del estudio TOCICOV, compuesto por pacientes con infección grave por COVID-19. El hecho de que se incluyesen pacientes graves con elevación de los marcadores inflamatorios podría justificar que no se encontraran diferencias significativas en parámetros que han demostrado ser marcadores pronósticos como los linfocitos, el DD o la LDH [79,188]. Por otra parte, aunque es cierto que la significación estadística en los análisis habla a favor de una asociación potente, dado que los pacientes están uniformemente graves y en una situación equivalente, la uniformidad de los criterios de inclusión probablemente justifique también que los valores de los AUC-ROC de los parámetros estudiados estuviese por debajo de 0,700, el cual, como se ha indicado, se considera el umbral de la adecuada capacidad predictora o discriminativa de un parámetro.

Otra potencial limitación podría haber sido el hecho de que más del 90% de los pacientes de este estudio recibió o estaba recibiendo esteroides, con los efectos que esto puede conllevar sobre muchos parámetros de la inflamación [228]. Si bien es verdad que estrictamente, estos datos se deben interpretar en pacientes que reciben esteroides, este es probablemente el escenario habitual y esperable de los pacientes con infección COVID-19 tras los resultados de diversos estudios [71,72].

6.5.3. Pacientes ingresados por neumonía por COVID-19

Idóneamente, el análisis de prevalencia y el posible papel de las infecciones bacterianas en pacientes COVID como factor de confusión de la capacidad pronóstica del ADE en pacientes con COVID-19 debería haberse planteado en la misma cohorte en la que se analizaron los

parámetros inflamatorios. Sin embargo, se realizó sobre una población más amplia y menos restrictiva con el objetivo de evaluar con mayor potencia y precisión el objetivo planteado.

El principal factor limitante de este análisis fue la ausencia de los datos relacionados con el antibiótico recibido, tanto empírico como ajustado a los resultados microbiológicos. En la primera ola de la pandemia, y tal y como muestran los resultados de la población del estudio TOCICOV, la mayoría de los pacientes con neumonía intersticial recibían azitromicina además de hidroxicloroquina y lopinavir-ritonavir. La indicación de antibioterapia empírica con cefalosporinas dependía del criterio del médico tratante para cada caso en particular. Sin embargo, y a pesar de la uniformidad de estos criterios, la falta de esta información no nos ha permitido esclarecer ninguna conclusión o recomendación a este respecto; a pesar de que como se ha identificado, la prevalencia de infecciones fue baja y se atribuyeron a la gravedad de la infección por COVID-19 y al ingreso en UCI.

7. CONCLUSIONES

1. El ancho de distribución del volumen corpuscular de los hematíes es un marcador de fácil obtención que resulta muy útil en la predicción de gravedad y mortalidad en pacientes ingresados en la unidad de cuidados intensivos por sepsis y en pacientes hospitalizados con infección grave por COVID-19.

2. En ambas poblaciones, los mayores niveles del ancho de distribución eritrocitario se producen durante las primeras 72 horas del deterioro clínico (ingreso en la unidad de cuidados intensivos o deterioro respiratorio, respectivamente); momento en el que el ancho de distribución eritrocitario presenta la mayor capacidad predictiva de mortalidad.

3. La capacidad predictiva de mortalidad del ancho de distribución eritrocitario en pacientes ingresados en la unidad de cuidados intensivos por sepsis es independiente de la concentración sanguínea de hemoglobina, de las comorbilidades o de la gravedad clínica, estimada por las escalas pronósticas.

4. La capacidad predictiva de mortalidad en pacientes con infección por COVID-19 es independiente de la hemoglobina o del tratamiento con tocilizumab.

5. En los pacientes con sepsis, el análisis longitudinal de los biomarcadores mostró una mejor utilidad del ancho de distribución eritrocitario en comparación con la proteína C reactiva sérica y con la procalcitonina sérica.

6. En los pacientes con infección por COVID-19, la proteína C reactiva y el ancho de distribución eritrocitario tienen una correlación estrecha y parecen reflejar los efectos pleiotrópicos y sistémicos de la Interleukina 6, a pesar del bloqueo de su receptor con tocilizumab.

7. La incorporación del ancho de distribución eritrocitario a las principales escalas pronósticas de sepsis supone una mejora de su capacidad predictora. El modelo resultante de la combinación del SOFA con el ancho de distribución eritrocitario al ingreso en la unidad de cuidados intensivos, a su vez las dos únicas variables asociadas con la mortalidad de forma independiente, presentó la mejor capacidad predictiva de mortalidad.

8. El desarrollo de infecciones bacterianas durante el ingreso por neumonía por COVID-19 no es un factor independiente de mortalidad, y, dado que su presencia esta íntimamente relacionada con la gravedad del síndrome de distrés respiratorio agudo, consideramos que la relación entre el ancho de distribución eritrocitario y el pronóstico de la infección por SARS-CoV-2 se debe a la propia tormenta de citoquinas y al ambiente inflamatorio.

8. ANEXO

Tabla 1. Quick-SOFA (qSOFA)

2 o más de los siguientes

Alteración del nivel de consciencia	Glasgow menor o igual a 13 puntos
Tensión arterial sistólica	Menor o igual a 100 mmHG
Frecuencia respiratoria	Mayor o igual a 22 respiraciones por minuto

Tabla 2. Síndrome de respuesta inflamatoria sistémica (SIRS).

2 o más de los siguientes

Temperatura	Mayor de 38ºC o menor de 36ºC
Frecuencia cardíaca	Mayor de 90 latidos por minuto
Taquipnea	Mayor de 20 respiraciones por minuto o hiperventilación (PaCO2 menor de 32).
Alteración del recuento leucocitario	Leucocitos mayores de 12.000 o menor de 4.000 x1000µ/L

Tabla 3. National Early Warning Score 2 (NEWS2)

Variable	Puntuación
Frecuencia respiratoria (respiraciones por minuto)	
<8	3
9-11	1
12-20	0
21-24	2
>25	3
Saturación oxígeno (pulsioximetría)	
Sin antecedente de insuficiencia respiratoria hipercápnica	
<91%	3
92-93%	2
94-95%	1
>96%	0

Con antecedente de insuficiencia respiratoria hipercápnica	
≤83%	3
84-85%	2
86-87%	1
88-92% sin oxigenoterapia	0
≥ 93% sin oxigenoterapia	0
93-94% con oxigenoterapia	1
95-96% con oxigenoterapia	2
≥97% con oxigenoterapia	3
Soporte respiratorio	
Si	2
No (aire ambiente)	0
Temperatura (ºC)	
≤35	3
35.1-36	1
36.1-38	0
381-39	1
≥39.1	2
Tensión arterial sistólica (mmHg)	
≤90	3
91-100	2
101-110	1
111-219	0
≥220	3
Frecuencia cardíaca (latidos por minuto)	
≤40	3
41-50	1
51-90	0
91-110	1
111-130	2
≥131	3
Nivel de consciencia	
Normal	0
Confusión/desorientación/agitación	3

Tabla 4. Logistic Organ Dysfunction Score (LODS).

Sistema/órgano Parámetro	5	3	1	0	1	3	5
Neurológico							
Escala de coma de Glasgow	3-5	6-8	9-13	14-15			
Cardiovascular							
Frecuencia cardíaca (latidos por minuto)	<30			30-139	>140		
		o			y	o	
Presión arterial sistólica (mmHG)	<40	40-69	70-89	90-239	240-259	>270	
Renal							
BUN (g/l)				<36	36-59	60-119	>120
			y	o	o		
Creatinina (mg/dl)				<1,20	1,20-1,59	≥1,60	
			y		o		
Diuresis (litros)	<0,5	0,5-0,74		0,75-99		≥10	
Pulmonar							
Pa02/FiO2 (mmHG/FiO2 con VM o VMNI) / KPa/FiO2		<150	≥150	No VM o CPAP			
PaO2 KPa/FiO2		<19,9	≥19,9	No IPAP			
Hematológico							
Leucocitos (x10 ^{9}/L)		<1,0	1,0-2,4	2,5-49,9	≥50,0		
			o	y			
Plaquetas (x10 ^{9}/L)				<50	≥50		
Hepático							
Bilirrubina (mg/dl)				<2,0	≥2,0		
			Y	O			
Actividad protrombina (segundos)				≤3	>3		
Actividad protrombina (sobre control)			<25	25			

BUN= Nitrógeno ureico en sangre, PaO2= Presión arterial de oxígeno, PaFiO2= Ratio Presión arterial de oxígeno-Fracción inspirada de oxígeno, VM= Ventilación mecánica, VMNI= Ventilación mecánica no invasiva, CPAP= Presión positiva continua, IPAP= Presión positiva fase inspiratoria.

Tabla 5. Sepsis-related Organ Failure Assessment (SOFA)

Sistema	0	1	2	3	4
Respiratorio					
Pa02/Fi02, mmHg (kPa)	≥ 400 (53,3)	< 400 (53,39	< 300 (40)	<200 (26,7) con soporte	<100 (13,3) con soporte
Coagulación					
Plaquetas (x10 9/L)	>150	<150	<100	<50	<20
Hígado					
Bilirrubina (g/dl)	<1,2	1,2-1,9	2-5,9	6-11,9	>12
Cardiovascular*					
	Tensión arterial media ≥ 70 mm Hg	Tensión arterial media < 70 mm Hg	Dopamina <5 o Dobutamina (cualquier dosis)	Dopamina 5,1-1,5 o Epinefrina (≤0,1) o Norepinefrina (≤0,1)	Dopamina >15 o Epinefrina (>0,1) o Norepinefrina (>0,1)
Sistema nervioso central					
Escala de Glasgow	15	13-14	10-12	6-9	<6
Renal					
Creatinina (mg/dl)	<1,2	1,2-1,9	2,0-3,4	3,5-4,9	>5
Diuresis (ml/24 horas)				<500	<200

PaFiO2= Ratio Presión arterial de oxígeno-Fracción inspirada de oxígeno.

* Las dosis de catecolaminas se refieren a ug/kg/min.

Tabla 6. Acute Physiology and Chronic Health Evaluation-II (APACHE-II)

Variable	4	3	2	1	0	1	2	3	4
Temperatura rectal (ºC)	>40,9	39-40,9		38,5-38,9	36-38,4	34-35,9	32-33,9	30-31,9	<30
TAM (mmHG)	>159	130-159	110-129		70-109		50-69		<50
Frecuencia cardíaca (lpm)	>179	140-179	110-129		70-109		55-69	40-54	<40
Frec, respiratoria (rpm)	>49	35-49		25-34	12-24	10-11	6-9		
Oxigenación									
Si FiO2 ≥0,5 (AaD02)	>499	350-499	200-349		<200				
Si FiO2 ≤0,5 (pAO2)					>70	61-70		56-60	<56
pH arterial	>7,69	7,60-7,69		7,50-7,59	7,33-7,49		7,25-7,32	7,15-7,24	<7,15
Sodio (mmol/L)	>179	160-179	155-159	150-154	130-149		120-129	111-119	<111
Potasio (mmol/L)	>6,9	6-6,9		5,5-5,9	3,5-5,4	3,0-3,4	2,5-2,9		<2,5
Creatinina (mg/dl)	>3,4	2-3,4	1,5-1,9		0,6-1,4		<0,6		
Hematocrito (%)	>59,9		50-59,9	46-49,9	30-45,9		20-29,9		<20
Leucocitos (x1000)	>39,9		20-39,9	15-19,9	3-14,9		1-2,9		<1

Total puntos APS	
15- Glasgow	

EDAD	Puntuación
≤44	0
45-54	2
55-64	3
65-74	5
≥75	6

ENFERMEDAD CRÓNICA	
Postoperatorio programado	2
Postoperatorio urgente o médico	5

Puntos APS (A)	Puntos GCS (B)	Puntos edad (C)	Puntos enfermedad previa (D)
Total puntos APACHE- II (A+B + C+ D)			

Enfermedad crónica

Hepática: Cirrosis (biopsia) o HT portal o episodio previo fallo hepático
Cardiovascular: Disnea o angina de reposo (clase IV de la NYHA)
Respiratoria: EPOC grave, con hipercapnia, policitemia o HT pulmonar
Renal: Diálisis crónica
Inmunocomprometido: Tratamiento inmunosupresor, inmunodeficiencia

TAM= Tensión arterial media, lpm= latidos por minuto, rpm= respiraciones por minuto, FiO2= Fracción inspirada de oxígeno, GA-a02= Gradiente alveolo-arterial de oxígeno, HT= Hipertensión.

Tabla 7. Simplified Acute Physiology Score-II (SAPS-II)

Variable	Rango	Puntos
Edad	<40	0
	40-59	7
	60-69	12
	70-74	15
	75-79	16
	≥80	18
Tipo de ingreso	Cirugía programada	0
	Médico	6
	Cirugía no programada	8
Temperatura (ºC)	<39	0
	≥39	3
Presión arterial sistólica (mm Hg)	≥220	2
	100-199	0
	70-99	5
	<70	13
Frecuencia cardíaca (lpm)	≥160	7
	120-159	4
	70-119	0
	40-69	2
	<40	11
Escala de coma de Glasgow	14-15	0
	11-13	5
	9-10	7
	6-8	13
	<6	26
Diuresis (L/24h)	≥1	0
	0,5-0,999	4
	<0,5	11
Leucocitos (mm3)	<1,000	12
	1,000-19,000	0
	≥20,000	3
BUN (mg/dL)	≥84	10
	28-83	6
	<28	0
Potasio (mEq/L)	<3	3
	3-4,9	0
	≥5	3
Sodio (mEq/L)	<125	5
	125-144	0
	≥145	1
Bicarbonato (mEq/L)	<15	6
	15-19	3
	≥20	0
PaO2/FiO2 (si VM o CPAP) (mmHg)	<100	11
	100-199	9
	≥200	6
SIDA	Sí	17
Carcinoma metastástico	Sí	9
Neoplasia hematológica	Sí	10

Lpm= latidos por minuto, BUN= Nitrógeno ureico en sangre, Pa02/FiO2, Ratio Presión arterial de oxígeno-Fracción inspirada de oxígeno, VM= Ventilación mecánica, CPAP= Presión positiva continua, SIDA= Síndrome de inmunodeficiencia adquirida.

Tabla 8. Índice de comorbilidad de Charlson

Variable		Puntuación
Edad		
	0-49	0
	50-59	1
	60-69	2
	70-79	3
	80-89	4
	90-99	5
Enfermedad coronaria		1
Insuficiencia cardíaca		1
Enfermedad vascular periférica		1
Enfermedad cerebrovascular		1
Demencia		1
Úlcera péptica		1
Enfermedad hepática leve		1
Diabetes		1
Hemiplejía		2
Enfermedad renal crónica moderada-grave		2
Diabetes con lesión de órganos		2
Cualquier tumor, leucemia, linfoma		2
Enfermedad hepática moderada-grave		3
Metástatasis de tumor sólido		6
SIDA		6

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Red blood cell distribution width as prognostic factor in sepsis: A new use for a classical parameter.

RDW as a prognostic factor in sepsis

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Funding: This work has been supported by a grant from Instituto de Salud Carlos III (Expedient number PI16-01480). The funding source had no involvement in the study.

Declaration of Interest: None.

Highlights.

- In septic patients admitted to the ICU, non-survivors presented higher RDW values during the first week after admission while CRP and PCT did not.
- Along with SOFA, RDW was the only independently factor associated with mortality after adjustment by Charlson comorbidity index, immunosuppression, nosocomial infection, NEWS2, SAPS-II and hemoglobin.
- When added to the prognostic scores, 24-hours RDW and mostly admission RDW improved their discrimination ability. Admission RDW with SOFA presented the better discrimination ability for hospital mortality.
- RDW is an easy to determine and widely available parameter that reflects the dysregulated inflammatory response and systemic dysfunction.

Abstract

Purpose. To evaluate Red blood cell distribution width (RDW) performance as a sepsis prognostic biomarker.

Methods. 203 septic patients admitted to the ICU. Analysis of RDW dynamics, hospital mortality discrimination ability and the added value when incorporated to the SOFA, LODS, SAPS-II and APACHE-II scores using the AUC-ROC.

Results. Non-survivors presented higher RDW values during the first week after ICU admission (p=0.048). Only SOFA and RDW were independently associated with mortality when adjusted by Charlson, immunosuppression, nosocomial infection, NEWS2, SAPS-II and haemoglobin (p<0.05). After adjustment, AUC-ROC was 0.827, 0.821, 0.826, 0.831 and 0.812 for each model including admission, 24, 48 and 72-hours and 7-days RDW, respectively. When added to the prognostic scores, 24-hours RDW and mostly admission RDW improved their discrimination ability (SOFA AUC-ROC=0.772 vs 0.812 SOFA + admission RDW, p=0.041; LODS AUC-ROC=0.687 vs 0.710, p=0.002; SAPS-II AUC-ROC=0.734 vs 0.785, p=0.021; APACHE-II AUC-ROC=0.672 vs 0.755, p=0.003). Admission RDW with SOFA presented the better discrimination ability for mortality.

Conclusion. RDW is an independent prognostic marker of death in septic patients admitted in the ICU that improves SOFA, LODS, APACHE-II and SAPS-II scores discrimination ability. This easily parameter could be incorporated to the prognostic scores as a marker of systemic dysfunction and dysregulated inflammatory response.

Keywords. Sepsis, Red blood cell distribution width, SOFA, LODS, SAPS-II, APACHE-II.

Abbreviations

APACHE-II. Acute Physiology and Chronic Health Evaluation-II

ARDS: Acute Respiratory Distress Syndrome

AUC-ROC: Area Under the Receiver Operating Characteristic Curve

DAG: Directed Acyclic Graphs

CRP: C reactive protein.

FiO2: Fraction of Inspired Oxygen

HIV: Human immunodeficiency virus

ICU: Intensive Care Unit

LODS: Logistic Organ Dysfunction System.

MAP: Mean arterial pressure.

NEWS2: National Early Warning Score 2

PaO2: Arterial oxygen tension

PCT: Procalcitonin.

RDW: Red blood cell distribution width.

SAPS-II. Simplified Acute Physiology Score-II.

SIRS: Systemic Inflammatory Response Syndrome

SOFA: Sequential Organ Failure Assessment.

SOT: Solid organ transplantation.

SCT: Stem cell transplantation.

qSOFA: quick-SOFA.

BACKGROUND

Sepsis remains an important cause of death worldwide [1]. As a matter of fact, it could be considered one of the main causes of hospital death, with mortality rates reaching 18.7% in hospitalized patients and up to 55.7% in the Intensive Care Units (ICUs) [2-4]. Despite the acknowledgment represented by the Third Consensus Definition for Sepsis and Septic shock (Sepsis-3) and the Surviving Sepsis Campaign, there are still important gaps in the diagnosis and identification of sepsis [5,6]. In this setting, several markers and molecules have been assessed to anticipate sepsis recognition, to confirm the diagnosis and to guide the management of these patients, but unfortunately, to date there are no parameters consolidated for this role [7].

Red blood cell distribution width (RDW) is a parameter routinely reported as part of a complete blood count. It measures the size variability of circulating erythrocytes (anisocytosis) and has traditionally been used in the differential diagnosis of iron deficiency anaemia and anaemia of chronic disease [8]. Anisocytosis reflects the dysregulation of the iron metabolism and inhibition of erythropoiesis resulting in chronic disease anaemia, mediated by diverse cytokines, mainly IL-6 (9, 10). In fact, Allen et al described the association between RDW and a biochemical profile suggestive of impaired iron mobilization, typical of anaemia of chronic disease, in a cohort of patients with heart failure [11]. Moreover, these changes in the red blood cell physiology are particularly common in critical illness, in patients admitted to the ICU and in hyperinflammatory states [12]. In consequence, and since it is a fast and available parameter, several studies have considered RDW as an inflammatory marker or a predictor of mortality in diverse clinical settings including chronic inflammatory diseases, cardiovascular disease, infections and acute respiratory distress syndrome (ARDS) [13]. Therefore, and based on the aforementioned pathophysiologic considerations, the aim of the present study was to understand RDW dynamics and to better analyze its prognostic role in sepsis, when compared to other biomarkers.

MATERIAL AND METHODS

Study population

The study population consisted of a retrospective cohort of consecutive patients admitted to the ICU due to sepsis at a 620-bed tertiary University Hospital from January 1st to 31st December 2019. Patients were included if sepsis was the reason for admission and/or sepsis criteria were met during ICU admission. Sepsis was

considered if the patient presented a confirmed or suspected infection with organ dysfunction represented by an increase of SOFA score of 2 or more points, according to Sepsis-3 criteria [5]. The study was approved by the local Research Ethics Committee (PI_222-20). According to Spanish law and the Ethics Committee, a waiver for informed consent was granted.

Data collection and scores calculation

The following data were extracted from medical records using a standardised data collection form: epidemiological and baseline conditions and comorbidities, physiological, laboratory and microbiological parameters, supportive treatment received, ICU and hospital stay. Furthermore, RDW, C-reactive protein (CRP) and procalcitonin (PCT) values were analysed at ICU admission, at 24, 48, 72 hours and 7 days later; and considering the maximum values identified during the ICU and hospital admission if the patient was discharged from the ICU.

The prognostic scores (quick-SOFA, National Early Warning Score 2 [NEWS2], Logistic Organ Dysfunction System [LODS], Sequential Organ Failure Assessment [SOFA], Acute Physiology and Chronic Health Evaluation-II [APACHE-II] and Simplified Acute Physiology Score-II [SAPS-II]) were calculated considering the worse or more relevant values within the first 24 hours of ICU admission [5, 14-20]. If the patient suffered from chronic conditions with previously altered values, scores were calculated considering the difference between baseline and admission values (ie. creatinine or bilirubin in kidney or liver disease, respectively). Furthermore, if any parameter or value was missing, no contribution was made to the score and thus it was considered in the normal range. According to their definition, patients met the qSOFA criteria if 2 of the 3 variables were present (Glasgow Coma Score of 13 or less, systolic blood pressure of 100 mmHg or less and respiratory rate of 22 per minute or greater) [5,15] and Systemic inflammatory response syndrome (SIRS) was considered if at least 2 of the following 4 criteria were met (temperature >38°C or < 36°C, heart rate > 90 beats per minute, respiratory rate > 20 breaths per minute or $PaCO_2$ < 32 mmHg and white blood count > 12,000/mm³, < 4,000/mm³, or > 10% band) [14].

On the other hand, septic shock was defined by the vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L in the absence of

hypovolemia, according to Sepsis-3 criteria [5] while Acute Respiratory Distress Syndrome was (ARDS) was defined according to the Berlin definition [21].

Statistical analysis

In-hospital mortality was the main study outcome, and the comparison between the groups and the statistical analyses were performed accordingly. Quantitative variables were expressed as mean and standard deviation or as median and p25-p75 as appropriate; qualitative variables were expressed as frequency and percentage. Survivors were compared to non-survivors. Numerical variables were compared using the t-test or Mann-Whitney´s U where appropriate, and categorical variables were compared using the chi-square test. In addition, significant variables were entered in the different multivariable logistic regression analyses to identify factors associated with hospital mortality. The discrimination ability was evaluated following an approach based on the area under the curve (AUC) - "receiver operating characteristic"- ROC. A value of AUC-ROC of 0.5 indicates random predictions, and a value of 1 indicates perfect discrimination. A model with an AUC-ROC roughly above 0.7 is considered to be useful to predict the responses of individual subjects [22]. Stata's *roccomp* command was used to compare more than two ROC areas.

Regarding analysis of RDW dynamics, firstly, we studied the association of RDW with hospital mortality along the different time points (admission, 24, 48,72 hours and 7 days), using a generalized estimating equation (GEE) analysis. As the dependent variable was mortality, we used the binomial family and the logit as the link function. RDW at the different time points as well as the time points variable were introduced as independent variables. Secondly, we performed different mortality multivariable logistic analyses considering a pool with the next variables: Charlson Comorbidity Index, inmunocompromise, nosocomial infection, NEWS2, SOFA, SAPS-II and haemoglobin; and testing one of the measures for RDW variable each time point (at admission, 24, 48,72 hours and 7 days after in addition to the maximum values identified). These variables were chosen to evaluate age and comorbid conditions (Charlson Comorbidity Index, immunocompromise and nosocomial infection), clinical situation at admission (NEWS2), organ damage (SOFA) and disease severity (SAPS-II). NEWS2, SOFA and SAPS-II were used over qSOFA, LODS and APACHE-II according to a discrimination ability analysis described elsewhere [23]. Besides, both haemoglobin and blood transfusions before and during admission were considered, since they could modify RDW values and significance [8,24]. However, when the

causal pathways between haemoglobin, transfusion and mortality were evaluated by DAG (Directed Acyclic Graphs), we confirmed that transfusion is a consequence of the haemoglobin levels or the anaemia status. Therefore, its inclusion in the model would not be appropriate and was consequently discarded. Accordingly, we evaluated the discrimination ability for each one of the models by AUC-ROC. Finally, we explored the discrimination ability of SOFA, LODS, APACHE-II and SAPS-II scores by themselves alone, or adding the RDW at admission or at 24h after ICU admission. For all the analyses, a significance level of 0.05 was set. Stata v16 software was used (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.)

RESULTS

Patient's characteristics

203 patients were admitted to the ICU due to sepsis during the study period. Baseline characteristics and the site of infection are shown in **table 1**. 63.9% were males with a mean age of 63.1 years (SD=14.3%). 78.3% were outpatients, 19.2% were transferred from another institution and 2% from nursing homes. Nosocomial infections were identified in 39.9% of patients. Fifteen patients (7.4%) presented more than one site of infection. The most frequent source of the infection was respiratory (38.9%), followed by abdominal (36%), urinary (12.3%), skin or soft tissue infection (6.9%), catheter-related bacteraemia (3.9%) and endocarditis (3.9%).79.3% of patients met the septic shock criteria and 14.3% the criteria for ARDS. Mean ICU stay was 16 days and mean hospitalization length of-stay was 38 days. Hospital mortality was 31.5%.

Table 2 shows clinical parameters and analytical values at ICU admission. The different diagnostic and prognostic scales were calculated accordingly. 33.5% of patients met qSOFA criteria (30.2 % among survivors versus 37.5% among non-survivors, p=0.304) while 77.8% met SIRS criteria (79.1% among survivors vs 75% among non-survivors, p=0.510). NEWS2, SOFA, LODS, APACHE-II and SAPS-II were significantly higher in non-survivors (p<0.01). The prognostic scores mortality discrimination ability was evaluated by an AUC-ROC comparison for the subsequent analysis. NEWS2 presented significantly higher AUC-ROC than qSOFA (0.615 vs 0.536, p=0.039) and SOFA than LODS (0.776 vs 0.693, p=0.01). SAPS presented higher discrimination ability than APACHE-II but without statistical significance (AUC-ROC= 0.738 vs 0.673, p=0.08).

RDW dynamics and hospital mortality.

Table 3 shows RDW values at the different time points and its dynamics during the first 30 days of ICU admission. The highest RDW values were seen on 16.1 days after ICU admission (mean RDW 17.70%), while during the first week the highest average RDW values were at 48-hours (mean RDW 16.09%) and 72-hours (mean RDW 16.02%) (**figure 1. A**). RDW was higher in non-survivors at all the time points (p<0.001), (**figure 1.B**). RDW mortality discrimination ability, evaluated through the AUC-ROC analysis, showed that AUC-ROC was above 0.700 for all the measures. The longitudinal analysis confirmed that, during the first week after ICU admission, RDW was associated with a higher in-hospital mortality risk (**OR= 1.05, 95% CI 1.01; 1.10, p=0.048**). Neither CRP (OR=0.99, 95% CI 0.99-1.00, p=0.957) nor PCT (OR=1.01, 95% CI 0.99-1.02, p=0.130) were associated with hospital mortality along time.

In order to identify at which time point RDW was a better predictor of hospital mortality, different multivariable logistic regression analyses were tested. A pool of independent variables, such as the Charlson comorbidity index, immunocompromise, nosocomial infection, NEWS2 score, SOFA score, SAPS-II score and haemoglobin were entered into the models, in addition to RDW at admission, 24, 48 and 72 hours and 7 days after ICU admission and maximum RDW, one by one. In the different multivariable logistic regression analysis for each time point RDW value, only SOFA and RDW were statistically associated with a higher hospital mortality risk (**table 4)**. AUC-ROCs for the adjusted models were as follows: 0.827 for the model considering admission RDW, 0.821 for he model considering 24-hours RDW, 0.826 for the model considering 48-hours RDW, 0.831 for the model considering 72-hours RDW and 0.812 for the model considering 7-days RDW (**figure 2**).

RDW discrimination ability when added to the prognostic scores

Finally, we explored the discrimination ability of RDW when added to the widely used prognostic scores SOFA, LODS, APACHE-II and SAPS-II (**table 5, figure 3**). When RDW values at admission and 24 hours later were added to the scores, their discrimination ability uniformly improved (p<0.05). The prognostic model comprised by SOFA score and RDW at admission presented the higher discrimination ability for mortality (AUC-ROC=0.812).

DISCUSSION

The present study shows that RDW is a prognostic biomarker of hospital mortality in septic patients admitted to the ICU, since RDW was independently associated with mortality throughout time. On top of that, RDW improved the discrimination ability of SOFA, LODS, APACHE-II and SAPS-II, placing it as a potential parameter to be included in these prognostic scores.

Previous reports have already found that RDW independently predicts mortality in septic patients, in the emergency department and ICUs [15, 25-43]. As a matter of fact, some authors have confirmed that RDW has better discrimination ability than CRP, PCT or even lactate, wide applied parameters in sepsis [28,37,38]. Our results confirm that RDW is a strong and independent predictor of mortality during the first week of ICU admission while CRP and PCT were not. Despite the large use of CRP and PCT among other biomarkers, to date there is still conflicting evidence to support any of these parameters in sepsis [6,7,44]. Our findings, in parallel to the previous studies, identify RDW as a potential marker of inflammation and prognosis in this setting.

Of note, RDW is a dynamic parameter whose variations have shown to predict mortality, running in parallel to other parameters such as malondialdehyde (MDA), tumor necrosis alpha factor or SOFA score (31,34,40,41). In our cohort, we show that in addition that RDW values during the first week present a robust and independent association with mortality, RDW values 48 and 72 hours after admission were the highest observed during the first week after admission and the strongest predictors of mortality. Yi Lang *et al*. described that RDW increased on day 3 after ICU admission in non-survivors patients with sepsis along with other inflammatory anemia-associated parameters such as ferritin, IL-6 or hepcidin [26]. Similarly, Kim *et al.* identified a higher mortality risk in septic patients whose RDW increased within 72 hours of the emergency department admission, while Ku *et al*. observed that RDW 72 hours after the onset of the gram-negative bacteraemia was higher in non-survivors (34, 45). These data highlight that RDW reflects the afore-mentioned systemic inflammation and the pleiotropic effects of the cytokines during the altered immune response after the sepsis onset. Consequently, the dynamic character of RDW turns it into an appropriate and useful biomarker.

Some authors have studied and compared RDW with prognostic scores such as SOFA, SIRS or APACHE-II, showing that RDW correlates with APACHE-II and SOFA [26,29,31,40,41] and that it could even be a better prognostic tool than APACHE-II or SIRS [28,35,37,42]. Besides, Sadaka *et al*. demonstrated that, when adding RDW to APACHE-II, the AUC-ROC for hospital mortality in septic shock significantly increased [30]. In the present study, we analysed the role of RDW not only compared to SOFA and LODS, prognosis scores that define and quantify organ dysfunction, but to the APACHE-II and SAPS-II scores, predictors of hospital and ICU mortality. Our results demonstrate that RDW improves the mortality discrimination ability of the SOFA, LODS and SAPS-II scores. We therefore believe that these novel findings are promising and relevant because they confirm that RDW is a robust marker of the dysregulated inflammatory response that conveys systemic organ dysfunction.

RDW has been criticised because of its lack of specificity, since its values rise related to inflammation, regardless of the cause [13,46,47]. However, we understand that RDW is not a marker of infection itself but an easily obtained parameter that reveals the altered inflammatory response secondary to the infection. Therefore, we believe that its main strength, besides the promptness and the availability, is that it measures and determines the systemic dysfunction related to the mentioned inflammatory environment. As a result, we consider that it could be a marker of organ dysfunction, similar to creatinine, bilirubin or platelets, other unspecific markers for sepsis or infection but which define the organ dysfunction included in the SOFA, LODS, APACHE-II and SAPS-II scores. In this sense, RDW and SOFA, that were the only two independent factors related to mortality, had the better discrimination ability when the scores were compared, possibly because they represent sepsis dysfunction in a more comprehensive way. Since the SOFA score is the tool to assess organ dysfunction or failure, according to the Third Consensus Definition for Sepsis and Septic shock (Sepsis-3), adding RDW could result in a better performance of the score. Further studies are needed to confirm and validate these findings.

However, our study has several limitations. In addition to being a single-centre, observational and retrospective study, the population size was relatively small. In spite of that, statistical significance with clinical relevance was reached in the analysis. Secondly, the study population consisted only of patients admitted to the ICU, instead of a general population of septic patients. However, almost all septic patients have

unequivocally ICU admission criteria if the disease is evolving. Thirdly, RDW was considered and analysed as a continuous variable and not as a categorical, grouped in quartiles or considering a cut-off value. Our analysis allows a more exact, precise and rigorous analysis of a variable such as RDW, but it limits the determination of a pathological threshold or value that defines a patient or situation as pathological [48]. Therefore, our findings must be evaluated in a prospective cohort, ideally in a multicenter registry, and considering RDW cut-off points that define the severity of the organic damage; similar to how organic damage in SOFA is determined by bilirubin, creatinine, or platelet count.

CONCLUSION

In summary, our study suggests that RDW values and their variations along time are an independent prognostic marker of death in septic patients admitted in the ICU and that, when added to SOFA, LODS, APACHE-II and SAPS-II scores, it could contribute to improve their discrimination ability. RDW is an easy to determine and widely available parameter that reflects the dysregulated inflammatory response and systemic dysfunction in septic patients.

Figure legends.

Figure 1. Red blood cell distribution width (RDW) dynamics. The figure shows RDW dynamics 30 days after ICU admission among the cohort (A) and among survivors and non-survivors (B). Data are expressed as mean and standard error.

Figure 2. Discrimination ability for the hospital mortality adjusted models. The figure shows the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) for the models including admission Red blood cell distribution width (RDW) (A), 24-hours RDW (B), 48-hours RDW, 72-hours RDW (D) and 7-days RDW (E). All the analyses were adjusted by the Charlson Comorbidity Index, immunosuppression, nosocomial infection, National Early Warning Score 2, Sequential Organ Failure Assessment, Simplified Acute Physiology Score-II and haemoglobin.

Figure 3. Discrimination ability for the hospital mortality prognostic model comprised by RDW when added to SOFA, LODS, SAPS-II and APACHE-II. The figure shows the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) for SOFA score (A) and LODS (B) and the added value of admission Red blood cell distribution width (RDW) and 24-hours RDW.

Table 1. Patient´s characteristics.

Characteristic	Global (n=203)	Survivors (n=139)	Non-survivors (n=64)	p-value
Male gender N (%)	129 (63.6%)	91 (65.5%)	38 (64.9%)	0.402
Age Years (SD)	63.1 (14.3)	61.8 (14.5)	65.9 (11.6)	0.147
Nosocomial N (%)	81 (39.9%)	46 (33.1%)	35 (54.7%)	**0.004**
Surgical intervention N (%)	111 (54.7%)	73 (52.5%)	38 (59.3%)	0.362
Septic shock N (%)	161 (79.31%)	103 (74.1%)	58 (90.6%)	**0.007**
ARDS N (%)	29 (14.29%)	18 (12.9%)	11 (17.2%)	0.423
Comorbidities				
Heart disease N (%)	65 (32.0%)	36 (25.9%)	29 (45.3%)	**0.001**
Peripheral artery disease N (%)	28 (13.8%)	21 (15.1%)	7 (10.9%)	0.413
Connective tissue disease N (%)	25 (12.3%)	12 (8.6%)	13 (20.3%)	**0.019**
Cerebrovascular disease N (%)	25 (12.3%)	13 (9.4%)	12 (18.8%)	0.058
Pulmonary disease N (%)	66 (32.5%)	39 (28.1%)	27 (42.2%)	**0.028**
Chronic liver disease N (%)	30 (14.8%)	16 (11.5%)	14 (21.9%)	0.120
Chronic kidney disease N (%)	43 (21.2%)	26 (18.7%)	17 (26.6%)	0.270
Neoplasm N (%)	86 (42.4%)	55 (39.6%)	31 (48.4%)	0.109
Haematological disease N (%)	37 (18.2%)	21 (15.1%)	16 (25%)	0.084
Diabetes N (%)	64 (31.5%)	39 (28.1%)	25 (39.1%)	0.116
Obesity N (%)	25 (12.3%)	18 (12.6%)	7 (10.9%)	0.911
Alcoholism N (%)	26 (12.8%)	17 (12.2%)	9 (14.1%)	0.298
Immunocompromise N (%)	74 (36.5%)	43 (30.9%)	31 (48.4%)	**0.018**
SOT N (%)	24 (11.8%)	13 (9.4%)	11 (17.2%)	0.108
SCT N (%)	14 (6.9%)	7 (5%)	7 (10.9%)	0.123
Charlson comorbidity index Mean (SD)	6.17 (3.98)	5.78 (4.31)	7.03 (3.01)	**0.005**
Sources of infection				
Respiratory	79 (38.9%)	54 (38.8%)	25 (39.1%)	0.977
Abdominal	73 (36%)	49 (35.3%)	24 (37.5%)	0.756

	Urinary	25 (12.3%)	20 (14.4%)	5 (7.8%)	0.185
	Skin and soft tissue	14 (6.9%)	10 (7.2%)	4 (6.3%)	0.805
	Catheter-related bacteremia	8 (3.9%)	3 (2.2%)	5 (7.8%)	0.054
	Endocarditis	8 (3.9%)	3 (2.2%)	5 (7.8%)	0.054
	Others/unknown				

SD: Standard deviation, ICU: Intensive care unit, HIV: Human immunodeficiency virus, SOT: Solid organ transplantation, HSCT: Hematopoietic stem cell transplantation.

Table 2. ICU admission clinical parameters, analytical values, and prognostic scores.

Parameter	Overall (n=203)	Survivors (n=139)	Non-survivors (n=64)	p-value
Glasgow coma score Mean (SD)	14 (2.5)	14 (2.2)	13 (2.9)	**<0.001**
Temperature (°C) Mean (SD)	37.1 (1.6)	37.4 (1.5)	36.5 (1.7)	**0.002**
MAP (mmHg) Mean (SD)	70 (20.7)	70 (21.8)	69 (18.2)	0.829
Heart rate (bpm) Mean (SD)	107 (23)	107 (21)	107 (27)	0.862
Respiratory rate (breaths per minute) Mean (SD)	28 (8)	27 (8)	30 (7)	**0.026**
Sp02 (%) Mean (SD)	93 (6)	93 (6)	93 (7)	0.280
Vasopressors N (%)	164 (80.8)	105 (75.5)	59 (92.2)	**0.007**
Mechanical ventilation N (%)	91 (44.8)	46 (33.1)	45 (70.3)	**<0.001**
PaO2/FiO2 ratio (Mean,SD)	232 (157)	230 (155)	237 (163)	0.793
Diuresis (ml/24h) N (%)				**<0.001**
<200	33 (16.3)	15 (10.8)	18 (12.5)	
200-500	35 (17.3)	16 (11.5)	19 (29.7)	
>500	134 (66.3)	108 (77.7)	26 (40.63)	
Renal replacement therapy N (%)	57 (28.1)	24 (17.3)	33 (51.6)	**<0.001**
Blood transfusion N (%)	50 (24.6)	27 (42.2)	23 (16.5)	**<0.001**
Arterial pH (Mean, SD)	7.30 (0.11)	7.319 (0.11)	7.260 (0.11)	**<0.001**
Bicarbonate (mmol/L) (Mean, SD)	19.9 (4.5)	20.2 (4.2)	19.1 (5.1)	0.145
Lactate (mmol/L) (Mean, SD)	3.8 (3.1)	3.5 (2.3)	4.7 (4.4)	0.505
pO2 (mmHg) (Mean, SD)	94 (37.5)	91 (38.1)	100 (35.6)	**0.047**
pCO2 (mmHg) (Mean, SD)	40 (12.2)	39 (11.6)	43 (13.1)	**0.034**
Urea (mg/dL) (Mean, SD)	93 (61)	85 (60)	111(60)	**<0.001**
Creatinine (mg/dl) (Mean, SD)	2.15 (1.66)	2.06 (1.72)	2.35 (1.51)	**0.037**
Glucose (mg/dl) (Mean, SD)	173 (90)	175 (81)	169 (107)	0.278
Sodium (mmol/L) (Mean, SD)	138 (6)	137 (5)	139 (8)	0.249
Potassium (mmol/L) (Mean, SD)	4.39 (0.95)	4.31 (0.90)	4.56 (1.04)	0.150
Bilirrubin(mg/dL) (Mean, SD)	2.20 (3.79)	1.92 (3.21)	2.81 (4.78)	0.114
Leukocytes (x10E3/mm3)(Mean, SD)	16.645 (14.595)	16.914 (11.525)	16.061 (19.788)	**0.041**
Neutrophils (x10E3/mm3) (Mean, SD)	13.954 (12.564)	14.228 (10.112)	13.359 (16.784)	0.052

Lymphocytes (x10E3/mm3) (Mean, SD)	961 (2109)	884 (896)	1129 (3530)	**0.013**
Platelets (x10E3/mm3) (Mean, SD)	185.305 (138.294)	196.878 (127.674)	160.172 (157.095)	**<0.001**
Haemoglobin (g/dL) (Mean, SD)	11.60 (2.65)	12.00 (2.53)	10.73 (2.72)	**<0.001**
Haematocrit (%) (Mean, SD)	35.15 (8.05)	36.33 (7.42)	32.57 (8.81)	**<0.001**
Prothrombin activity (%)(Mean, SD)	53.96 (21.12)	56.23 (20.77)	49.01 (21.17)	**0.014**
RDW (%) (Mean, SD)	15.66 (2.88)	15.06 (2.82)	16.95 (2.58)	**<0.001**
CRP (mg/L) (Mean, SD)	166 (90)	167 (86)	162 (98)	0.820
PCT (ng/mL) (Mean, SD)	18.9 (32.3)	17.8 (27.2)	21.8 (42)	0.513
Score				
qSOFA N (%)	66 (32.51)	42 (63.64%)	24 (36.36)	0.304
NEWS2 Mean (SD)	9.72 (3.36)	9.21 (2.91)	10.83 (3.98)	**0.008**
SIRS N (%)	158 (77.83)	110 (69.62)	48 (30.38)	0.510
SOFA Mean (SD)	9.06 (3.58)	7.99 (3.24)	11.39 (3.19)	**<0.001**
LODS Mean (SD)	7.92 (2.181)	7.37 (1.69)	9.09 (2.64)	**<0.001**
APACHE-II Mean (SD)	19.94 (5.94)	18.65 (5.14)	22.72 (6.62)	**<0.001**
SAPS-II Mean (SD)	51.42 (13.93)	47.68 (12.13)	59.53 (14.22)	**<0.001**

SD: Standard deviation, MAP: Mean arterial pressure, bpm: beats per minute, ICU: Intensive Care Unit, SD: Standard deviation, RDW: Red blood cell distribution width, CRP: C reactive protein, PCT: Procalcitonin, qSOFA: quick-SOFA, NEWS2: National Early Warning Score 2, SIRS: Systemic Inflammatory Response Syndrome, SOFA: Sequential Organ Failure Assessment, LODS: Logistic Organ Dysfunction System, APACHE-II: Acute Physiology and Chronic Health Evaluation-II, SAPS-II: Simplified Acute Physiology Score-II.

Table 3. Red blood distribution width values.

	Overall (n=203)	Survivors (n=139)	Non-survivors (n=64)	p-value for hospital mortality	AUC-ROC
RDW at admission (Mean %) (SD)	15.66 (2.88)	15.06 (2.82)	16.95 (2.58)	<0.001	0.737
RDW 24 hours (Mean %) (SD)	15.90 (2.76)	15.35 (2.73)	17.15 (2.41)	<0.001	0.737
RDW 48 hours (Mean %) (SD)	16.09 (2.80)	15.49 (2.66)	17.54 (2.61)	<0.001	0.750
RDW 72 hours (Mean %) (SD)	16.02 (2.50)	15.48 (2.18)	17.55 (2.74)	<0.001	0.747
RDW 7 days (Mean %) (SD)	15.81 (2.66)	15.40 (2.45)	17.33 (2.86)	<0.001	0.740

RDW: Red blood cell distribution width, SD: Standard deviation, AUC-ROC: area under the curve receiving operating characteristic.

Table 4. Factors statistically associated with a higher hospital mortality after adjustment

	OR*	95% Confidence interval
Analysis considering admission RDW		
SOFA	1.29	1.11-1.49
RDW	1.17	1.03-1.34
Analysis considering 24-hours RDW		
SOFA	1.28	1.11-1.49
RDW	1.16	1.01-1.34
Analysis considering 48-hours RDW		
SOFA	1.30	1.11-1.52
RDW	1.20	1.04-1.39
Analysis considering 72-hours RDW		
SOFA	1.30	1.09-1.55
RDW	1.30	1.09-1.55
Analysis considering 7-days RDW		
SOFA	1.30	1.08-1.57

SOFA: Sequential Organ Failure Assessment, RDW: Red blood cell distribution width. OR= Odds ratio.
* All the analyses were adjusted by the Charlson Comorbidity Index, immunosuppression, nosocomial infection, National Early Warning Score 2, Simplified Acute Physiology Score-II and haemoglobin.

Table 5. Discrimination ability for the hospital mortality prognostic model comprised by RDW when added to the prognostic scores

	AUC-ROC	95% Confidence interval	p-value for comparison between AUC-ROCs
SOFA	0.772	0.700-0.844	
+ Admission RDW	0.812	0.748-0.876	0.041
+ 24-hours RDW	0.808	0.744-0.873	0.042
LODS	0.687	0.605-0.768	
+ Admission RDW	0.771	0.702-0.839	0.002
+ 24-hours RDW	0.770	0.701-0.839	0.002
SAPS-II	0.734	0.660-0.809	
+ Admission RDW	0.785	0.721-0.849	0.021
+ 24-hours RDW	0.781	0.716-0.846	0.030
APACHE-II	0.672	0.588-0.756	
+ Admission RDW	0.755	0.682-0.827	0.003
+ 24-hours RDW	0.752	0.678-0.826	0.004

SOFA: Sequential Organ Failure Assessment, RDW: Red blood cell distribution width, LODS: Logistic Organ Dysfunction System, SAPS-II: Simplified Acute Physiology Score-II, APACHE-II: Acute Physiology and Chronic Health Evaluation-II., AUC-ROC: Area Under the Receiver Operating Characteristic Curve.

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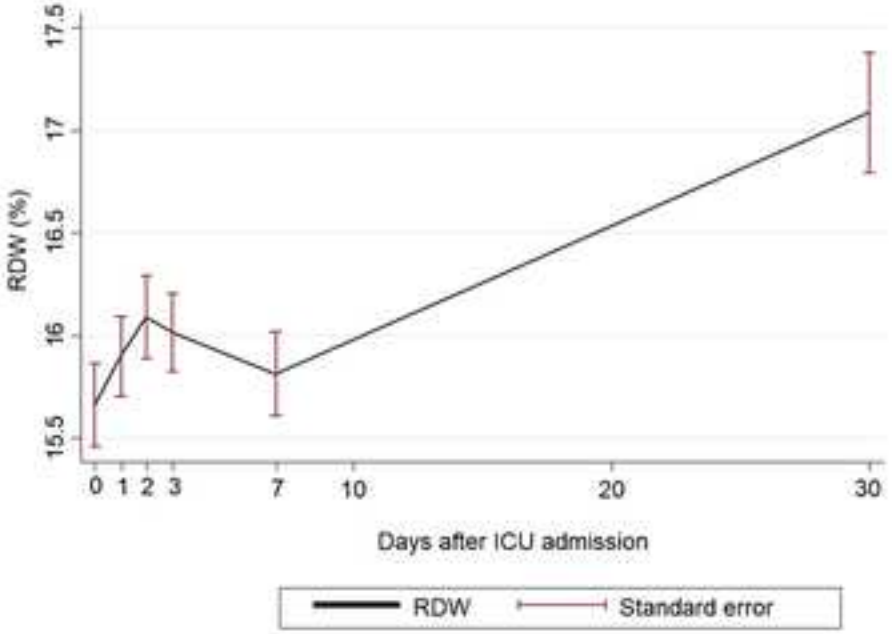
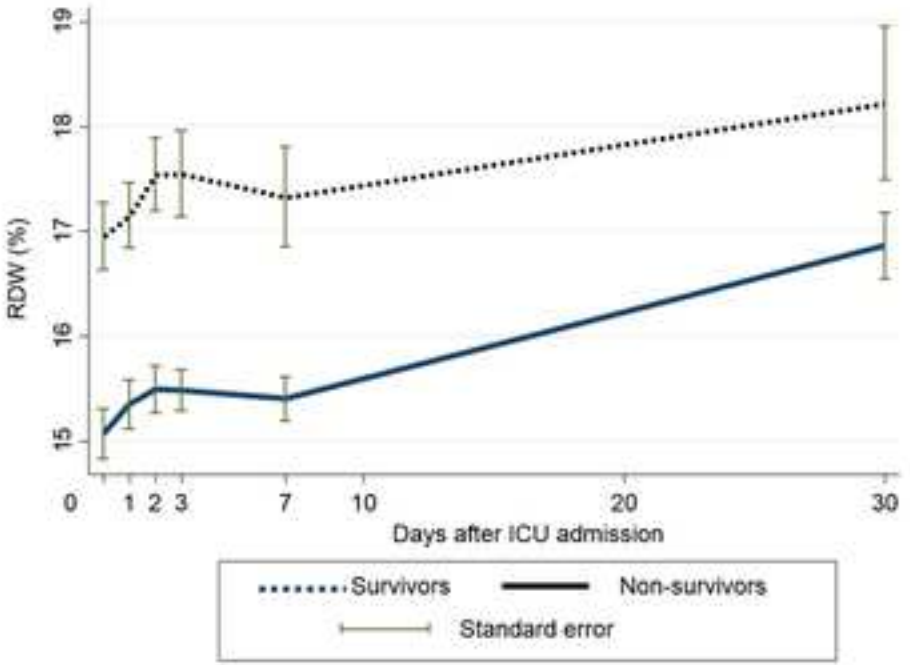
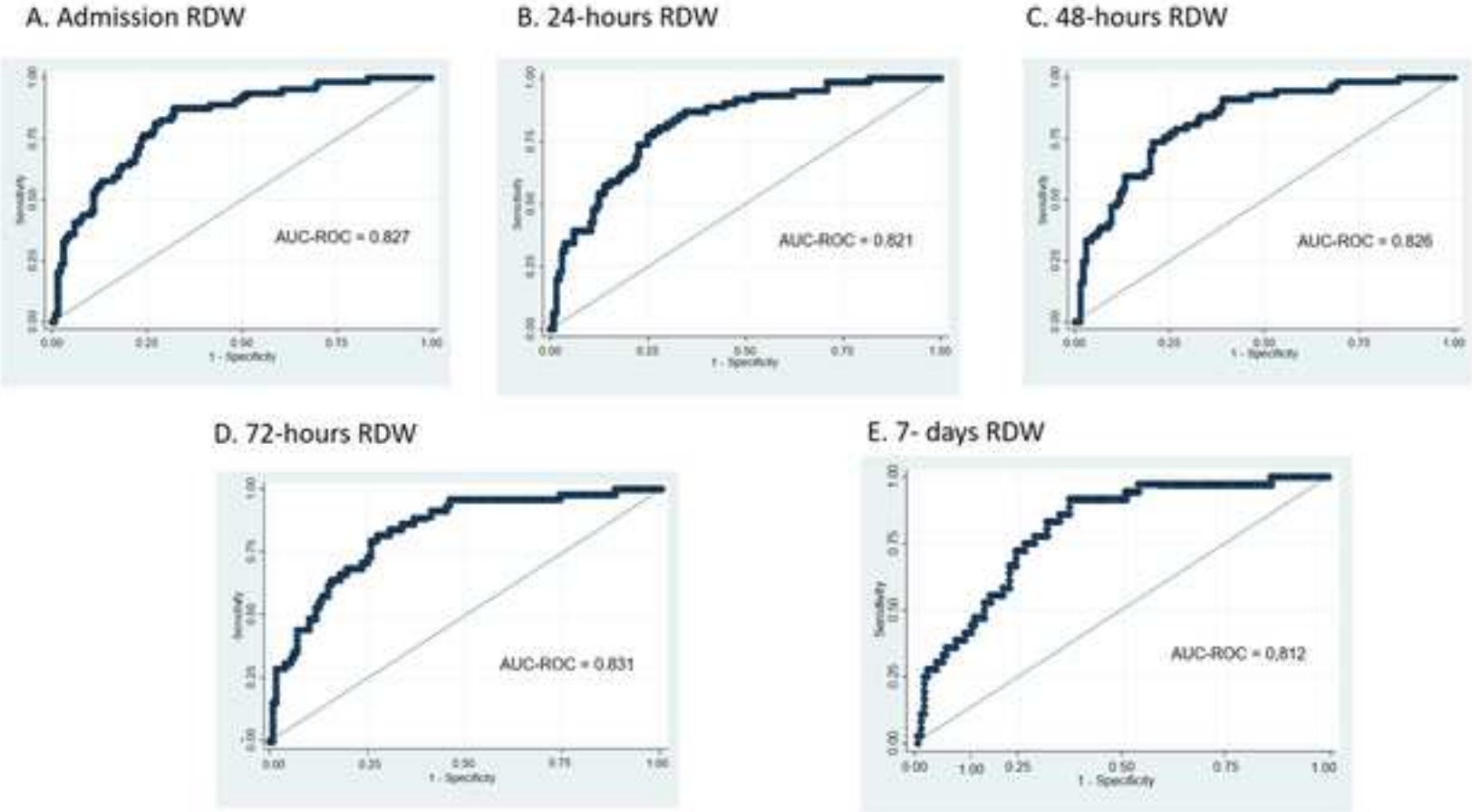


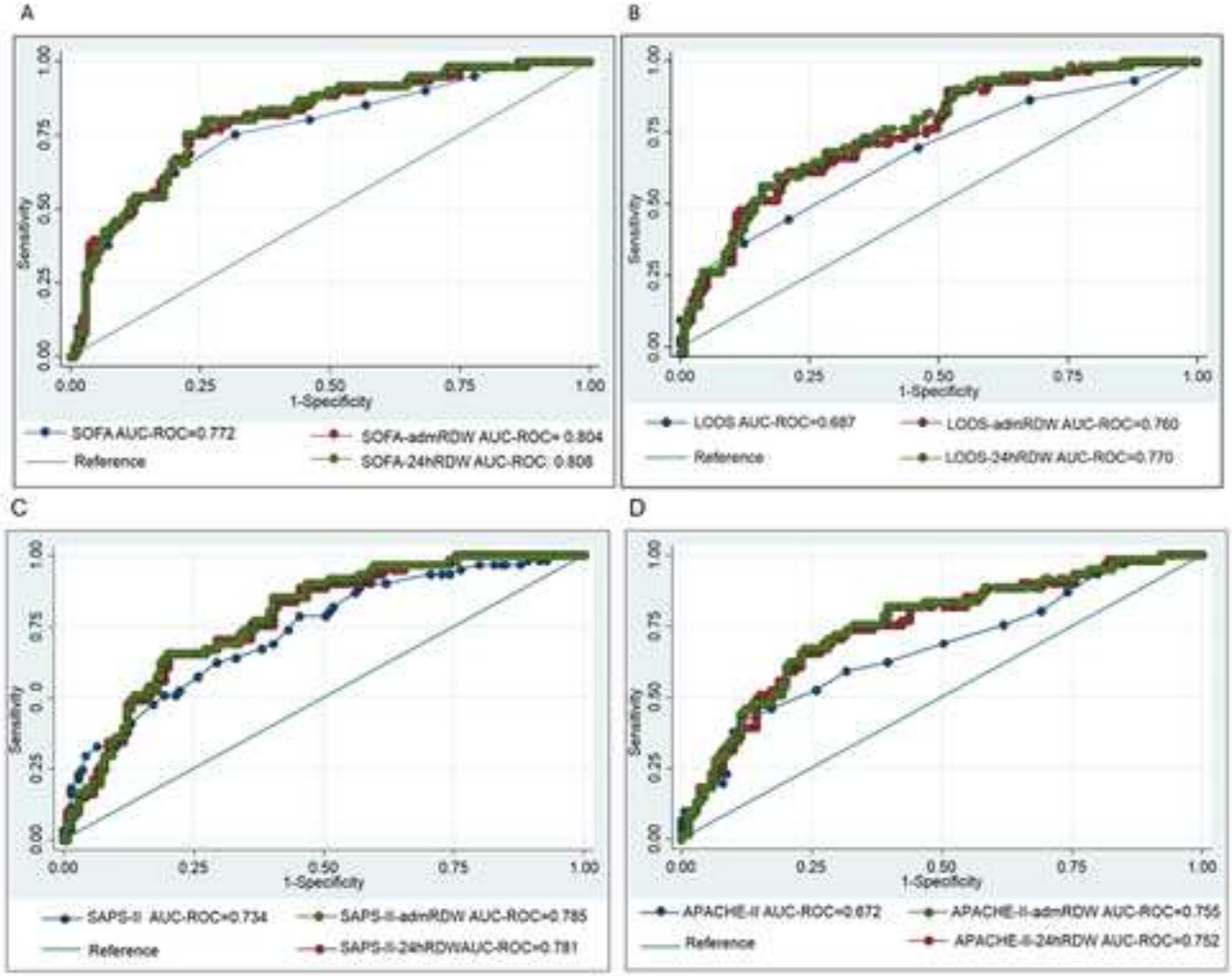
A. Red blood cell distribution width (RDW) dynamics 30 days after ICU admission. Data are expressed as mean and standard error.



B. Red blood cell distribution width (RDW) dynamics 30 days after ICU admission among survivors and non-survivors. Data are expressed as mean and standard error.



The figure shows the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) for the models including admission Red blood cell distribution width (RDW) (A), 24-hours RDW (B), 48-hours RDW, 72-hours RDW (D) and 7-days RDW (E). All the analyses were adjusted by the Charlson Comorbidity Index, immunosuppression, nosocomial infection, National Early Warning Score 2, Sequential Organ Failure Assessment, Simplified Acute Physiology Score-II and haemoglobin.



The figure shows the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) for SOFA score (A), LODS (B), SAPS-II score (C) and APACHE-II (D) and the added value of admission Red blood cell distribution width (RDW) and 24-hours RDW.

Medicina Clínica xxx (xxxx) xxx–xxx

MEDICINA CLINICA

www.elsevier.es/medicinaclinica

Original

Mejor capacidad pronóstica de NEWS2, SOFA y SAPS-II en pacientes con sepsis

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INFORMACIÓN DEL ARTÍCULO

Historia del artículo:
Recibido el 16 de agosto de 2021
Aceptado el 28 de octubre de 2021
On-line el xxx

Palabras clave:
Sepsis
NEWS2
SOFA
SAPS-II

RESUMEN

Antecedentes y objetivos: Comparar la capacidad de las escalas qSOFA, NEWS2, SOFA, LODS, SIRS, APACHE-II y SAPS-II.
Materiales y métodos: Análisis de mortalidad hospitalaria de 203 pacientes ingresados en la UCI por sepsis. Las escalas se compararon de acuerdo a su aplicación. La capacidad predictiva se evaluó mediante el análisis del AUC-ROC y el rendimiento con los criterios de información de Akaike (AIC) bayesiano (BIC).
Resultados: La mortalidad hospitalaria fue del 31,5%. NEWS2 mostró mejor capacidad pronóstica y rendimiento según los criterios AIC/BIC que qSOFA (AUC-ROC = 0,615 vs. 0,536; p = 0,039). SOFA presentó mejor rendimiento y AUC-ROC que LODS (0,776 vs. 0,693; p = 0,01) y ambos demostraron una mejor capacidad pronóstica que SIRS (AUC-ROC = 0,521; p < 0,003). Finalmente, SAPS-II predijo con mayor rendimiento la mortalidad que APACHE-II y presentó mayor capacidad discriminante, aunque sin significación estadística (AUROC = 0,738 vs. 0,673; p = 0,08).
Conclusiones: La escala NEWS2 mostró mayor capacidad pronóstica que qSOFA en pacientes sépticos ingresados en la UCI, por lo que su aplicación para el reconocimiento precoz del paciente con sepsis o en riesgo debería plantearse en los servicios de urgencias y hospitalización. Por otra parte, dado que SOFA y SAPS-II mostraron mejor rendimiento y simplicidad que LODS y APACHE-II, respectivamente, deberían considerarse las escalas de elección en este contexto.

Better prognostic ability of NEWS2, SOFA and SAPS-II in septic patients

Keywords:
Sepsis
NEWS2
SOFA
SAPS-II

ABSTRACT

Background and objectives: To compare the ability of qSOFA, NEWS2, SOFA, LODS, SIRS, APACHE-II and SAPS-II scores.
Material and methods: Analysis of in-hospital mortality of 203 patients admitted to the ICU because of sepsis. The scores were compared according to their application. Discrimination was evaluated with AUC-ROC curve and performance with the Akaike's (AIC) and Bayesian information criterion (BIC).
Results: In-hospital mortality was 31.53%. NEWS2 showed better mortality discrimination ability and better performance considering the AIC/BIC criterion for mortality tan qSOFA (AUC-ROC = .615 and .536; *P* = .039). SOFA presented higher performance and AUC-ROC tan LODS (.776 vs .693; *P* = .01) and both showed higher discrimination ability than SIRS (AUC-ROC = .521; *P* < .003). Finally, SAPS-II was able to predict mortality with better performance than APACHE-II and presented higher discrimination capacity but without statistical significance compared (AUROC = .738 for SAPS-II and AUROC = .673 for APACHE-II; *P* = .08).

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https://doi.org/10.1016/j.medcli.2021.10.021

Cómo citar este artículo: V. Moreno-Torres, A. Royuela, E. Múñez et al., Mejor capacidad pronóstica de NEWS2, SOFA y SAPS-II en pacientes con sepsis, Med Clin (Barc)., https://doi.org/10.1016/j.medcli.2021.10.021

Conclusion: NEWS2 is a better predictor of mortality than qSOFA and its implementation for the early recognition of the septic patient or the patient with higher risk in the emergency and hospitalization wards should be addressed. In addition, given that SOFA and SAPS-II showed better performance and are simpler than LODS and APACHE-II, respectively, both should be considered the scores of choice in this setting.

Introducción

Actualmente, y a pesar de los avances que se han producido en la identificación, diagnóstico y manejo del paciente séptico, la sepsis sigue siendo una importante causa de mortalidad en todo el mundo[1,2]. De hecho, puede considerarse una de las primeras causas de muerte hospitalaria con tasas de mortalidad de hasta el 18,7 y el 55,7% en el paciente hospitalizado y en las unidades de cuidados intensivos (UCI), respectivamente[3].

El tercer documento de consenso de definición de la sepsis y *shock* séptico (Sepsis-3) supuso no solo un cambio en la definición de la sepsis, basada en el concepto de daño orgánico y en la escala SOFA (Sequential Organ Failure Assessment), si no que enfatizó la importancia de la detección precoz y *screening* de pacientes que pueden presentar una sepsis o que están en riesgo de presentarla mediante la utilización de la escala quick-SOFA (qSOFA)[4–6].

Dentro de las iniciativas y de las estrategias de identificación precoz y manejo empírico de la sepsis a raíz de este documento y durante el estudio de los factores pronósticos de la sepsis; en los últimos años se han desarrollado y perfeccionado una serie de escalas pronósticas, que por otra parte, también se han utilizado para monitorizar la evolución y evaluar la respuesta del tratamiento[1].

Con esta premisa, y además de la ya mencionada escala SOFA, la escala National Early Warning Score 2 (NEWS2) se diseñó para que la primera evaluación y «triage» de los pacientes agudos en el National Healthcare Service (NHS) del Reino Unido se realizase de forma estandarizada y protocolizada[7]. A su vez, la escala Logistic Organ Dysfunction System (LODS), define y cuantifica la disfunción orgánica de forma similar al SOFA, y ambas sustituyeron a los criterios Systemic Inflammatory Response Syndrome (SIRS) durante la actualización de la definición de sepsis[4,8,9]. Por otra parte, la escalas Acute Physiology and Chronic Health Evaluation-II (APACHE-II) y Simplified Acute Physiology Score-II (SAPS-II) son herramientas pronósticas muy útiles y ampliamente utilizadas para predecir mortalidad hospitalaria y en la UCI, independientemente de la causa o enfermedad que condicione el ingreso[10,11].

Por consiguiente, el objetivo del presente estudio es comparar y analizar el rendimiento y la capacidad pronóstica de estas escalas en pacientes con sepsis que ingresan en la UCI.

Pacientes y métodos

Población de estudio

La población de estudio fueron los pacientes adultos ingresados por sepsis en el Hospital Puerta de Hierro-Majadahonda entre el 1 de enero de 2028 y el 31 de diciembre de 2019. Los pacientes se incluyeron si la sepsis se diagnosticaba o se cumplían los criterios de sepsis durante el ingreso en la UCI. El estudio se aprobó por el Comité Ético de Investigación del centro (PI 80-21).

Recolección de datos y cálculo de las escalas

Los datos epidemiológicos, clínicos, hemodinámicos, de laboratorio o microbiológicos se recogieron mediante un formulario estándar. El valor de las escalas se determinó de acuerdo con estos datos, y en el caso de que el paciente presentase una enfermedad crónica con valores basales alterados, las escalas se calculaban considerando la diferencia entre el valor basal y el presentado durante el ingreso en la UCI (por ejemplo, creatinina o bilirrubina en caso de enfermedad renal o hepática, respectivamente)[4–11]. La ausencia de valor de algún parámetro se consideraba como cero o equivalente, de forma que no suponía una variación o valor en la escala.

Comparación de las escalas pronósticas

Las escalas pronósticas se analizaron entre distintos grupos de acuerdo con su aplicación y diseño. La escala qSOFA se comparó con la escala NEWS2 puesto que ambas se diseñaron como herramientas de detección precoz basados únicamente en datos clínicos. La escala LODS se comparó con la escala SOFA y SIRS ya que se han planteado o utilizado en las definiciones de la sepsis como criterio del daño orgánico[4,9]. Finalmente se analizaron las escalas APACHE-II y SAPS-II. La variable objetivo fue la mortalidad hospitalaria.

Análisis estadístico

En el análisis descriptivo se utilizaron la media y la desviación estándar (DE) para las variables numéricas y las frecuencias absolutas o relativas para las categóricas.

El valor de cada una de las escalas, determinado de acuerdo con la bibliografía[4–11], se introdujo en un análisis de regresión logística univariable para evaluar su capacidad predictiva de mortalidad. Posteriormente, se realizó un análisis comparativo de la capacidad discriminante de mortalidad y del rendimiento de distintas escalas. La discriminación estadística es la capacidad que tiene un modelo o parámetro para diferenciar un evento en un paciente. Se evalúa mediante el análisis del área bajo la curva característica operativa del receptor (AUC-ROC). Un valor cercano al 1 indica una discriminación perfecta, pero un modelo con un AUC-ROC mayor de 0,7 se considera con una capacidad discriminante suficiente para diferenciar un evento en un sujeto[12]. El comando *roccomp* de Stata se utilizó para comparar las AUC-ROC. Se presentan los correspondientes intervalos de confianza al 95% de cada una de las AUC-ROC.

A su vez, los criterios de información de Akaike (AIC) y bayesiano (BIC) son medidas de la calidad relativa de un modelo estadístico y proporcionan un medio para la selección del mismo. Suponen un balance entre la bondad de ajuste del modelo y la complejidad del mismo. En ambos casos, el mejor modelo será aquel que resulte de un AIC/BIC menor sobre el conjunto de modelos considerados[13].

Para todos los análisis, el nivel de significación estadística se fijó en 0,05. El análisis se realizó mediante el *software* (StataCorp. 2019. Stata® Statistical Software: Release 16. College Station, TX: StataCorp LLC) y SPSS® version 15.0 (IBM).

Resultados

Características de los pacientes

Durante el periodo de estudio, 203 pacientes ingresaron en la UCI debido a la sepsis. Sus características se muestran en la tabla 1.

Tabla 1
Características de los pacientes

Características	Global (n = 203)	Supervivientes (n = 139)	No supervivientes (n = 64)
Varones, n (%)	129 (63,6)	91 (65,5)	38 (64,9)
Edad, años (DE)	63,1 (14,3)	61,81 (14,5)	65,94 (11,6)
Paciente ambulatorio, n (%)	159 (78,3)	112 (80,6)	47 (73,4)
Nosocomial, n (%)	81 (39,9)	46 (33,1)	35 (54,7)
Estancia en la UCI, días (DE)	16 (34,1)	10,8 (46,6)	15,8 (48,8)
Estancia hospitalaria, días (DE)	37.6 (52.1)	32.4 (55.9)	37.5 (66.1)
Intervención quirúrgica, n (%)	111 (54,7)	73 (52,5)	38 (59,3)
Cardiopatía, n (%)	65 (32,0)	36 (25,9)	29 (45,3)
Enfermedad arterial periférica, n (%)	28 (13,8)	21 (15,1)	7 (10,9)
Conectivopatía, n (%)	25 (12,3)	12 (8,6)	13 (20,3)
Enfermedad cerebrovascular, n (%)	25 (12,3)	13 (9,4)	12 (18,8)
Hemiplejía, n (%)	6 (3)	4 (2,9)	2 (3,1)
Neumopatía, n (%)	66 (32,5)	39 (28,1)	27 (42,2)
Hepatopatía, n (%)	30 (14,8)	16 (11,5)	14 (21,9)
Enfermedad renal crónica, n (%)	43 (21,2)	26 (18,7)	17 (26,6)
Neoplasia, n (%)	86 (42,4)	55 (39,6)	31 (48,4)
Enfermedad hematológica, n (%)	37 (18,2)	21 (15,1)	16 (25)
Úlcera péptica, n (%)	9 (4,5)	4 (2,9)	5 (7,8)
Diabetes, n (%)	64 (31,5)	39 (28,1)	25 (39,1)
Obesidad, n (%)	25 (12,3)	18 (12,6)	7 (10,9)
Alcoholismo, n (%)	26 (12,8)	17 (12,2)	9 (14,1)
VIH, n (%)	3 (1,5)	2 (1,4)	1 (1,6)
Trasplante (TOS/TPH), n (%)	38 (18,7)	20 (14,4)	18 (28,1)
Inmunosupresión, n (%)	74 (36,5)	43 (30,9)	31 (48,4)
Demencia, n (%)	5 (2,5)	2 (1,4)	3 (4,7)
Índice de comorbilidad de Charlson, media (DE)	6,17 (3,98)	5,78 (4,31)	7,03 (3,01)

DE: desviación estándar; TOS: trasplante de órgano sólido; TPH: trasplante de precursores hematopoyéticos; UCI: unidad de cuidados intensivos; VIH: virus de la inmunodeficiencia humana.

El 63,6% eran varones, con una edad media de 63,1 años. El 78,3% procedía de domicilio o en régimen ambulatorio, y el 52,7% ingresó desde el servicio de urgencias. En el 39,9% de los pacientes la infección tenía origen nosocomial. La estancia media en la UCI fue de 16 días, y la mortalidad hospitalaria del 31,5%.

Características durante el ingreso en la unidad de cuidados intensivos

En la tabla 2 se muestran los parámetros clínicos y analíticos de los pacientes al ingreso en la UCI. En el global, el 79,3% cumplía criterios de *shock* séptico (74,1% de los supervivientes vs. 90,6% de los no supervivientes). En relación con el foco de la infección (tabla 3), el más frecuente fue el foco respiratorio (38,8% de los supervivientes vs. 39,1% de los no supervivientes), seguido del foco abdominal (35,3 y 37,5%), urinario (14,4 y 7,8%), piel y partes blandas (2,2 y 6,3%), bacteriemia asociada a catéter (2,2 y 7,8%) y la endocarditis (2,2 y 7,8%). En 5 pacientes el foco de la infección no se pudo identificar (1,4% de los supervivientes vs. 4,7% de los no supervivientes).

Escalas pronósticas

El valor de las escalas se determinó con los datos referido (tabla 4). El 33,5% cumplía los criterios qSOFA (30,2% de los supervivientes y 37,5% de los no supervivientes) y el 77,8% los criterios SIRS (79,1 vs. 75%). La media del valor de la escala NEWS fue de 9,72 (9,21 para los supervivientes y 10,83 para los no supervivientes), de 9,06 para la escala SOFA (7,99 vs. 11,39), 7,92 para la escala LODS (7,37 vs. 9,09), 18,65 para la escala APACHE-II (18,65 vs. 22,72) y 47,68 para la escala SAPS-II (12,23 vs. 59,63).

La capacidad discriminante de mortalidad y el rendimiento de las escalas se comparó teniendo en cuenta la aplicación, diseño y las variables que consideran cada una: qSOFA se comparó con NEWS2, SOFA con LODS y SIRDS y APACHE-II con SAPS-II (tabla 5). En primer lugar, la escala NEWS2 obtuvo un AUC-ROC igual a 0,615 y el qSOFA obtuvo un AUC-ROC de 0,536. La diferencia entre ambas curvas fue estadísticamente significativa (p = 0,039). En cuanto al AIC y BIC, fueron mejores en la escala NEWS2 (tabla 5). En segundo lugar, las escalas SOFA y LODS presentaron mayor AUC-ROC (0,776 y 0,693, respectivamente) que los criterios SIRS (0,521), con una diferencia significativa entre las 3 (p < 0,001). Además, la escala SOFA mostró un mejor rendimiento de acuerdo con los AIC y BIC. Finalmente, SAPS-II mostró mejor capacidad discriminante para mortalidad, aunque no se alcanzó la significación estadística (AUROC = 0,738 para SAPS-II y AUROC = 0,673 para APACHE-II). El rendimiento en la predicción de mortalidad de SAPS-II fue superior, al obtenerse AIC y BIC más bajos que APACHE-II.

Discusión

Nuestros resultados confirman que las escalas NEWS2, SOFA, LODS, SAPS-II y APACHE-II son adecuados predictores de mortalidad en pacientes sépticos que ingresan en la UCI. Además, la comparación de estas escalas ha demostrado que NEWS2, SOFA y SAPS-II tienen una mejor capacidad pronóstica en comparación con las escalas equivalentes.

Las escalas qSOFA, NEWS y NEWS2 se diseñaron y validaron con el objetivo de identificar al paciente séptico de forma precoz y para evaluar la gravedad del paciente agudo[4,6,7,14]. Ambas se basan únicamente en parámetros clínicos y se pueden calcular rápidamente a pie de cama, lo que las hace muy útiles y aplicables en la práctica clínica. Tanto es así que el qSOFA se ha convertido en una herramienta ampliamente utilizada en los servicios de urgencias y en las plantas de hospitalización como parte de las estrategias del reconocimiento precoz de la sepsis. Además, Canet et al. demostraron que la escala qSOFA permite identificar a los pacientes con sospecha de infección en el ámbito de urgencias con mayor riesgo de mortalidad y estancia en la UCI[15]. Sin embargo, confirmaron que una de las principales limitaciones de esta herramienta, a pesar de un alto valor predictivo positivo que permite predecir la mortalidad hospitalaria con exactitud, es su escaso valor predictivo negativo. Por otra parte, la escala NEWS ha demostrado ser más precisa que el qSOFA en la predicción de la mortalidad hospitalaria y la mortalidad atribuida a la

Tabla 2
Parámetros clínicos y analíticos al ingreso en la UCI

Parámetros	Global (n = 203)	Supervivientes (n = 139)	No supervivientes (n = 64)
Escala de coma de Glasgow, media (DE)	14 (2,5)	14 (2,2)	13 (2,9)
Temperatura (°C), media (DE)	37,1 (1,6)	37,4 (1,5)	36,5 (1,7)
PAM (mmHg), media (DE)	70 (20,7)	70 (21,8)	69 (18,2)
Frecuencia cardíaca (lpm), media (DE)	107 (23)	107 (21)	107 (27)
Frecuencia respiratoria (rpm), media (DE)	28 (8)	27 (8)	30 (7)
SpO_2 (%), media (DE)	93 (6)	93 (6)	93 (7)
Vasopresores, n (%)	164 (80,8)	105 (75,5)	59 (92,2)
Dopamina	23 (11,3)	18 (12,9)	5 (7,8)
Dobutamina	12 (6,1)	7 (5)	5 (7,8)
Noradrenalina	164 (80,8)	105 (74,8)	59 (92,2)
Ventilación mecánica, n (%)	91 (44,8)	46 (33,1)	45 (70,3)
Ratio PaO_2/FiO, media (DE)	232 (157)	230 (155)	237 (163)
Diuresis (ml/24 h), n (%)			
< 200	33 (16,3)	15 (10,8)	18 (12,5)
200-500	35 (17,3)	16 (11,5)	19 (29,7)
> 500	134 (66,3)	108 (77,7)	26 (40,63)
Terapia renal sustitutiva, n (%)	57 (28,1)	24 (17,3)	33 (51,6)
pH arterial, media (DE)	7,30 (0,11)	7,32 (0,11)	7,26 (0,11)
Bicarbonato (mmol/l), media (DE)	19,9 (4,5)	20,2 (4,2)	19,1 (5,1)
Lactato (mmol/l), media (DE)	3,8 (3,1)	3,5 (2,3)	4,7 (4,4)
pO_2 (mmHg), media (DE)	94 (37,5)	91 (38,1)	100 (35,6)
pCO_2 (mmHg), media (DE)	40 (12,2)	39 (11,6)	43 (13,1)
Urea (mg/dl), media (DE)	93 (61)	85 (60)	111 (60)
Creatinina (mg/dl), media (DE)	2,15 (1,66)	2,06 (1,72)	2,35 (1,51)
Glucosa (mg/dl), media (DE)	173 (90)	175 (81)	169 (107)
Sodio (mmol/l) (media, DE)	138 (6)	137 (5)	139 (8)
Potasio (mmol/l), media (DE)	4,39 (0,95)	4,31 (0,90)	4,56 (1,04)
Cloro (mmol/l), media (DE)	103 (8)	103 (8)	104 (7)
Bilirrubina (mg/dl), media (DE)	2,20 (3,79)	1,92 (3,21)	2,81 (4,78)
Leucocitos ($\times 10^3/mm^3$), media (DE)	16.600 (14.600)	16.900 (11.500)	16.000 (19.800)
Neutrófilos ($\times 10^3/mm^3$), media (DE)	14.000 (12.600)	14.200 (10.100)	13.400 (16.800)
Linfocitos ($\times 10^3/mm^3$), media (DE)	961 (2.109)	884 (896)	1.129 (3.530)
Plaquetas ($\times 10^3/mm^3$), media (DE)	185.300 (138.300)	196.900 (127.700)	160.200 (157.100)
Hemoglobina (g/dl), media (DE)	11,60 (2,65)	12,00 (2,53)	10,73 (2,72)
Hematocrito (%), media (DE)	35,15 (8,05)	36,33 (7,42)	32,57 (8,81)
Actividad de protrombina (%), media (DE)	53,96 (21,12)	56,23 (20,77)	49,01 (21,17)

DE: desviación estándar; lpm: latidos por minuto; PAM: presión arterial media; PaO_2/FiO: presión arterial de oxígeno/fracción inspirada; pCO_2: presión parcial de dióxido de carbono; pO_2: presión parcial de oxígeno; rpm: respiraciones por minuto; SpO_2: saturación de oxígeno.

Tabla 3
Foco de la infección

	Global (n = 203)	Supervivientes (n = 139)	No supervivientes (n = 64)
Respiratorio, n (%)	79 (38,9)	54 (38,8)	25 (39,1)
Urinario, n (%)	25 (12,3)	20 (14,4)	5 (7,8)
Piel y partes blandas, n (%)	14 (6,9)	10 (7,2)	4 (6,3)
Abdominal, n (%)	73 (36)	49 (35,3)	24 (37,5)
Bacteriemia asociada a catéter, n (%)	8 (3,9)	3 (2,2)	5 (7,8)
Desconocido, n (%)	5 (2,5)	2 (1,4)	3 (4,7)
Endocarditis, n (%)	8 (3,9)	3 (2,2)	5 (7,8)
Otros, n (%)	15 (7,4)	9 (6,5)	6 (9,4)

Tabla 4
Mortalidad hospitalaria y escalas pronósticas

	Global (n = 203)	Supervivientes (n = 139)	No supervivientes (n = 64)
QSOFA, n (%)	66 (32,5)	42 (30,2)	24 (37,5)
NEWS2, media (DE)	9,72 (3,36)	9,21 (2,91)	10,83 (3,98)
SIRS, n (%)	158 (77,8)	110 (79,1)	48 (75)
SOFA, media (DE)	9,06 (3,58)	7,99 (3,24)	11,39 (3,19)
LODS, media (DE)	7,92 (2,181)	7,37 (1,69)	9,09 (2,64)
APACHE-II, media (DE)	19,94 (5,94)	18,65 (5,14)	22,72 (6,62)
SAPS-II, media (DE)	51,42 (13,93)	47,68 (12,13)	59,53 (14,22)

APACHE-II: Acute Physiology and Chronic Health Evaluation-II; DE: desviación estándar; LODS: Logistic Organ Dysfunction System; NEWS2: National Early Warning Score 2; qSOFA: quick-SOFA; SAPS-II: Simplified Acute Physiology Score-II; SIRS: Systemic Inflammatory Response Syndrome; SOFA: Sequential Organ Failure Assessment.

sepsis[16,17], superioridad que por el momento no se ha confirmado en la escala NEWS2, fruto de la actualización de NEWS en 2017. Únicamente Mellhammar et al. constató que NEWS2 era superior al qSOFA en la detección de una variable compuesta por sepsis con disfunción orgánica, mortalidad relacionada con la infección o ingreso en la UCI debido a la infección[18]. Nuestro estudio, basado en una cohorte homogénea de pacientes sépticos ingresados en la UCI de acuerdo con los criterios Sepsis-3, demuestra que NEWS2, que no solo incluye la frecuencia respiratoria, la presión arterial y la conciencia, sino también la temperatura, la frecuencia cardíaca

Tabla 5
Comparación de la capacidad discriminante de mortalidad y rendimiento de las escalas

	MortalidadOR (IC 95%)	AUC-ROC (IC 95%)	Comparación de escalas (valor de p AUC-ROC)	AIC	BIC
qSOFA	1,39 (0,74-2,58)	0,536 (0,465-0,607)	0,039	255,994	262,621
NEWS2	1,16 (1,06-1,27)	0,615 (0,526-0,704)		246,765	253,391
SOFA	1,39 (1,24-1,55)	0,776 (0,705-0,846)	0,011*	213,479	220,106
LODS	1,48 (1,26-7,74)	0,693 (0,613-0,772)	0,001**	229,361	235,988
SIRS	0,79 (0,39-1,59)	0,521 (0,457-0,584)	< 0,001***	256,613	263,239
APACHE-II	1,13 (1,07-1,20)	0,673 (0,592-0,755)	0,084	236,216	242,842
SAPS-II	1,07 (1,04-1,10)	0,738 (0,665-0,811)		223,191	229,817

AIC: criterio de información de Akaike; APACHE-II: Acute Physiology and Chronic Health Evaluation-II; AUC-ROC: área bajo la curva operativa característica del receptor; BIC: criterio de información bayesiano; IC 95%: intervalo de confianza del 95%; LODS: Logistic Organ Dysfunction System; NEWS2: National Early Warning Score 2; OR: *odds ratio;* qSOFA: quick-SOFA; SAPS-II: Simplified Acute Physiology Score-II; SIRS: Systemic Inflammatory Response Syndrome; SOFA: Sequential Organ Failure Assessment.
* Comparación de SOFA con LODS.
** Comparación de LODS con SIRS.
*** Comparación de SOFA con SIRS.

y la saturación de oxígeno, tiene mejor capacidad pronóstica de mortalidad que los criterios qSOFA. Estos hallazgos confirman que la valoración clínica completa, pero al mismo tiempo factible, de todos los signos vitales durante la evaluación inicial de un paciente en riesgo, permite mejorar la identificación del paciente grave y con mayor riesgo de mortalidad por sepsis y que, por lo tanto, debería plantearse su uso sistemático en los servicios de urgencias y hospitalización más allá del NHS.

Por otro lado, el documento de consenso Sepsis-3 consolidó al SOFA como criterio de disfunción orgánica que define a la sepsis[4]. Dado que tanto las escalas SOFA como LODS eran superiores a los criterios SIRS en la discriminación de la mortalidad hospitalaria en pacientes de la UCI con sospecha de infección, el grupo de trabajo seleccionó al SOFA sobre LODS por su mayor simplicidad y amplio uso. Sin embargo y aunque Wu et al demostraron, en pacientes con traumatismos graves, que la escala LODS presentaba un mayor rendimiento pronóstico en comparación con el SOFA[19], otros estudios en pacientes sépticos no han conseguido confirmar cuál es más precisa en términos de predicción de mortalidad[20–24]. Nuestros hallazgos muestran que el SOFA tiene una mejor capacidad discriminante y predictiva de mortalidad que LODS en este grupo de pacientes, además de que confirman que los criterios SIRS son poco precisos en este contexto. Aunque equivalentes, las escalas SOFA y LODS no se calculan con las mismas variables, lo que puede justificar esta diferencia, ya que mientras que el SOFA considera la necesidad y las dosis de vasopresores, la escala LODS incluye parámetros de coagulación y leucocitos[5,8]. El uso de vasopresores es un indicador robusto de gravedad, *shock* séptico y necesidad de ingreso en la UCI, probablemente con un papel pronóstico más potente que las pruebas de sangre o coagulación, parámetros a su vez menos específicos de sepsis y/o daño orgánico. Además, y reforzando la definición del Consenso de Sepsis-3, el uso y aplicación de la escala SOFA es más sencillo y está generalizado en el ámbito de urgencias y cuidados críticos[4].

En último lugar, las escalas APACHE-II y SAPS-II son herramientas predictoras de gravedad y mortalidad del paciente crítico muy utilizadas en la atención al paciente crítico, y en su cálculo se considera el peor valor de cada variable durante las primeras 24 h de ingreso del paciente[10,11,25]. A pesar de que actualmente existen versiones más recientes de estas escalas, en la práctica el uso de APACHE-II y SAPS-II se ha mantenido por su mayor accesibilidad y aplicabilidad.

Estudios previos han comparado la capacidad discriminante y calibración de estas dos escalas sin hallar diferencias significativas a pesar de que ambas predicen la mortalidad con precisión, incluyendo el trabajo de, Kadziolka et al. en el que no se demostraron diferencias en el AUC-ROC de APACHE-II y SAPS-II a pesar de que la calibración de la escala con la prueba de Hosmer-Lemeshow arrojó resultados favorable para SAPS-II[26–29]. En nuestro estudio, el rendimiento de la escala SAPS-II, analizado mediante los AIC y BIC, fue superior con respecto al APACHE-II y, aunque solo se logró una significación marginal, la capacidad discriminante de mortalidad analizada mediante el AUC-ROC fue también superior.

Nuestro estudio presenta algunas limitaciones puesto que se trata de un estudio observacional, retrospectivo y unicéntrico y que presenta un tamaño muestral que, aunque ha permitido demostrar significación estadística y relevancia clínica en el análisis estadístico, puede considerarse pequeño. En segundo lugar, la población de estudio fueron únicamente pacientes ingresados en la UCI. En nuestra opinión esto no supone necesariamente una limitación en la práctica clínica puesto que el curso evolutivo de la sepsis y *shock* séptico supone en una alta proporción de pacientes criterios de ingresos en la UCI. No obstante, este criterio de inclusión podría suponer un sesgo en la comparación de las escalas NEWS2 y qSOFA, ya que los pacientes incluidos están intrínsecamente graves dado el propio ingreso en la UCI. Además, el circunscrito ámbito del estudio conlleva a que deban realizar más estudios en los servicios de urgencias y en las plantas de hospitalización para validar nuestros resultados.

En conclusión, nuestro estudio demuestra que la escala NEWS2 es una herramienta que predice con mayor capacidad la mortalidad en pacientes sépticos que ingresan en la UCI que los criterios qSOFA, motivo por el que debería evaluarse y plantearse su uso para la detección precoz del paciente séptico en los ámbitos de urgencias y hospitalización. Además, y dado que SOFA y SAPS-II tienen mejor rendimiento, son más aplicables y más simples en comparación con LODS y APACHE-II, deben ser consideradas las escalas de elección para definir el daño orgánico, gravedad y el riesgo de mortalidad hospitalaria.

Consideraciones éticas

El estudio se desarrolló de acuerdo con el Código de Ética de la Asociación Médica Mundial (Declaración de Helsinki y se aprobó por el Comité Ético de Investigación del centro (PI 80-21).

Financiación

Este trabajo ha sido financiado con ayuda del Instituto de Salud Carlos III (contrato Río-Hortega del primer autor, expediente CM19/00223).

Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses.

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Internal and Emergency Medicine
https://doi.org/10.1007/s11739-021-02824-7

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Bacterial infections in patients hospitalized with COVID-19

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Received: 19 May 2021 / Accepted: 2 August 2021

Abstract

Bacterial infections may complicate the course of COVID-19 patients. The rate and predictors of bacterial infections were examined in patients consecutively admitted with COVID-19 at one tertiary hospital in Madrid between March 1st and April 30th, 2020. Among 1594 hospitalized patients with COVID-19, 135 (8.5%) experienced bacterial infectious events, distributed as follows: urinary tract infections (32.6%), bacteremia (31.9%), pneumonia (31.8%), intra-abdominal infections (6.7%) and skin and soft tissue infections (6.7%). Independent predictors of bacterial infections were older age, neurological disease, prior immunosuppression and ICU admission ($p < 0.05$). Patients with bacterial infections who more frequently received steroids and tocilizumab, progressed to lower Sap02/FiO2 ratios, and experienced more severe ARDS ($p < 0.001$). The mortality rate was significantly higher in patients with bacterial infections as compared to the rest (25% vs 6.7%, respectively; $p < 0.001$). In multivariate analyses, older age, prior neurological or kidney disease, immunosuppression and ARDS severity were associated with an increased mortality ($p < 0.05$) while bacterial infections were not. Conversely, the use of steroids or steroids plus tocilizumab did not confer a higher risk of bacterial infections and improved survival rates. Bacterial infections occurred in 8.5% of patients hospitalized with COVID-19 during the first wave of the pandemic. They were not independently associated with increased mortality rates. Baseline COVID-19 severity rather than the incidence of bacterial infections seems to contribute to mortality. When indicated, the use of steroids or steroids plus tocilizumab might improve survival in this population.

Keywords COVID-19 pneumonia · Bacterial infections · Steroids

Abbreviations

ARDS	Acute respiratory distress syndrome
DTR	Difficult- to-treat resistance
HBP	High blood pressure
MDR	Multidrug resistant bacteria.
SapO2	Oxygen saturation by pulse oximetry
TNFi	Tumor necrosis factor inhibitors
DM	Diabetes mellitus
FiO2	Fraction of Inspired Oxygen
ICU	Intensive care Unit
PaO2	Partial pressure of oxygen
SOT	Solid organ transplantation

The members of the Puerta de Hierro COVID-19 working group are listed in "Acknowledgements" section.

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Introduction

Already in the middle of 2021, the SARS-CoV-2 infection continues, being the largest health problem worldwide [1]. Since its first outbreak in December 2019 and the official consideration as a pandemic by the WHO, the disease has spread through the world affecting practically every community. COVID-19 disease occurs in several phases in which

Published online: 18 August 2021

some patients require hospitalization due to acute respiratory distress (ARDS) after the so-called cytokine storm or cytokine release syndrome [2]. Among different therapeutic options, treatment with corticosteroids and tocilizumab has been widely used with conflicting results for the latter [3–5]. In addition, the use of these immunosuppressants could increase the risk of secondary infections [6, 7]; not to forget that respiratory viral infections may also predispose to bacterial [8].

In the present study, our objective was to describe and analyze the prevalence of bacterial infections and the main risk factors for infections, other than SARS-CoV-2, in patients admitted due to COVID-19 pneumonia during the first period of the pandemic. We analyzed the rate of patients with bacterial infections as well as their impact on OVID-19 morbidity and mortality. Knowing the characteristics of bacterial infections in patients with COVID-19 could help us optimize therapeutic options, and corticosteroids and/or antibiotherapy use in patients at risk.

Patients and methods

Study design and patients

This retrospective observational cohort study was performed at Hospital Puerta de Hierro-Majadahonda, a large tertiary university hospital located in Madrid, one of the most affected regions by COVID-19 during the first wave. The study population consisted of adult patients who were admitted because of interstitial pneumonia due to suspected or confirmed SARS-CoV-2 between March 1st and April 30th, coinciding with lockdown and the first pandemic wave. According to this, both RT-PCR confirmed SARS-CoV-2 infection and suspected SARS-CoV-2 interstitial pneumonia (in the absence of other causes) were included. Follow-up continued to June 30th, 2020. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and was approved by the hospital's Research Ethics Commission. All patients were requested their consent to register their clinical information into a database for epidemiological studies.

Local treatment protocol

During the first pandemic wave, immunosuppressive and antibiotic therapy was protocolized in our center. All patients with interstitial pneumonia received azithromycin and hydroxicloroquine during 3 and 5 days, respectively. Lopinavir/ritonavir was used if the patient presented hypoxemia during the first 10 days after symptom onset while interferon-beta (IFN-β) use was determined by the presence of respiratory insufficiency. Steroids and/or tocilizumab were considered in case of ARDS 7 days after symptom onset and in the absence of data suggestive of bacterial superinfection. Empirical antibiotic therapy with cephalosporins in addition to azithromycin relied on the physician's criteria for each situation.

Data collection and outcomes

Electronic medical records for all hospital admitted patients with COVID-19 pneumonia were reviewed. The main demographics, the baseline comorbidities including immunosuppression and immunosuppressive treatment, microbiological tests (respiratory samples, urinary antigen test, blood, urine and other sites cultures depending on the foci), immunosuppressive treatment received to treat COVID-19 ARDS and outcomes, were collected directly from the electronic medical records. All data were registered by a primary reviewer and subsequently checked by at least two senior physicians.

Definitions

Immunosuppression was defined either as the presence of hematological disease (active lymphoproliferative, myeloproliferative disorders or bone marrow transplantation), solid organ transplantation, active and disseminated solid organ neoplasm or any condition, including autoimmune disease (e.g., Systemic Lupus Erythematosus, Sjögren Syndrome or Inflammatory Bowel Disease…) that had required immunosuppressive treatment for at least 3 months. Immunosuppressive treatment was considered when the patient was either receiving active treatment at the time of admission, including equivalent doses of prednisone above 5 mg, or had received chemotherapy or immunotherapy 6 months before disease onset.

Acute Respiratory Distress Syndrome (ARDS) and its severity were defined according to the Berlin definition [9]. In patients whose partial pressure of oxygen (PaO2) was unavailable; SapO2/FiO2 ratio was used to assess ARDS and severity [10]. Mild ARDS was considered when PaO2/FiO2 ratio was > 200 mmHg or SapO2/FiO2 > 235 mmHg, moderate when PaO2/FiO2 ratio was > 100 mmHg or SapO2/FiO2 > 160 mmHg and severe when PaO2/FiO2 ratio was ≤ 100 mmHg.

Bacterial infection was documented as the presence of one of the following: fever or chills in the absence of other etiologies, purulent sputum, catheter swelling, inflammatory diarrhea or abdominal pain, along with microbiologic results including blood and urine cultures, upper and lower respiratory samples, cerebrospinal fluid, urinary antigen tests, intraoperative samples, glutamate dehydrogenase test or Clostridioides toxin in stool test. Bacterial pathogen evaluation in blood, fluids, sputum and other samples was performed according to standard microbiological procedures during hospital admission. In addition, laboratory parameters (neutrophilia or procalcitonin elevation) along

with radiological and intraoperative findings were considered, particularly in those patients whose microbiological confirmation was not possible. Every suspected infection and its pathogen were carefully and individually assessed by two senior physicians to determine clinical relevance and to avoid selection bias.

Microorganisms were considered multidrug resistant (MDR) if they were resistant to one or more agents in at least three antibiotic classes (beta-lactams, fluoroquinolones, macrolides, aminoglycosides or sulfamides), while difficult-to-treat resistance (DTR) was defined by the resistance to all first-line agents, including all beta-lactams and fluoroquinolones [11, 12].

Statistical analysis

Descriptive analyses were performed through the mean (standard deviation, SD) for quantitative variables and absolute (and relative) frequencies for the categorical. An univariate analysis was performed comparing those characteristics for patients who suffered bacterial infections vs those who did not, and also between survivors and non-survivors by means of chi-square test in case of categorical variables and Mann–Whitney's *U* or Student *t*-test for numerical variables depending on their distributions and performing the Levène test. Potential confounding variables were entered into two multivariable logistic regression analyses. The objective was to identify factors related with the risk of bacterial infection and mortality, respectively. For all analyses, significance level was defined as a *p* value below 0.05. Statistical analysis was performed using SPSS software version 26.0 (IBM, Spain).

Results

Patients characteristics

A total of 1594 patients admitted because of suspected or confirmed SARS-CoV-2 pneumonia between March and April 2020 were analyzed. Mean age was 65 years old, 62.1% were male and 87.2% had a positive PCR for SARS-CoV-2 at the time of admission. Overall, 135 patients (8.5%) developed a bacterial infection during admission (Table 1). Patients with infections were significantly older (mean age 68 vs 64.5, $p=0.007$) and presented higher rates of baseline

Table 1 Baseline characteristics of the study population

	Total *N* (%)	Bacterial infections		*p* value*
		Yes *n* (%)	No *n* (%)	
COVID-19 hospitalized patients	1594	135	1459	–
Mean age (mean, SD)	65 (15.0)	68 (14.3)	64.5 (14.9)	0.007
Male sex	990 (62.1)	87 (64.4)	903 (61.9)	Ns
High blood pressure	699 (43.9)	65 (48.1)	634 (43.5)	Ns
Diabetes mellitus	281 (17.6)	37 (27.4)	244 (16.7)	0.002
Obesity	424 (26.6)	36 (30)	388 (35.7)	Ns
Heart disease	270 (16.9)	28 (20.7)	242 (16.6)	Ns
Neurological disease	225 (14.1)	28 (20.7)	197 (13.5)	0.021
Lung disease	248 (15.6)	26 (19.3)	222 (15.2)	Ns
Kidney disease	112 (7)	18 (13.3)	94 (6.4)	0.003
Liver disease	48 (3)	6 (4.4)	42 (2.9)	Ns
Immunosuppression	166 (10.4)	39 (28.9)	127 (8.7)	<0.0001
Autoimmune disease	65 (4.1)	12 (8.9)	53 (3.6)	0.01
Solid organ transplantation	30 (1.9)	6 (4.4)	24 (1.6)	0.036
Hematological disease	35 (2.2)	10 (7.4)	25 (1.7)	0.000
Solid organ neoplasm	32 (2)	8 (5.9)	24 (1.6)	0.004
Others	4 (0.25)	–	–	–
Immunosuppressive treatment	139 (8.7)	34 (25.2)	105 (7.2)	0.000
Steroids	68 (4.3)	15 (11.1)	53 (3.6)	0.000
Calcineurin inhibitors	25 (1.6)	6 (4.4)	19 (1.3)	0.005
Mycophenolate	25 (1.6)	8 (5.9)	17 (1.2)	0.000
Biologicals	30 (1.9)	6 (4.4)	24 (1.6)	0.022
Chemotherapy	16 (1)	6 (4.4)	10 (0.7)	0.000
Others	6 (0.4)	–	–	–

SD Standard deviation, *NS* Non-significant

comorbidities as diabetes (27.4% vs 16.7%, $p=0.002$), neurological disease (20.7% vs 13.5%, $p=0.021$) and kidney disease (13.3% vs 6.4%, $p=0.021$). In addition, 28.9% of patients with bacterial infection were immunosuppressed (vs 8.7%, $p<0.0001$), being the main causes: autoimmune disease (8.9%), hematological disease (7.4%), solid organ neoplasm (5.9%) and solid organ transplantation (4.4%). As a result, 25.2% of infected individuals were receiving immunosuppressive treatment.

A total of 156 bacterial infections occurred in 135 patients, with significant microbiological isolation in 91.9% of them (Table 2). The main sites of infection were urinary tract (32.6%), lung (31.8%) and bacteremia (31.9%), related to catheter in 67.4% of them. In addition, nine patients (6.7%) presented intra-abdominal or skin and soft tissue infection. Other foci were meningitis, endocarditis, otorhinolaryngologycal site, tuberculosis or septic shock (5.9%).

Regarding the observed microorganisms, gram-positive cocci were the most frequent isolation (54.1%). On the other hand, gram-negative bacteria were documented in 40 patients (29.6%) while non-fermentative bacteria were identified in 13 patients (9.6%). Species are also shown in Table 2.

MDR were isolated in 26 patients (19.3%); due to *E. Coli* spp (30.8%), *Pseudomonas* (15.4%), resistant-*staphylococci* (15.4%), *Stenotrophomonas* (15.4%), *Enterobacter* (7.7%), *Klebsiella* (7.7%), *Achromobacter* (3.8%) and *Acinetobacter* species (3.8%). 63.3% of MDR infections happened in ICU admitted patients. In parallel, Gram-negative-DTR were identified in 18 (13.3%), being *E. Coli (*27.8%*), Stenotrophomonas* (22.2%*), Pseudomonas (*16.7%*), Enterobacter* (11.1%), *Klebsiella* (11.1%), *Achromobacter* (5.5%) and *Acinetobacter* species (5.5%). 77.8% of these isolates were observed in patients admitted to the ICU.

11 patients had no isolate. Foci were: nosocomial pneumonia (four patients), skin and soft tissue (three patients), urinary tract (two patients), diverticulitis (one patient) and septic shock in an immunosuppressed patient with hematological disease.

Table 2 Bacterial infections in 135 COVID-19 patients. Anatomic site and microorganism

	N (%)
Site of infection	
Lung	43 (31.8)
Community-acquiredy/superinfection	22/43 (51.2)
Nosocomial	21/43 (48.8)
Bacteremia	43 (31.9)
Catheter related	29/43 (67.4)
Primary	14/43 (32.6)
Urinary tract	44 (32.6)
Intra-abdominal	9 (6.7)
Skin and soft tissue	9 (6.7)
Others*	8 (5.9)
Microorganism	
Gram-positive cocci	73 (54.1)
MRSA	11 (8.2)
MSSA	2 (1.5)
CNS	32 (23.7)
Enterococci	34 (25.2)
Streptococci	16 (11.9)
Enterobacterales	40 (29.6)
E. Coli	30 (22.2)
Klebsiella spp.	16 (11.9)
*Enterobacter s*pp.	3 (2.2)
Others	2 (1.5)
Non-fermentative gram-negative	13 (9.6)
P. aeruginosa	12 (8.9)
Stenotrophomonas maltophilia	4 (3)
Acinetobacter spp.	1 (1)
Achromobacter spp.	1 (1)
Anaerobic bacteria	9 (6.7)
Clostridium difficile	4 (3)

MRSA Methicillin-resistant *Staphylococcus aureus*, *MSSA* Methicillin-sensitive *Staphylococcus aureus*, *CNS* Coagulase-Negative Staphylococci

*Included meningitis (2 cases), endocarditis (2 cases), otorhinolaryngology site (2 cases), tuberculosis (1 case), or septic shock from unknown foci (1 case)

Disease severity, treatment and outcomes.

Overall, 90.4% of patients with any bacterial infection have had ARDS in the context of COVID-19 disease vs 74.8% of patients without infectious complications ($p<0.001$). In addition, these patients had lower SapO2/FiO2 ratios (198 vs 280, $p<0.001$). Consequently, the patients with bacterial infections had more severe ARDS (40.7% vs 5.1%, $p<0.001$) when compared with the rest. The immunosuppressive treatment used to treat ARSD was also analyzed. Patients with infections had received more steroids (76.1% vs 56.5%, $p<0.0001$) and more tocilizumab (40% vs 16.9%, $p<0.001$).

Considering outcomes, patients who had suffered bacterial infection had longer hospital stay (19.5 vs 8.4 days, $p<0.001$), had been more frequently admitted to the ICU (40% vs 3.8%, $p<0.001$) and ICU stays had been significantly higher (27.2 vs 10.4 days, $p<0.001$). In addition, these patients had higher readmission rates after discharge (14.2% vs 4.9%, $p<0.001$), 57.9% of them motivated by bacterial infections acquired during first admission. Overall mortality was 15.1%, being significantly higher in patients with bacterial infection (25.03% vs 6.70%, $p<0.001$).

Risk factors for bacterial infection

To identify the risk factors associated with bacterial infections in the context of COVID-19, a multivariable analysis was performed considering the patient's baseline characteristics, previous treatments, disease severity and the treatment used for ARDS (Table 3). Independent factors related with bacterial infection were: age (OR 1.02, 95% CI 1.01–1.04, $p = 0.009$), neurological disease (OR 1.69, 95% CI 1.01–2.82 ($p = 0.046$)), immunosuppression (OR 4.41, 95% CI 2.76–7.06, $p < 0.001$) and ICU admission (OR 21.36, 95% CI 13.21–34.55, $p < 0.001$). Neither steroid nor tocilizumab combined with steroid treatment for ARDS were significantly associated with a higher risk of infection after variable adjustment.

Mortality risk factors

Since a higher proportion of individuals with bacterial infection died during admission in the univariate analysis, a multivariable analysis was performed to identify mortality risk factors (Fig. 1). Mortality was determined by baseline comorbidities, including age (OR 1.13, 95% CI1.10–1.16, $p < 0.0001$), neurological disease (OR 2.77, 95% CI 1.77–4.34, $p < 0.0001$), kidney disease (OR 3.46, 95% CI 1.92–6.24, $p < 0.0001$), previous immunosuppression (OR 3.33, 95% CI 1.91–5.81, $p < 0.0001$) and by the presence and severity of ARDS: mild ARDS (OR 4.67, 95% CI 1.50–14.54, $p = 0.008$), moderate ARDS (OR 93.88, 95% CI 29.27–301.08, $p < 0.0001$) and severe ARDS (OR 282.10, 95% CI 79.18–1005.33), $p < 0.0001$). By contrast, bacterial infections were not independently associated with mortality (OR 0.85, CI 0.47–1.53, $p > 0.05$). Steroid treatment (OR 0.35, 95% CI 0.20–0.60, $p < 0.0001$) and the combination of steroids with tocilizumab (OR 0.56, 95% CI 0.34–0.93, $p < 0.024$) showed lower mortality rates.

Discussion

In this study, we aimed to describe and analyze the burden and risk factors of bacterial infections in patients with COVID-9 disease, since the main therapeutic approaches to date are corticosteroids and tocilizumab, both with recognized potential to develop infections.

We documented a prevalence of 8.5% of bacterial infections, a slightly higher proportion compared to nosocomial and health-care associated infection rates before the pandemic [13, 14]. However, our results are similar to those reported in other COVID-19 cohorts [15, 16]. In addition, we also confirmed that infections are not only caused by respiratory superinfection. There is an important rate of primary and catheter-related bacteremias, urinary tract and abdominal infections, with a significant rate of gram-positive cocci, enterobacterial, non-fermentative and multiresistant pathogens [14, 17, 18]. These are not surprising data since COVID-19 actually can result in long hospital stays, ICU admission, vascular and respiratory devices, malnutrition and a wider use of empirical antibiotherapy, all well-known risk factors for nosocomial infection [19, 20]. Furthermore, SARS-CoV-2 infection could result in a systemic hyperinflammatory disease that carries a state of

Table 3 Risk factors for bacterial infections in COVID-19 hospitalized patients

	Univariate analysis*		Multivariate analysis**	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Baseline conditions				
Age	1.02 (1.01–1.03)	**0.007**	**1.02 (1.01–1.04)**	**0.009**
DM	1.88 (1.26–2.81)	**0.002**	1.60 (0.99–2.49)	0.059
Neurological disease	1.68 (1.08–2.60)	**0.022**	**1.69 (1.01–2.82)**	**0.046**
Kidney disease	2.23 (1.30–3.82)	**0.003**	1.221 (0.64–2.29)	0.555
Active neoplasm	2.66 (1.52–4.65)	**0.001**	1.047 (0.50–2.20)	0.903
Immunosuppression	4.26 (2.82–6.45)	**< 0.001**	**4.41 (2.76–7.06)**	**< 0.001**
Outcome				
ARDS	3.17 (1.77–5.68)	**< 0.001**	1.34 (0.70–2.55)	0.375
ICU admission	16.69 (10.80–25.81)	**< 0.001**	**21.36 (13.21–34.55)**	**< 0.001**
Treatment				
Steroids	2.46 (1.63–3.70)	**< 0.001**	0.94 (0.55–1.61)	0.828
Tocilizumab	3.29 (2.27–4.76)	**< 0.001**	0.66 (0.12–3.75)	0.636
Steroids* + Tocilizumab^{T}	2.43 (1.66–3.58)	**< 0.001**	1.31 (0.80–2.16)	0.282

Ods ratio, confidence intervals and p-values marked with bold indicate statistically significance

OR Odds ratio, *CI* Confidence interval, *DM* Diabetes mellitus, *ARDS* Acute respiratory distress syndrome, *ICU* Intensive care Unit

^{T}: Product term between steroids and tocilizumab treatment

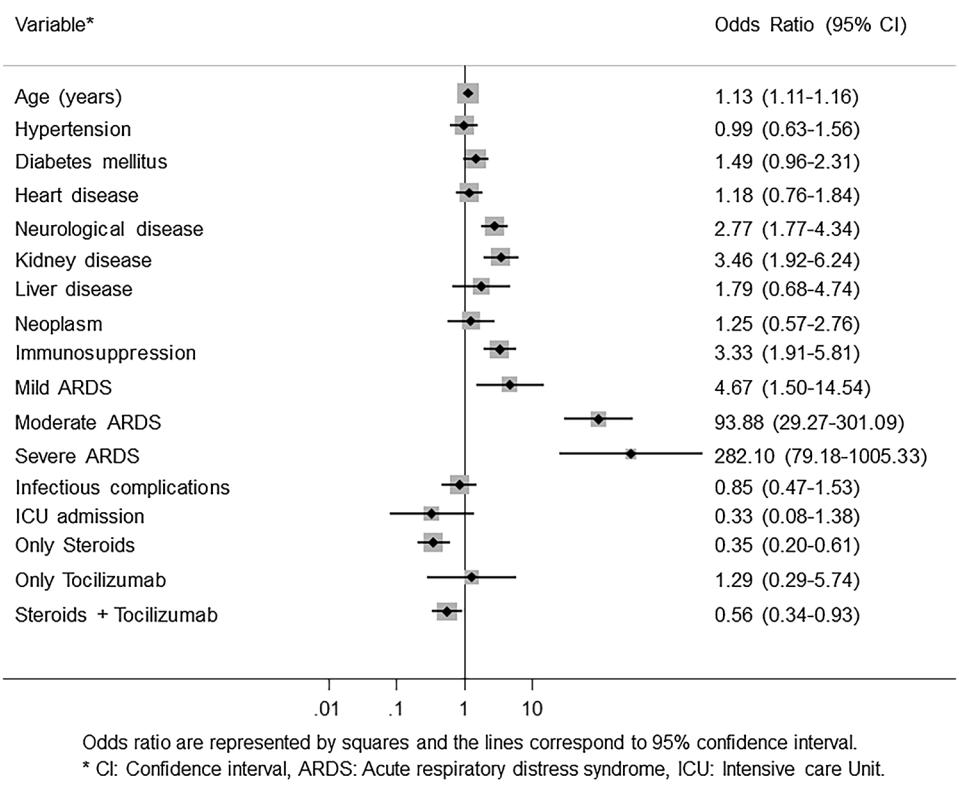

Fig. 1 Predictors of mortality in COVID-19 hospitalized patients. Odds ratio are represented by squares and the lines correspond to 95% confidence interval. *CI* Confidence interval, *ARDS* Acute respiratory distress syndrome, *ICU* Intensive care Unit

immunosuppression beyond lung injury [21, 22], contributing to a significant number of infectious complications affecting other sites.

Our data show that, in addition to age and baseline comorbidities, ICU admission was the main risk factor for the development of bacterial infection. At the same time, ARDS severity and the respiratory worsening of patients during the disease mainly determined ICU admission. Other authors have described significantly higher rates of secondary infections in critically ill patients with severe ARDS [23–25]. Moreover, Bardi et al. identified disease severity as the only factor associated with the development of infection in the ICU [26]. The pandemic situation of the first wave implied a lack of material and staff, infrastructural changes and a significant work overload that altered the normal organization of the ICU. As a result, standards of prophylactic care and aseptic conditions of the procedures were difficult to fulfill as usual, justifying the higher rates of infectious complications.

On the other hand, steroid treatment was initially criticized during the first months of the pandemic since there were no robust evidence to support its use. Several reports informed that early treatment with steroids might extend SARS-CoV-2 RNA replication [27, 28]. In addition, bacterial and opportunistic infections during or after steroid treatment are a major concern and recognized side effect, even at low doses and short courses [6, 29, 30], being a possible limitation for its use in COVID-19 patients. As a matter of fact, Obata et al. found that steroids but not tocilizumab were associated with higher rates of bacterial and fungal infection in COVID-19 hospitalized patients [31]. However, in our cohort neither steroid treatment nor tocilizumab exposure carried a significant risk of bacterial infection when adjusted in the multivariate analysis. Consequently, our study confirms the benefit of the anti-inflammatory effect of steroids in COVID-19, overcoming the potential risk of infections in this scenario [3, 32, 33].

In parallel, similar concerns have affected the use of tocilizumab in autoimmune diseases [7, 34], revealing an even higher risk of bacterial infections with tocilizumab than with tumor necrosis factor inhibitors (TNFi) [35]. In our cohort, tocilizumab use was limited (one or two doses), therefore not conditioning the maintained blockage of IL-6R and avoiding the possible long-term immunosuppression and risk of infection [34]. To note, Stone et al. documented fewer serious infections in patients treated with tocilizumab [4] and the recent trial from Veiga et al. showed no differences in the secondary infection rates when tocilizumab was compared with standard care [5]

Finally, the main related mortality factors were again age, comorbidities and ARDS, while bacterial infections were not an independent factor, reinforcing the hypothesis that infections are a surrogate marker of the most fragile or most severely affected patients with COVID-19. Furthermore, steroid and the combination of steroid with tocilizumab provided a protective effect, supporting its role

in COVID-19, since they did not lead to more bacterial infections.

According to our findings, questions about antibiotic therapy during COVID-19 disease arise again. Others have observed that, to date, there is no evidence enough to support empirical antimicrobial due to data absence [36]. In parallel, our results support that antibiotic prescription should not be generalized and might be carefully evaluated; above all if we consider that infections are the consequence of severe disease. Consequently, the best approach seems to be ARDS treatment with clinical and microbiological surveillance; waiting to microbiological definite diagnosis rather than early empirical prescription when suspected.

The main limitation of our study was the absence of data regarding the empirical and targeted antibiotic treatment in the cohort. We were not able to understand their role in the risk and courses of bacterial infections. Unfortunately, and despite that treatment during this period of the pandemic was carefully standardized; no information, conclusions or recommendations could be elucidated in this regard.

In summary, nearly 9% of individuals hospitalized with COVID-19 developed bacterial infection during the first pandemic wave. Although this population exhibited an unfavorable clinical profile, bacterial infections were not independently associated with increased mortality rates or with steroid and tocilizumab treatment for ARDS. This result suggests that bacterial infections reflect disease severity rather than contribute to mortality. Immunosuppressive treatment should be used when indicated given that it did not implied higher bacterial infection rates and resulted beneficial for patients with SARS-CoV-2 pneumonia in terms of survival.

Acknowledgements We thank the members of the Puerta de Hierro COVID-19 working group for their contribution: A. Fernández-Cruz, E. Múñez, R. Malo de Molina, I. Pintos, A. Díaz de Santiago, A. Ramos, P. Mills, P. Laguna, G. Vázquez, M. Valle, A. Muñoz, B. Cantos, J. Calderón, A Ángel-Moreno, I. Baños, E Montero, M. C. Carreño, Y. Romero, R. Muñoz, P. Durán, S. Mellor-Pita, P. Tutor, M. Aguilar, G. Díaz, C. García, B. Jara, R. Laporta, M. T. Lázaro, C. López, P. Mínguez, A. Trisán, R. Carabias, M. Erro, B. Agudo, J. Aller, R. Benlloch, M. R. Blasco, M. A. Brito, V. Calvo, M. Calvo, J. Campos, R. Cazorla, M. Cea, H. Cembrero, E. Colino, S. Córda, S. Cruz, G. Del Pozo, C. Del Pozo, M. Elosua, M. Espinosa, C. Fernández, C. Ferre, M. García-Espantaleón, E. A. García-Izquierdo, B. Gil, P. Gómez-Porro, S. González, I. González, G. Escalera, A. I. López, A. Losa, M. E. Marín, I. Martínez, M. E. Martínez, C. Maximiano, M. Méndez, S. Mingo, C. Mitroi, B. Núñez, P. Ortega, J. F. Oteo, N. Pérez, L. Prieto, L. Relea, G. Rodríguez, J. Sabín, J. Sáenz, A. Sánchez, A. Sánchez, J. Sanz, J. Segovia, L. Silva, J. Toquero, M. E. Velasco, S. Villaverde, A. Andrés, S. Blanco, I. Diego, I. Donate, G. Escudero, E. Expósito, A. Galán, S. García, J. Gómez, A. Gutiérrez, V. Edith, .I Gutiérrez, F. Martínez, A. Mora, I. Morrás, A. Muñoz, A. Valencia, J. M. Vázquez, A. Arias, J. Bilbao, A. M. Duca, M. A. García-Viejo, J. M. Palau, A. Roldán, R. Castejón, M. J. Citores, S. Rosado, J. A. Vargas, P Ussetti.

Funding This work has been supported by a grant from Instituto de Salud Carlos III (Expedient number PI16-01480).

Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Stata v16 software (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

Declarations

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

Ethical approval The study was approved by the Research Ethics Committee of our center.

Consent to participate and for publication Consent was requested for all patients to include their clinical information within a database for epidemiological and clinical studies.

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Infect Dis Ther (2021) 10:347–362
https://doi.org/10.1007/s40121-020-00373-8

ORIGINAL RESEARCH

Combination of Tocilizumab and Steroids to Improve Mortality in Patients with Severe COVID-19 Infection: A Spanish, Multicenter, Cohort Study

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Received: October 7, 2020 / Accepted: November 16, 2020 / Published online: December 6, 2020

ABSTRACT

Background: We aimed to determine the impact of tocilizumab use on severe COVID-19 (coronavirus disease 19) pneumonia mortality.

Methods: We performed a multicentre retrospective cohort study in 18 tertiary hospitals in Spain from March to April 2020. Consecutive patients admitted with severe COVID-19 treated with tocilizumab were compared to patients not treated with tocilizumab, adjusting by inverse probability of the treatment weights (IPTW). Tocilizumab's effect in patients receiving steroids during the 48 h following inclusion was analysed.

Results: During the study period, 506 patients with severe COVID-19 fulfilled the inclusion

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s40121-020-00373-8) contains supplementary material, which is available to authorized users.

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criteria. Among them, 268 were treated with tocilizumab and 238 patients were not. Median time to tocilizumab treatment from onset of symptoms was 11 days [interquartile range (IQR) 8–14]. Global mortality was 23.7%. Mortality was lower in patients treated with tocilizumab than in controls: 16.8% versus 31.5%, hazard ratio (HR) 0.514 [95% confidence interval (95% CI) 0.355–0.744], $p < 0.001$; weighted HR 0.741 (95% CI 0.619–0.887), $p = 0.001$. Tocilizumab treatment reduced mortality by 14.7% relative to no tocilizumab treatment [relative risk reduction (RRR) 46.7%]. We calculated a number necessary to treat of 7. Among patients treated with steroids, mortality was lower in those treated with tocilizumab than in those treated with steroids alone [10.9% versus 40.2%, HR 0.511 (95% CI 0.352–0.741), $p = 0.036$; weighted HR 0.6 (95% CI 0.449–0.804), $p < 0.001$] (interaction $p = 0.094$).

Conclusions: These results show that survival of patients with severe COVID-19 is higher in those treated with tocilizumab than in those not treated and that tocilizumab's effect adds to that of steroids administered to non-intubated patients with COVID-19 during the first 48 h of presenting with respiratory failure despite oxygen therapy. Randomised controlled studies are needed to confirm these results.

Trial registration: European Union electronic Register of Post-Authorization Studies (EU PAS Register) identifier, EUPAS34415

Keywords: COVID-19; Mortality; SARS-CoV-2; Steroids; Tocilizumab

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Key Summary Points

Over-exuberant cytokine release occurred in some patients infected by SARS-CoV-2. Treatment strategies aimed to break down this hyper-inflammatory response were considered.

This study describes the experience with a series of consecutive COVID-19 patients treated with TCZ in 18 tertiary hospitals in Spain and compares the outcomes of this cohort with those observed in a similar cohort of SARS-CoV-2 infected patients who did not receive tocilizumab.

Tocilizumab treatment reduced mortality by 14.7% relative to no tocilizumab treatment (RRR 46. 7%). Tocilizumab with steroid-48 h treatment reduced mortality by 29.1% relative to no tocilizumab treatment (RRR 72.8%).

Mortality is improved when tocilizumab is used concomitantly or shortly followed by steroids (first 48 h). These results contribute to the body of evidence supporting the use of tocilizumab in SARS-CoV-2 infection.

Randomised controlled studies are needed to confirm these results and establish the potential place of tocilizumab in the treatment of COVID-19.

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DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.13228859.

INTRODUCTION

In the past few decades, newly evolved coronaviruses have posed a global threat to public health.

The most recent one, SARS-CoV-2 coronavirus, has produced a pandemic infecting more than 10 million people and causing more than 500,000 deaths worldwide, challenging not only healthcare systems but also the culture and economy of the population [1].

SARS-CoV-2 causes COVID-19 (coronavirus disease 19), which in most cases is an asymptomatic or mildly symptomatic condition that resolves without therapy or with minor supportive treatment. In some cases, SARS-CoV-2 can cause pneumonia, which in 20% may be moderate in severity. However, a subgroup of patients with COVID-19 pneumonia develops rapidly progressing respiratory failure that may necessitate mechanical ventilation and support in an intensive care unit (ICU) and/or result in multi-organ and systemic manifestations in terms of sepsis, septic shock, and multiple organ dysfunction syndromes [2].

Available evidence suggests that a hyper-inflammatory syndrome (HIS) that resembles secondary hemophagocytic lymphohistiocytosis (sHLH) may have a pathogenic role [3, 4]. This is consistent with the general knowledge about coronavirus (CoV) infections, where the immune response has been shown to play an essential role in controlling and eliminating CoV infections, and maladjusted immune responses may result in immunopathology and impaired pulmonary gas exchange [5].

At the time of conducting the study, treatment was mainly symptomatic, with oxygen therapy representing the major treatment intervention for patients with severe infection. Several antiviral treatments have been used

such as lopinavir/ritonavir, chloroquine, hydroxychloroquine, remdesivir, and alpha-interferon. No vaccine is currently available. Based on the observation that acute, life-threatening respiratory injury associated with an over-exuberant cytokine release occurred in some patients infected by SARS-CoV-2, treatment strategies aiming to break down this hyper-inflammatory response were considered. Elevated levels of blood interleukin-6 (IL-6) have been identified as a one of the risk factors associated with severe COVID-19 [5].

In this context, tocilizumab, a humanized IgG1 monoclonal antibody targeting the IL-6 receptor, was postulated as a suitable treatment option [6]. Preliminary experiences with tocilizumab showed promising results with improvements seen in clinical symptoms, oxygenation status, and inflammatory laboratory parameters [7]. This limited evidence together with a strong mechanistic rationale, plus current knowledge on the role of anti-IL6 inhibitors in the treatment of cytokine release syndromes, supported the progressively extended use of tocilizumab in clinical practice. Simultaneously numerous clinical studies, including clinical trials, were initiated worldwide with the aim to assess the potential efficacy of tocilizumab in preventing the fatal consequences of acute respiratory and multi-organ failure associated with acute respiratory syndrome due to severe COVID-19.

At the time of launching our study (3 March 2020), many uncertainties existed about the actual benefits and risks of tocilizumab in the treatment of SARS-CoV-2 pneumonia. To increase knowledge on the actual role of tocilizumab in SARS-CoV-2 pneumonia, we conducted a retrospective multicentre cohort study. Our study aimed to describe our experience with a series of consecutive COVID-19 patients treated with tocilizumab in 18 tertiary hospitals in Spain and compare the outcomes of this cohort with those observed in a similar cohort of SARS-CoV-2 infected patients who did not receive tocilizumab to identify patients who will most benefit from its use.

METHODS

Design, Study Period, and Subjects

This was a retrospective, observational cohort study performed in 18 tertiary Spanish hospitals including patients with severe COVID-19.

The study population were adult patients ($\geq$ 18 years) with COVID-19, confirmed by PCR on nasopharyngeal swab, who were consecutively admitted to the participating hospitals between 3 March and 20 April 2020.

Patients were included consecutively according to the date of admission to hospital until the planned sample size was met in a competitive manner. Eighteen centres contributed data to both cohorts (Online Appendix Table 1). Eligible patients were hospitalised patients outside the intensive care units (ICU) with documented pneumonia (by either imaging and/or the presence of rales/crackles on physical examination) with severe respiratory failure. Severe respiratory failure was defined by presence of a Brescia-COVID Scale score of 2 (i.e. patients on oxygen therapy plus one of the following criteria: (1) the patient had dyspnoea or staccato speech at rest or after minimum activity; (2) respiratory rate > 22 with > 6 l/min O_2; (3) significant worsening of the chest x-ray) or an score of 3 (i.e. the patient required high-flow nasal ventilation, continuous positive airway pressure (CPAP), or non-invasive ventilation (NIV) because of rapid deterioration of respiratory exchanges without immediate possibility of invasive ventilation (Fig. 1) [8]; and at least one of these parameters: IL6 > 40 pg/ml, or increasing lactate dehydrogenase (LDH) or LDH > twice the upper limit of normal, increasing C reactive protein (CRP), D-dimer (DD) > 1500 ng/ml, lymphocytes < 1200/µl, or ferritin > 500 ng/ml. We excluded patients < 18 years old and those who died within 24 h after admission to hospital or after developing inclusion criteria. Of these, patients who received tocilizumab therapy according to clinical practice were assigned to the tocilizumab cohort, whilst patients who did not were assigned to the control cohort.

Brescia-COVID respiratory severity scale	
0	Ambient air
1	Oxygen therapy
2	Oxygen therapy plus 1 of the following criteria: a) The patient has dyspnoea or **STACCATO SPEECH** (inability to count rapidly to 20 after taking a deep breath) at rest or after minimum activity (sitting down on bed, standing up, speaking, swallowing, coughing) b) Respiratory rate > 22 with >6L/minute O2 c) PaO2 <65mmHg with >6L/minute O2 d) Significant worsening of chest x-ray (increased compactness and extension of infiltrate)
3	**The patient requires high-frequency nasal ventilation (HFNC), CPAP or NIV**
4	The patient is intubated for **CPAP** or pressure support
5	The patient is under controlled mechanical ventilation; PaO2/FiO2 >150 mmHg
6	The patient is under controlled mechanical ventilation; PaO2/FiO2 ≤150 mmHg
7	The patient is under controlled mechanical ventilation; PaO2/FiO2 ≤150 mmHg ***and*** intravenous infusion of neuromuscular blockers
8	The patient is under controlled mechanical ventilation; PaO2/FiO2 ≤150 mmHg ***and*** one of the following: a) Prone position b) ECMO

Fig. 1 Brescia-COVID respiratory severity scale

Data Collection

Epidemiological, clinical, pharmacological, laboratory, and radiological data were extracted from medical records using a standardised data collection form. The patients were followed according to clinical practice. Data were collected from days 1, 3, 7, 15, and 28 post-inclusion. Adverse events possibly or probably related to tocilizumab treatment were collected for treated patients. All data were included by a primary reviewer and subsequently checked by two senior physicians.

Laboratory Procedures

Routine blood examinations included a complete blood count, coagulation profile, serum biochemical tests (including LDH), CRP, DD, IL-6, and serum ferritin. Chest radiographs or computed tomography (CT) scans were also done for all inpatients.

Definition of the Outcome

The primary outcome of the study was in-hospital mortality. The outcome of patients treated with tocilizumab was compared to that of those who did not receive tocilizumab. In patients from the non-treated cohort, baseline day (day 0) was defined as the first day that the patient fulfilled the inclusion criteria established in the protocol. In patients from the tocilizumab cohort, baseline day was considered the day of initiation of treatment with tocilizumab.

Definition of the Exposure

Exposure to tocilizumab was defined as the use of intravenous tocilizumab at any time during the hospital admission. The decision to prescribe tocilizumab was at the discretion of the treating physician.

Details of tocilizumab use (including the timing of initiation, dosing, and number of doses) were recorded. Likewise, the choice of COVID-19 treatments other than tocilizumab was at the discretion of the treating physician,

although based on national and local recommendations for COVID-19 management.

For the main analysis, we generated a variable with the following mutually exclusive categories: "non-use of tocilizumab drug" (control cohort) and "use of tocilizumab drug" (treatment cohort). Subsequently, for the treatment cohort, we disaggregated the latter into two different subgroups: the 'with concomitant glucocorticoids or corticoids (steroids)' and 'no concomitant steroids' groups. Concomitant use of steroids was defined as initial use within the first 48 h after tocilizumab administration (steroid-48 h).

Statistical Analysis

Categorical variables are described with frequencies and percentages and continuous variables with mean (standard deviation, SD) and median [interquartile range (IQR): 25th–75th percentiles], and the survival function is described using the Kaplan-Meier function.

We used standardised differences, defined as differences between groups divided by pooled standard deviation to assess heterogeneity between both cohorts for baseline covariates. The inverse probability of the treatment weights (IPTW) approach [9] was used to create a pseudo-population in which the two groups were balanced across baseline covariates. The stabilised weights were calculated using propensity scores (PS) [10] obtained from a logistic regression model aimed to minimise the between arms standardised differences [11]. The baseline covariates included in the final model were gender, age, hypertension, neurological exploration, diabetes mellitus, WHO ordinal scale, time from symptoms, confirmed infection, lymphocytes, neutrophils, platelets, prothrombin activation, temperature, LDH, and baseline medication use of angiotensin converting enzyme (ACEs) inhibitors, lopinavir-ritonavir, hydroxychloroquine, corticosteroids, interferon, non-steroidal anti-inflammatory drugs, moxifloxacin, remdesivir, and azithromycin. Covariate balance was assessed using the standardised differences with the goal to achieve values < 0.10 using the IPTW to define insignificant differences in potential confounders, which was achieved by most, but not by all, baseline covariates. However, for some authors < 0.20 might also be acceptable [12, 13] and only IL-6 remained unbalanced (STD = 0.42) with no possible PS correction given the high number of missing data ($\approx$ 51%).

Since there were missing data for some key covariates needed for the PS calculations, the following variables (all with $<$ 10% missing data) were imputed using the EM algorithm, which relies on the flexible and reasonable missing at random assumption: WHO ordinal scale, time from symptoms, lymphocytes, neutrophils, platelets, haemoglobin, CRP, DD, temperature, LDH, and prothrombin activation.

Baseline categorical data were compared using the chi-square test and continuous variables using ANOVA with rank-transformed data for raw and IPTW adjusted analyses. Raw and IPTW-adjusted regression models were used to estimates risks [hazard ratio (HR) with 95%CI (95%CI)] for time-to-event variables.

In all statistical analyses, we applied a two-sided type I error of 5%. SPSS v.25 (IBM Corp., Armonk, NY) and SAS v9.4 (Cary, NC, USA) software was used.

Ethics

The study was approved by the Spanish Regulatory Authority (Spanish Agency of Medicines and Medical Devices, AEMPS) and by the Research Ethics Committee (REC) at Hospital Universitario Puerta de Hierro-Majadahonda (FIB-TOC-2020-01), and a waiver for informed consent was granted. The study protocol was submitted to the local RECs at each study site and in line with Spanish legislation; some provided their own additional approval while others did not need to undergo the full review and approval process, i.e. they recognised the initial REC approval. The study complied with the provisions in European Union (EU) and Spanish legislation on data protection and the Declaration of Helsinki 2013.

Table 1 Baseline characteristic of patients with SARS-CoV-2 infection according to tocilizumab exposure

	Raw analysis				IPTW analysis		
	Tocilizumab group (N = 268)	Control group (N = 238)	p value	Standardised difference (%)	Tocilizumab group (N = 254)	Control group (N = 235)	Standardised difference (%)
Gender (men), n (%)	184 (68.7)	140 (58.8)	0.021	− 20.6	165 (65.0)	150 (63.8)	0.9
Age, mean (SD)	65.0 (11.7)	71.3 (14.2)	< 0.0001	− 47.9	66.6 (10.7)	67.3 (14.8)	− 1.8
Underlying medical conditions, n (%)							
High blood pressure	130 (48.5)	145 (60.9)	0.001	− 25.1	132 (52.0)	126 (53.5)	− 0.4
Cardiovascular disease	65 (24.3)	74 (31.1)	0.084	− 15.3	68 (26.9)	61 (26.1)	3.5
Diabetes	78 (29.1)	68 (28.6)	0.894	1.2	75 (29.6)	66 (27.8)	5.2
Chronic kidney disease	23 (8.6)	30 (12.6)	0.140	− 13.1	23 (9.2)	23 (9.9)	− 0.9
Onco-hematologic	12 (4.5)	12 (5.0)	0.765	− 2.7	10 (3.8)	9 (4.0)	− 0.1
Chronic lung disease	46 (17.2)	49 (20.6)	0.324	− 8.8	47 (18.4)	43 (18.4)	− 0.3
Transplant (SOT/SCT)	6 (2.2)	4 (1.7)	0.652	4.0	6 (2.2)	7 (3.0)	− 4.0
Neurologic	15 (5.6)	42 (17.6)	< 0.0001	− 38.3	21 (8.2)	25 (10.4)	− 6.1
Liver disease	10 (3.7)	12 (5.0)	0.470	− 6.4	15 (5.9)	13 (5.5)	2.7
HIV	2 (0.7)	3 (1.3)	0.559	− 5.2	1 (0.5)	2 (0.7)	− 1.9
NSAIDs	19 (7.1)	18 (7.6)	0.838	− 1.8	22 (8.6)	16 (6.7)	8.5
ACE inhibitors	53 (19.8)	40 (16.8)	0.389	7.7	47 (18.4)	41 (17.4)	3.4
ARBs	49 (18.3)	56 (23.5)	0.146	− 12.9	61 (23.8)	50 (21.0)	8.9
Treatment, n (%)							
Hydroxychloroquine	262 (97.8)	220 (92.4)	0.004	24.9	247 (97.1)	223 (94.8)	11.0
Lopinavir/Ritonavir	240 (89.6)	164 (68.9)	< 0.0001	52.6	206 (81.0)	180 (76.3)	10.0
Azithromycin	163 (60.8)	132 (55.5)	0.222	10.9	150 (59.0)	138 (58.4)	− 2.5
Remdesivir	1 (0.4)	1 (0.4)	0.932	− 0.8	1 (0.4)	1 (0.4)	0.5
Interferon	124 (46.3)	72 (30.3)	< 0.0001	33.4	105 (41.2)	92 (39.0)	1.3

Table 1 continued

	Raw analysis				IPTW analysis		
	Tocilizumab group (*N* = 268)	**Control group (*N* = 238)**	***p* value**	**Standardised difference (%)**	**Tocilizumab group (*N* = 254)**	**Control group (*N* = 235)**	**Standardised difference (%)**
Steroids							
Steroids prior to D0	87 (32.5)	26 (10.9)	< 0.0001	54.1	61 (23.8)	51 (21.5)	− 1.2
WHO classification D0							
Admitted, no oxygen therapy	5 (1.9)	5 (2.1)	0.981	0.6	4 (1.6)	4 (1.6)	1.9
Admitted with oxygen therapy	244 (91.0)	216 (90.8)	0.981		234 (91.9)	217 (92.3)	
High flow	19 (7.1)	17 (7.1)	0.982		16 (6.5)	14 (6.1)	
Laboratory values, mean (SD)							
Lymphocytes (× 10E3/mm^3)	834.3 (469.7)	902.9 (580.9)	0.235	− 13.0	843.3 (420.5)	854.1 (501.8)	− 0.6
Lactate dehydrogenase (UI/l)	451.4 (186.2)	459.8 (339.8)	0.361	− 3.1	448.8 (179.6)	463.16 (288.2)	− 9.1
D-dimer (ng/ml)	2200.2 (5216.8)	1950.9 (2588.7)	0.334	6.1	2040.8 (4412.3)	1869.9 (2553.4)	− 1.5
C-reactive protein (mg/l)	149.5 (105.7)	148.0 (111.1)	0.584	1.4	148.54 (95.64)	146.0 (105.9)	4.6
Ferritin (ng/ml)	1682.6 (1427.9)	1363.7 (2953.5)	< 0.0001	13.7	1697.1 (1388.3)	1532.0 (2794.2)	− 0.6
Interleukin 6 (IL-6) (pg/ml)	206.5 (468.5)	78.8 (94.9)	< 0.0001	37.8	195.4 (375.2)	80.3 (93.7)	42.4
Chest CT (at hospital admission), *n* (%)							
Normal imaging	4 (1.6)	9 (4.1)	0.192		3 (1.3)	7 (3.2)	
Unilateral pneumonia	18 (7.3)	26 (11.9)			17 (7.2)	25 (12.3)	
Bilateral interstitial pneumonia	66 (26.9)	58 (26.6)			65 (27.7)	54 (24.7)	
Patchy bilateral pneumonia	100 (40.8)	77 (35.3)			91 (38.7)	80 (36.5)	
Confluent bilateral pneumonia	57 (23.3)	48 (22.0)			59 (38.7)	61 (23.3)	

Table 1 continued

	Raw analysis				IPTW analysis		
	Tocilizumab group ($N = 268$)	Control group ($N = 238$)	p value	Standardised difference (%)	Tocilizumab group ($N = 254$)	Control group ($N = 235$)	Standardised difference (%)
Tocilizumab treatment, n (%)							
Initial tocilizumab dose (mg)	532.4 (109.5)				538.8 (110.1)		
Cumulative tocilizumab dose (mg)	789.2 (354.8)				827.9 (366.1)		
Time from onset of symptoms to D0, mean (SD)	11.73 (5.20)	8.43 (4.67)	< 0.0001	66.7	10.41 (4.71)	10.12 (5.42)	− 0.2

RESULTS

During the study period, 506 consecutive patients with COVID-19, respiratory insufficiency, and increased inflammatory parameters were included. Among them, 268 were treated with tocilizumab and 238 patients were not. Twenty-four patients who died in the first 24 h after developing inclusion criteria were excluded.

Baseline Clinical Characteristics

Characteristics of both cohorts are shown in Table 1. Median time to tocilizumab from the onset of symptoms was 11 days (IQR 8–14 days).

Among 268 patients treated with tocilizumab, 22 (8.2%) received 3 doses, 92 (34.3%) received 2 doses, and the remaining 154 (57.4%) received only one dose. Median initial tocilizumab dose was 600 mg (IQR 400–600). Median time from first dose to second dose was 1 day (IQR 1–1) and from first dose to third dose was 2 days (IQR 1–3). Median cumulative dose of tocilizumab was 600 mg (IQR 600–1000).

A PS was developed to estimate each patient's probability of receiving tocilizumab given their baseline characteristics and reduce selection bias. Characteristics of the treatment and control cohort after adjustment by IPTW are shown in Table 1.

In-Hospital Mortality of Patients Treated with Tocilizumab Compared to Patients Not Treated with Tocilizumab

Global hospital mortality was 23.7%. Characteristics of survivors and non-survivors are shown in Online Appendix Table 2.

Mortality was lower in patients treated with tocilizumab than in controls [16.8% versus 31.5%, HR 0.514 (95% CI 0.355–0.744), $p < 0.001$] (Table 2). Tocilizumab treatment reduced mortality by 14.7% relative to no tocilizumab treatment (RRR 46.7%). We calculated a number needed to treat of 7. Differences in mortality persisted after applying the PS adjusted for tocilizumab treatment [weighted HR 0.741 (95% CI 0.619–0.887), $p = 0.001$]

Table 2 Association between tocilizumab treatment and mortality in patients with SARS-COV-2 infection, according to tocilizumab and steroid-48 h exposure

All	Number of events		Ratio		IPTW ratio	
	Tocilizumab, *N* = 268	No tocilizumab, *N* = 238	HR (95% CI)	*p* value	wHR (95% CI)	*p* value
Deaths	45 (16.8%)	75 (31.5%)	0.514 (0.355–0.744)	**< 0.001**	0.741 (0.619–0.887)	***0.001***
Steroid-48 h	*N* = 119	*N* = 87				
Deaths	13 (10.9%)	35 (40.2%)	0.511 (0.352–0.741)	**< 0.001**	0.600 (0.449–0.804)	***0.006***
No steroid-48 h	*N* = 149	*N* = 151				
Deaths	32 (21.5%)	40 (26.5%)	0.713 (0.447–1.137)	0.345	0.830 (0.650–1.048)	0.115

HR hazard ratio, *IPTW* inverse probability of treatment weighting, *wHR* weighted hazard ratio, *steroid-48 h* treated with steroids in the 48 h following inclusion

*Weighted hazard ratios and 95% confidence intervals were obtained by inverse probability treatment weighting

(Table 2). Figure 2 demonstrates differences in the probability for survival for patients with SARS-CoV2 infection, according to tocilizumab treatment (log-rank $p < 0.001$).

Tocilizumab Effect in Subgroups According to Baseline Characteristics

The effect of tocilizumab treatment on mortality in different subsets of patients was consistent with a protective effect. Figure 3 shows different effects of tocilizumab according to different values of variables that displayed significant interaction. Patients > 65 years old with lymphocyte counts < 1000 cells/μl, hypertension, and cardiovascular disease seem to benefit the most from tocilizumab treatment.

Effect of Tocilizumab in Patients with Post-Baseline Steroid Treatment

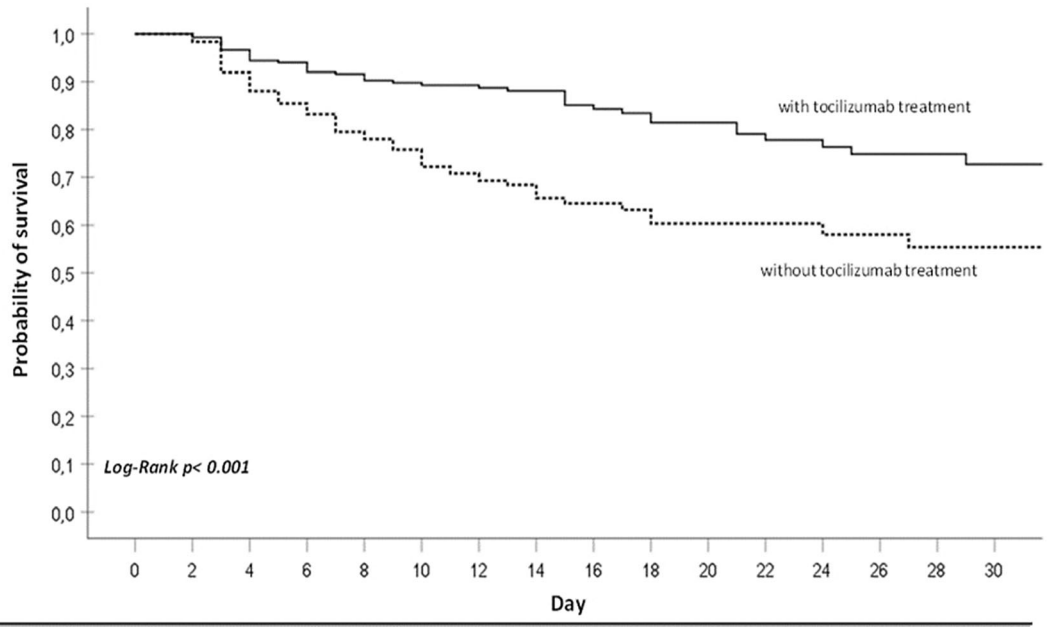
One-hundred nineteen (44.4%) patients in the tocilizumab arm and 87 (36.6%) in the control arm were treated with steroids in the 48 h following inclusion (steroid-48). Among them 71.3% patients received steroids in pulses, and 28.7% non-pulsed steroids. Characteristics of patients according to tocilizumab and steroid-48 h exposure are shown in Online Appendix Table 3.

An interaction was found between tocilizumab and steroid-48 h use (interaction $p = 0.094$). Among patients treated with steroid-48 h, mortality was lower in those treated with tocilizumab than in those treated with steroid-48 h alone [10.9% vs. 40.2%, HR 0.511 (95% CI 0.352–0.741), $p = 0.036$; weighted HR 0.6 (95% CI 0.449–0.804), $p < 0.001$] (Table 2).

Tocilizumab with steroid-48 h treatment reduced mortality by 29.1% relative to no tocilizumab treatment (RRR 72.8%). We calculated a number needed to treat of 4. Figure 4 shows differences in probability of survival according to steroid exposure in patients treated with tocilizumab.

Adverse Events

Adverse events were monitored during the study period in the tocilizumab group. Although up to 32.6% in the tocilizumab group compared to 30.3% in the control group had an increase in SGOT/AST above the upper limit of normal, there were no significant differences among the study groups. A total of 11 (4.1%) patients in the tocilizumab group had serious adverse reactions, including hepatotoxicity with increased liver enzymes (3 patients) or bilirubin (2 patients), thrombocytopenia (1 patient), catheter-related superficial thrombophlebitis (2 patients),



Patients at Risk																
Days	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Tocilizumab	268	266	245	220	192	172	144	118	95	77	69	54	51	41	35	25
No Tocilizumab	238	231	202	179	143	104	80	60	47	34	30	26	24	22	14	14

Fig. 2 Probability of survival of patients with SARS-COV-2 infection according to tocilizumab exposure. Descriptive raw analysis

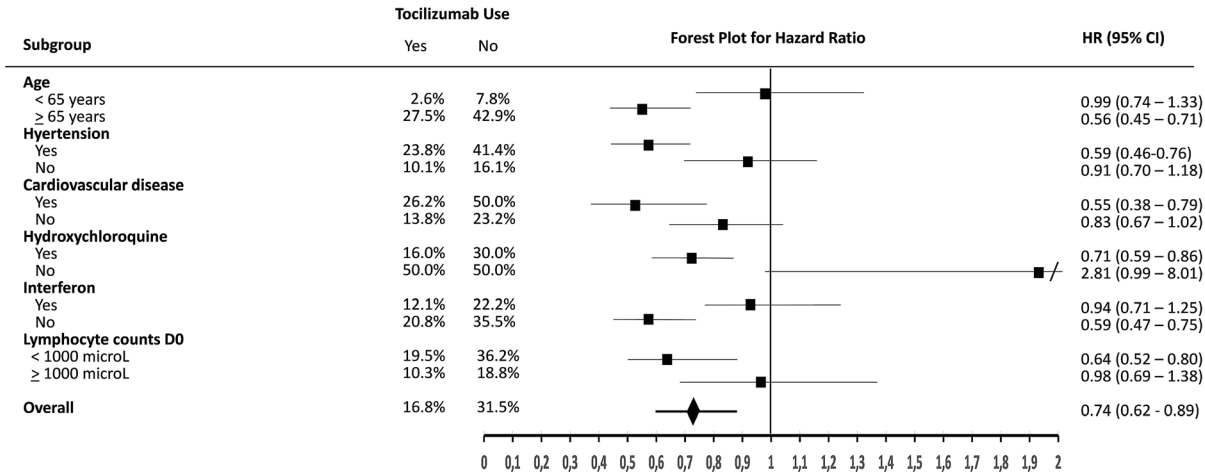
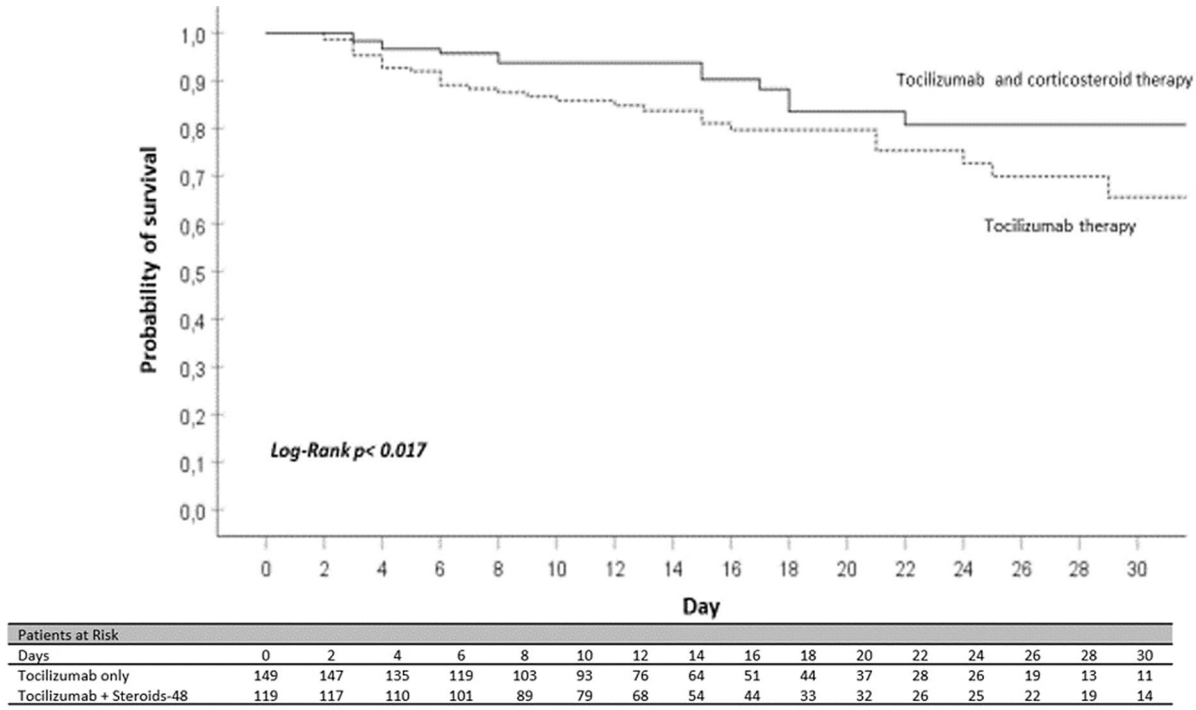


Fig. 3 Forest plot of stratified analyses for mortality showing the weighted hazard ratio from IPTW analysis of tocilizumab treatment

diarrhoea (1 patient), and headache and ocular phosphenes (1 patient). Bacteraemia without a source after tocilizumab administration was documented in one patient (0.4%). Median follow-up time was 12 days (7–18 days).

DISCUSSION

Our results show that survival of patients with severe COVID-19 is significantly higher in patients treated with tocilizumab than in those



Patients at Risk																
Days	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Tocilizumab only	149	147	135	119	103	93	76	64	51	44	37	28	26	19	13	11
Tocilizumab + Steroids-48	119	117	110	101	89	79	68	54	44	33	32	26	25	22	19	14

Fig. 4 Probability of survival of patients with SARS-COV-2 infection treated with tocilizumab according to steroid-48 h exposure. Descriptive raw analysis

not treated. In particular, mortality is reduced when tocilizumab is used concomitantly or shortly followed (first 48 h) by steroids. These results come from a large cohort of 506 consecutive patients with COVID-19 pneumonia, respiratory insufficiency, and increased inflammatory parameters recruited across 18 hospitals from different regions in Spain. Of these, 268 were treated with tocilizumab whilst 238 patients were not. These results are considered clinically relevant as they contribute to the growing body of evidence supporting the use of tocilizumab in SARS-CoV-2 infection.

Our data are consistent with previously published series that report a reduction in the risk of death in patients with severe COVID-19 treated with tocilizumab compared to standard of care. Most of these are observational studies, some of them comparing tocilizumab use with a control group [14, 15, 16].

At the time of planning our study, results from randomised controlled trials had not yet been published. Recently, the results of the first randomised, open label, controlled trial of tocilizumab versus standard of care have been published [17]. The study was prematurely interrupted because of futility (and recruitment difficulties) with one third of the initially planned study population. As recognised by the authors, this small open-label clinical trial still leaves important questions unanswered on the potential role of tocilizumab in the treatment of COVID-19.

Recently, steroid treatment has shown promise as a life-saving therapy in patients with COVID-19 [18, 19], and, indeed, dexamethasone has been granted EU regulatory approval for the treatment of COVID-19 patients on oxygen or mechanical ventilation, becoming the standard of care in clinical practice. Therefore, when analysing the effect of new treatments, it is important to consider the combined use of steroid therapy.

Prior studies do not report specifically on the combined effect of tocilizumab and post-baseline steroids, although in some of them steroids have been used concomitantly with tocilizumab in a considerable proportion of cases [17%

standard of care (SoC) and 30% tocilizumab] [14], close to 100% in some cases [20, 21]. Contrarily, in the only randomised clinical trial concomitant use was not allowed unless patients were on chronic treatment. A single-centre Italian study jointly analysed data regarding the administration of both tocilizumab and steroids, together or separately, and suggested the need for evaluating combined use of these therapies [22]. More recently, Narain et al. published the results of a retrospective cohort study and concluded that the combination of corticosteroids with tocilizumab showed superior survival outcome compared with SoC treatment and treatment with corticosteroids alone or in combination with anakinra [23]. These results are consistent with our findings.

The timing of steroid administration in relation to tocilizumab treatment might be decisive. A small non-controlled Spanish cohort study suggests that patients treated with prior or concomitant steroid therapy had better survival results than those with steroids added at a later stage [20]. Another short observational single-centre study shows an improvement in mortality in patients treated with tocilizumab when salvage steroids are added in a median of 2.3 days [24]. In our study, tocilizumab adds to steroid administered to non-intubated cases with severe COVID-19 during the first 48 h of presenting with respiratory failure despite oxygen therapy (Brescia-COVID respiratory severity scale 2 or worse).

The severity of the study population that could most benefit from treatment remains to be determined. Some authors suggest that the administration of tocilizumab is more effective in severe cases (PaO2/FiO2 < 150) [14], while others report better results in cases with less oxygen requirement [21].

In our study, tocilizumab showed an added benefit to that of steroids in reducing mortality of severe COVID-19. The major strength of this study was its multicentric nature, with a large number of patients included from different regions in Spain. The study also included a concurrent comparison with a non-treated cohort. The IPTW to adjust for indication bias adds to the strength of the results.

However, this is an observational study and, as such, has the inherent limitations of this kind of study. Indication bias for steroid treatment was not adjusted for. It is possible that steroid treatment was not evenly administered across the study population. This could possibly explain the high mortality in the subset of patients who received steroid-48 h, but not tocilizumab.

The short follow-up time prevented us from detecting important adverse events such as reactivation of latent infections in patients treated with tocilizumab, particularly in those treated with tocilizumab and steroids. Thus, safety concerns might have been underestimated. To overcome this problem, ongoing registries have been put in place to identify medium-term complications.

Although our cohort was limited to non-ICU patients, tocilizumab's effect on critical patients who already require mechanical ventilation or ICU admission deserves further investigation. One recent article by Somers et al. [25] suggests that tocilizumab in ICU patients is also beneficial, although the risk of infection is high.

CONCLUSION

Our study results show that survival of patients with severe COVID-19 is higher in patients that received tocilizumab as part of the standard of care than in those who did not. The benefits appear higher when tocilizumab is given concomitantly with steroids, mostly when given within the first 48 h of presenting with respiratory failure despite oxygen therapy. Nevertheless, relevant questions remain unanswered. Large, randomised, controlled studies are needed to convincingly establish the efficacy and safety of tocilizumab in the treatment of COVID-19.

ACKNOWLEDGEMENTS

We thank the members of the TOCICOV study group for their contribution to the work.

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Funding. No funding or sponsorship was received for this study or publication of this article. The Rapid Service Fee was funded by the authors.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Authorship Contribution. Conceptualization and study design: ASL, BRA, AFC. Methodology: BRA, AFC, ASL, F-T. Data collection: all authors. Data interpretation: ASL, BRA, AFC, FT. Writing first draft: ASL, BRA, AFC. Critical revision for important intellectual content: all authors. Final approval: All authors. All authors agree to be accountable for all aspects of the work by ensuring that questions related to the accuracy or integrity of any part of the work will be appropriately investigated and resolved. AFC and BRA had full access to all the data in this study and take complete responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. This manuscript is based on work that has been previously presented at medRxiv, posted 10 September 2020 (https://www.medrxiv.org/content/10.1101/2020.09.07.20189357v2).

Disclosures. Belén Ruiz-Antorán, Aránzazu Sancho-López, Ferrán Torres, Víctor Moreno-Torres, Itziar de Pablo-López, Paulina García-López, Francisco Abad-Santos, Clara M. Rosso-Fernández, Ana Aldea-Perona, Eva Montané, Ruth M. Aparicio-Hernández, Roser Llop-Rius, Consuelo Pedrós, Paloma Gijón, Carolina Hernández-Carballo, María J Pedrosa-Martínez, Consuelo Rodríguez-Jiménez, Guillermo Prada-Ramallal, Lourdes Cabrera-García, Josefa A. Aguilar-García, Rocío Sanjuan-Jimenez, Evelyn

I. Ortiz-Barraza, Enrique Sánchez-Chica and Ana Fernández-Cruz declare no conflicts of interest.

Compliance with Ethics Guidelines. The study was approved by the Spanish Regulatory Authority (Spanish Agency of Medicines and Medical Devices, AEMPS) and by the Research Ethics Committee (REC) at Hospital Universitario Puerta de Hierro-Majadahonda (FIB-TOC-2020-01), and a waiver for the informed consent was granted. The study protocol was submitted to the local RECs at each study site and in line with Spanish legislation, some provided their own additional approval while others did not need to undergo the full review and approval process, i.e. they recognised the initial REC approval. The study complied with the provisions in European Union (EU) and Spanish legislation on data protection and the Declaration of Helsinki 2013.

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

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Major determinants of death in patients hospitalized with COVID-19 during the first epidemic wave in Madrid, Spain

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Abstract

Spain is one of the European countries most largely affected by COVID-19, being Madrid the epicenter. A good knowledge of the main features of hospitalized patients during the complete lockdown should improve the management of new COVID-19 surges.

All patients hospitalized at one large tertiary hospital in Madrid for suspected COVID-19 pneumonia from March 1 to May 31 were retrospectively identified.

A total of 1752 patients were admitted with suspected pneumonia due to SARS-CoV-2 infection during the 3-month study period. The peak of daily admissions (n = 84) was reached on March 24, whereas the maximal cumulative number of hospitalized patients (n = 626) occurred on March 30. Overall, 85.3% had a positive PCR test for SARS-CoV-2 at least once during admission. Their median age was 65 (54–77) and 59.9% were male. The median length of hospitalization was of 7 (4–13) days. Roughly 6.5% were admitted at the intensive care unit.

Death occurred in 242 (13.8%). Overall, 75% of deaths occurred in patients older than 75 years-old. It was 38.2% in patients hospitalized older than 80 years-old versus 2.2% in patients younger than 60 years-old ($p < 0.001$). Up to 94 (38.8%) of deceased patients had been transferred from nursing homes. The median Charlson co-morbidity score was 6 in deceased patients.

The in-hospital mortality rate during the first wave of COVID-19 in Madrid was 14%. It was largely driven by older age, the presence of underlying chronic conditions (≥2) and living at nursing homes.

Abbreviations: CCI = Charlson co-morbidity Index, ICU = intensive care unit, IQR = interquartile range.

Keywords: coronavirus, COVID-19, mortality, pneumonia, SARS-CoV-2, Spain

1. Introduction

A novel human coronavirus, SARS-CoV-2, that causes pneumonia and other complications named as COVID-19 was first reported in Wuhan, China, at the end of 2019.[1] During the first trimester of 2020, worldwide spreading occurred and in March the WHO considered COVID-19 as pandemic. To date, more than 14 million confirmed cases have been reported globally, with nearly 600,000 deaths.[2]

Spain has been one of the European countries most largely affected by COVID-19.[3] The first death caused by COVID-19 was reported in Valencia on February 13. Rapid increases in local and community transmissions were soon recognized in large cities, such as Madrid and Barcelona. Complete nationwide lockdown measures were implemented on March 14th in order to reduce viral transmission and avoid the collapse of the health care system. The confinement was extended during 15 days and dragged on during Eastern until April 13th. The plan for easing lockdown restrictions begun on May 8th. By then, a nationwide epidemiological survey reported an overall 5% seroprevalence rate, although it was above 11% in Madrid.[4,5]

The region of Madrid has a population of 6.6 million and was particularly hit by COVID-19, with an official count of 79,884 cases reported up to June 30th with 9,193 deaths.[6] During the peak of the pandemic wave, hospital admissions due to COVID-19 collapsed most hospitals in the city,[3] very much resembling

Editor: Nesreen E. Morsy.

This work was funded in part by grants from ISCIII-Fondos Feder (CM19/00223; and CES12/003).

The authors have no conflicts of interest to disclose.

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

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How to cite this article: Moreno-Torres V, de la Fuente S, Mills P, Muñoz A, Muñez E, Ramos A, Fernández-Cruz A, Arias A, Pintos I, Vargas J, Cuervas-Mons V, de Mendoza C. Major determinants of death in patients hospitalized with COVID-19 during the first epidemic wave in Madrid, Spain. Medicine 2021;100:16(e25634).

Received: 25 September 2020 / Received in final form: 4 December 2020 / Accepted: 30 March 2021

http://dx.doi.org/10.1097/MD.0000000000025634

what occurred in Milan and New York city.[7,8] Information and knowledge derived from those days, in particular at reference hospitals, might help to preparedness for confronting new surges of SARS-CoV-2 infection. Herein, we analyze COVID-19 hospital admissions and deaths at one large tertiary hospital located in northern Madrid during the complete lockdown and the total period of the stay-home policy.

2. Patients and methods

This retrospective study included all patients initially attended at the emergency unit with suspected COVID-19 pneumonia that subsequently were admitted at the Puerta de Hierro-Majadahonda University Hospital from March 1 to May 31, 2020. This is a large tertiary university hospital located in Northwestern Madrid. Its catchment population approaches 375,000 persons, of whom 69% are aged 14 to 64 years-old. Overall, 13% are above 65 years-old. The health district includes 69 nursing homes that offer a total of 6,755 places. The hospital has 613 beds in individual rooms, including 22 intensive care unit (ICU) beds, and all medical and surgical specialties.

Our study was approved by the hospital review board (PI134–20) and consent was requested for all patients to include their clinical information within a database for epidemiological and clinical studies.

We reviewed electronic medical records and laboratory results for all hospital admitted patients with COVID-19. The main demographics (gender and age), admission to ICU, and diagnosis discharge (including death if so) were recorded for each subject. Deceased patients were subject to particular examination. The case definition for COVID-19 was made following the ECDC criteria.[9] In more detail, clinical criteria included at least one of the following symptoms: cough, fever, shortness of breath, sudden onset of anosmia, and ageusia or dysgeusia. Diagnostic imaging criteria included radiological evidence showing pulmonary infiltrates consistent with COVID-19. Lastly, laboratory criteria included the detection of SARS-CoV-2 nucleic acids in clinical specimens, generally nasopharyngeal and/or oropharyngeal swabs, following WHO protocol. All individuals with clinical symptoms of COVID-19 and compatible imaging criteria were considered as COVID-19 cases despite negative PCR.

For all deceased COVID-19 patients, the presence of co-morbidities was recorded, including arterial hypertension, dyslipidemia, diabetes, chronic kidney disease, cancer, neurocognitive impairment (Alzheimer, other dementias and other mental disorders), heart disease, and chronic obstructive pulmonary disease. The baseline Charlson co-morbidity Index (CCI) was used to estimate the risk of mortality, taking into consideration that the overall 10-year survival rate is 98.3% in patients with a CCI = 0, goes to 77.48% in those with a CCI of 3, and drops to 21.36% in those with a CCI of 5 or more.[10]

2.1. Statistical analysis

Continuous variables were expressed as mean and standard deviations (SD), and medians with interquartile ranges (IQR). Categorical variables were summarized as counts and percentages. Patients were grouped by range age and by median age. Age groups were stratified into 4 groups, as follows: 0 through 59 years; 60 through 69 years; 70 through 79 years; and ≥80 years. Time-to-events were measured in days from the date of hospital admission to the date of hospital discharge or in-hospital death.

The Student *t* test was used for the comparison of continuous variables and the Wilcoxon-Mann-Whitney tests was used for non-parametric variables. Categorical variables were compared using the chi-square test. All analyses were performed using the SPSS version 20.0 software statistical package (SPSS Inc., IBM, Armonk, NY).

3. Results

Between March 1 and May 31, 2020, a total of 1752 patients were hospitalized with suspected COVID-19 pneumonia. Overall, 85.3% of COVID-19 cases had a positive SARS-CoV-2 PCR at least once during admission.

The peak of daily admissions was reached on March 24, with 84 patients. The highest cumulative number of hospitalizations with COVID-19 was reached on March 30, with 626 patients (Fig. 1). It should be noted that hospital beds were doubled (two per room) once it became apparent that the emergency unit was overwhelmed by the continuous arrival of COVID-19 patients.

One month after lockdown begun, the number of hospitalizations fell to figures similar to those recorded at the time the confinement started. Hospital admissions due to COVID-19 reached the lowest levels at the end of April, and since then only sporadic admissions occurred until the end of May.

Overall, 59.9% of admitted patients were male. The median age (IQR) was 65 (54–77). There were no statistical significance differences between positive and negative SARS-CoV-2 PCR individuals for any of the demographics, clinical, radiological and laboratory parameters analyzed. Patients were hospitalized during a median (IQR) length of 7 days (4–13). A total of 116 (6.6%) required admission at the ICU. In this group of patients, the median (IQR) length of hospitalization extended to 36 (22–51) days.

Overall 648 (37%) of COVID-19 hospitalized patients were younger than 60 years-old. The median length of hospitalization was significantly shorter in this group compared to older patients (Table 1). Furthermore, only 6.6% of the younger COVID-19 patient group required admission at ICU, whereas it rose to 10% in the rest ($P < .001$).

Death occurred in 242 (13.8%) of COVID-19 hospitalized patients. The main predictor of in-hospital mortality was older age. The death rate in the younger group was only 2.2%, whereas it rose up to 38.2% in those older than 80 years-old ($P < .001$). Overall 75% of deaths occurred in patients older than 75 years-old.

The median age of deceased patients was 82 (73–87), being 58.7% male. Overall, 10.3% of deaths (25 patients) occurred in patients that had been admitted at the ICU. The median length of hospitalization before death was 6 (3–14) days, being significantly shorter in older than younger patients (Table 2). Overall 38.8% of deaths occurred in patients that were living at nursing homes before hospitalization. It should be noted that 28.6% of COVID-19 deaths in the younger group corresponded to institutionalized individuals, many of them suffering from serious conditions, mostly neurocognitive impairment, cancer and/or lung disease.

Among COVID-19 deceased individuals, a median of 2 (1–3) comorbidities were present before hospitalization, being the most prevalent: hypertension (62.2%), dyslipidemia (38.8%), heart disease (37.6%), diabetes (28.1%), cancer (20.2%), neurocognitive impairment (16.9%), lung disease (12.4%) and chronic kidney insufficiency (11.2%). As expected, hypertension,

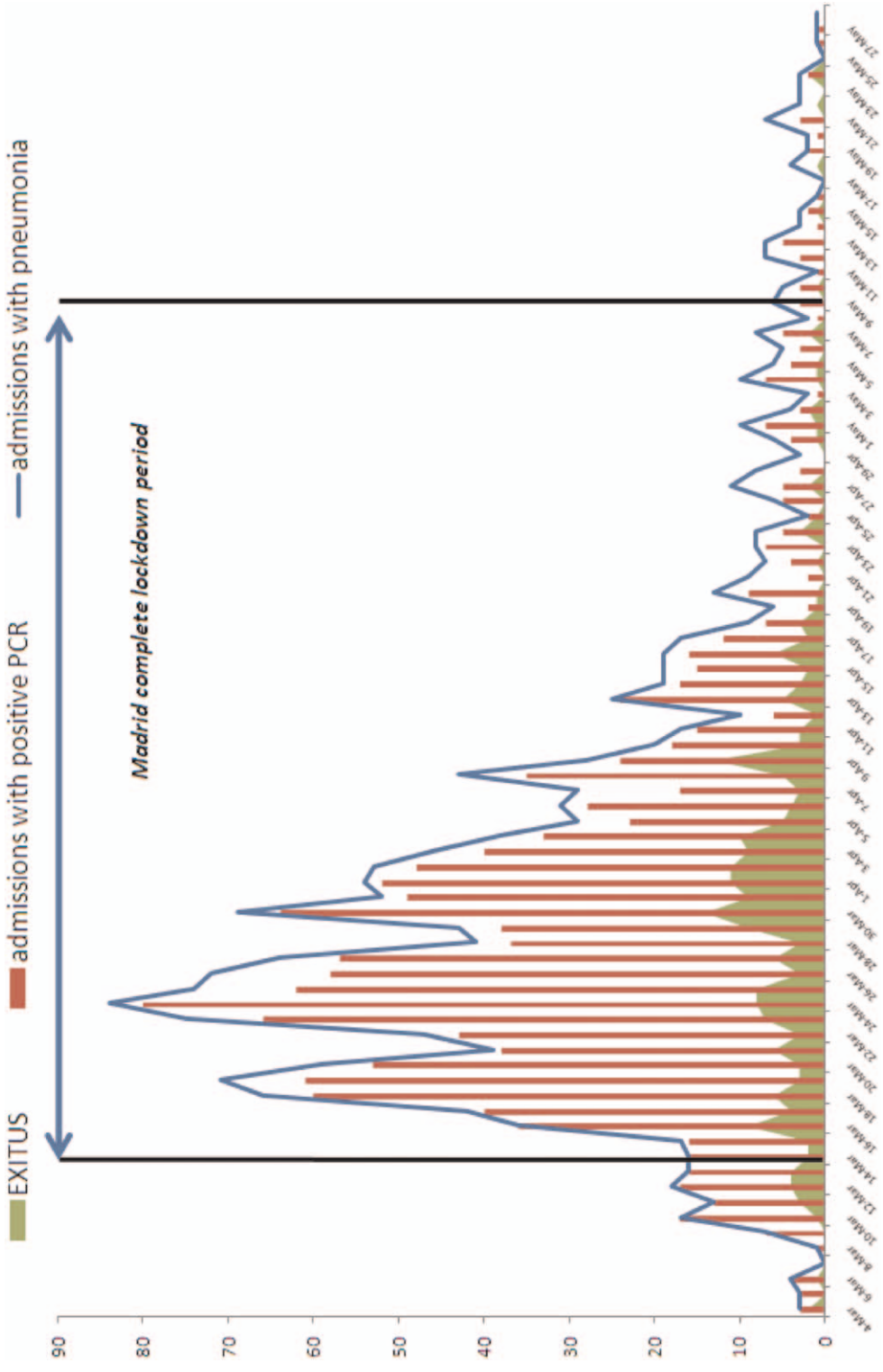

Figure 1. Daily hospital admissions with suspected COVID-19 from March 1st to May 31st at a tertiary Hospital (Madrid, Spain).

Table 1

Main characteristics of patients hospitalized with COVID-19 according to age.

		Age range (yr-old)				
	Total	**<60**	**60-69**	**70-79**	**≥80**	***P***
N (%)	1752	648 (37)	388 (22.1)	355 (20.3)	361 (20.6)	–
Male, N (%)	1050 (59.9)	397 (61.3)	252 (64.9)	222 (62.5)	179 (49.6)	<.01
SARS-CoV-2 PCR positive, N (%)	1495 (85.3)	554 (85.5)	347 (89.4)	307 (86.5)	287 (79.5)	ns
Median length of hospital admission (d) (IQR)	7 (4–13)	6 (3.75–10)	9 (5–15)	10 (6–16)	7 (4–13)	<.01
Intensive Care Unit, N (%)	116 (6.6)	43 (6.6)	35 (9.0)	38 (10.7)	–	<.001
Death, N (%)	242 (13.8)	14 (2.2)	26 (6.7)	64 (18)	138 (38.2)	<.001

IQR = interquartile range; ns = no significant.

dyslipidemia and heart disease were significantly more frequent in older than younger COVID-19 deceased patients (Table 2). Overall, the median Charlson co-morbidity index was 6, being 3 in the younger group and 7 in the older group. Unfortunately, body mass index was not recorded regularly in all hospitalized patients during the first months of COVID-19 pandemic. For this reason, it was not possible to evaluate this factor, which has been associated with higher mortality in previous studies.[11]

4. Discussion

The arrival of the first COVID-19 wave in Spain was very abrupt, with rapid surges in large cities such as Madrid. During the week that followed the nationwide lockdown on March 14th, the health system became stressed and overwhelmed as never before.[3] High pressure with more than 30 new daily hospitalizations was sustained for one month, with a peak of more than 80 admissions per day. The number of deaths due to COVID-19 rose with peaks of more than 10 per day, with an average of two weeks of delay after hospital admission. This unprecedented scenario was accompanied by a complete switch of allocation resources, doubling the number of beds per room and expanding and converting spaces as ICU and intermediate care units. Almost all available physicians and nurses were assigned to care for COVID-19 patients. Schedules were forced and protective equipment was often scarce. The hit was tremendous and encouraged us to assess what could we learn from this unique experience. In this study we aimed to identify the most relevant information that could be used for an adequate preparedness in case of new COVID-19 surges.

During the whole 3-month study period, a total of 1752 COVID-19 patients were admitted at our hospital. Infection with SARS-CoV-2 was confirmed using PCR in more than 85%. Clinical parameters as well as outcomes did not differ for the remaining 15% of suspected COVID-19 pneumonia cases. False negative PCR results have been reported occasionally, mainly as result of poor sampling or too long symptom deferral.[12,13] During the first days of the pandemic, we excluded influenza, syncytial respiratory virus and other potential etiological respiratory agents in the subset of non-confirmed but suspected COVID-19 cases.

The median age of patients hospitalized with COVID-19 was 65 years-old and 58% were male. Only 38% had less than 60 years of age. In the younger group of patients, most admissions were short with a median of 6 days, and only 6.5% required transfer to the ICU. Thus, critically ill COVID-19 patients younger than 60 years-old are rare. Indeed, the overall fatality rate in this group was low (2.2%) and mainly occurred in patients with serious pre-existing conditions and co-morbidities. Similar findings have been highlighted by others.[14–17] Therefore, gaps in knowledge about the coronavirus infection early on during the first days of the pandemic could have led to avoidable hospital admissions that would have benefited from adequate home care. In the future, a distinct consideration for younger patients with COVID-19 could relieve emergency units and hospital wards.

Table 2

Main characteristics of deceased patients with COVID-19 according to age.

		Age range (years-old)		
	Total	**≤ 65**	**> 65**	***P***
N	242	21	219	–
Male, N (%)	141 (58.3)	13 (61.9)	88 (40.2)	.06
Admission length (d) (median, IQR)	6 (3–14)	15 (6–38)	6 (3–11)	.002
Prior living at nursing homes (%)	94 (38.8)	6 (28.6)	88 (40.2)	ns
Charlson Co-morbidity Score (median, IQR)	6 (5–8)	3 (2.5–5.5)	7 (5–8)	<.001
o-morbidities (median, IQR)	2 (1–3)	1 (0–2)	2 (1–3)	<.001
Hypertension, N (%)	150 (62)	3 (14.3)	147 (67.1)	<.001
Dyslipidemia, N (%)	94 (38.8)	3 (14.3)	91 (41.5)	.018
Diabetes, N (%)	68 (28.1)	5 (23.8)	63 (28.8)	ns
Heart disease, N (%)	91 (37.6)	2 (9.5)	94 (42.9)	.004
Chronic kidney disease, N (%)	27 (11.2)	1 (4.8)	26 (11.9)	ns
Cancer, N (%)	49 (20.2)	3 (14.3)	46 (21)	ns
Lung disease, N (%)	30 (12.4)	5 (23.6)	25 (11.4)	.15
Neurocognitive impairment, N (%)	41 (16.9)	4 (19)	37 (16.7)	ns

IQR = interquartile range; ns = no significant.

The overall mortality rate at our hospital was 13.8%. By far, older age was the major determinant of death, rising up to 38.8% in the subset of patients above 80 years-old. Interestingly, higher mortality rates (28%) have been reported for COVID-19 patients admitted at other hospitals located in the Madrid region as well at other Spanish hospitals during the same period.[18,19] A recent study from 92 US hospitals reported 23% of deaths among 11,210 hospitalized adults with COVID-19.[20] Possible causes of this discrepancy may include the rapid initiation and broader use of corticosteroid therapy at our institution. In a recent report from our hospital,[20] patients received steroid treatment within a median of 10 days after the symptom onset, presumably during the inflammatory surge of the disease. Indeed, the survival of patients with SARS-CoV-2 pneumonia was significantly higher in the group that received corticoids compared to the rest.[21] In addition, a significant number of severely ill COVID-19 patients were treated with tocilizumab (data not shown), a monoclonal interleukin 6 blocker that recently has been shown to reduce mortality up to 45% in the subset of COVID-19 patients under mechanical ventilation.[22] Furthermore, early planning and the possibility for doubling the number of beds per room delayed the early overload of wards and ICUs, contributing to attenuate mortality rates. Finally, the health district covered by our hospital is the wealthiest in Madrid, with a disproportionate representation of patients with high socioeconomic status, hygiene and cleaning, homes with large spaces and garden areas, all of which could have contributed to infections with low inoculums and potentially less severe COVID-19 disease episodes.[23]

It is noteworthy that more than 80% of deaths occurred in individuals with at least one serious underlying illness, such as cancer, heart disease, diabetes and/or other metabolic diseases. In this sense, the median CCI of deceased patients index was 6, with an estimated 10-year survival of 2.25%, reflecting their fragility, comorbidities and low life expectancy. This observation is in agreement with data from China, Italy and the United States, reflecting the linkage between underlying cardiovascular conditions and COVID-19 severity.[24–26]

Finally, and even though there was very important medical and nursery support mobilized from the hospital to the nursing homes that managed the epidemic in these centers, it must be pointed out that 40% of deceased patients with COVID-19 at our hospital had been transferred from there. This was the case not only for the elderly population but also for a significant proportion of younger COVID-19 patients. The rapid spread of the coronavirus among residents within communities has been associated with close contact and prolonged viral exposure.[23,27] Given that the population living in nursing homes is extremely vulnerable due to COVID-19 as result of its dependency, older age and associated co-morbidities, it seems worthy to work providing adequate medicalization and improved protective procedures to reduce harm in these settings to minimize harm facing future COVID-19 surges.

All knowledge drawn from the first COVID-19 wave should encourage to build more efficient measures for reducing harm during a second wave of COVID-19 during fall or anytime. First, hospitals with the capacity to expand the number of beds and staff would be more prepared to contain the stressful flow of patients on need for hospitalization, particularly when the number of daily admissions surpasses 50 per day. Second, most patients under 60 years-old should be assessed carefully and when possible should be managed at home by well-trained primary care physicians and nurses. This decision will alleviate stress and pressure at hospitals. Third, efforts for protecting persons above 80-years-old should be maximized, especially if those living at nursing homes and with co-morbidities. In this regard, periodic SARS-CoV-2 antibody testing in these facilities might help to split out the population still susceptible to infection and focus to protective efforts in this subset of residents.[28]

In summary, the first wave of COVID-19 hit in an unprecedented manner all tertiary hospitals in Madrid. The mortality rate at our institution was 14%, lower than at other clinics in the city, most likely as result of earlier introduction of corticosteroids and tocilizumab in patients with most severe illness. In the absence of a protective vaccine, harm reduction plans confronting new COVID-19 surges should consider expanding home care management for younger patients and improving protective measures for the elderly at nursing homes.

Author contributions

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Mortality by Covid-19 Prior to Vaccination - One Year Experience of Hospitalized Patients in Madrid

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PII: S1201-9712(22)00049-2
DOI: https://doi.org/10.1016/j.ijid.2022.01.043
Reference: IJID 5965

To appear in: *International Journal of Infectious Diseases*

Received date: 6 December 2021
Revised date: 16 January 2022
Accepted date: 18 January 2022

Please cite this article as: Víctor Moreno-Torres MD , Alejandro Muñoz MD , Jorge Calderón-Parra MD , Patricia Mills-Sánchez MD , Ilduara Pintos-Pascual PhD, MD , Celia Rodríguez-Olleros MD , Fátima Ibánez-Estéllez MD , Yale Tung-Chen PhD MD , Antonio Ramos-Martínez PhD MD , Juan A Vargas-Núñez PhD MD , Prof. Valentín Cuervas-Mons PhD MD , Carmen de Mendoza PhD , Mortality by Covid-19 Prior to Vaccination - One Year Experience of Hospitalized Patients in Madrid, *International Journal of Infectious Diseases* (2022), doi: https://doi.org/10.1016/j.ijid.2022.01.043

Mortality by Covid-19 Prior to Vaccination - One Year Experience of Hospitalized Patients in Madrid

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Highlights.

1. A year after the start of the pandemic, mortality rates have remained unchanged.
2. Considering the three waves, the profile of non-survivors has not changed significantly.
3. Advanced age and multiple pathologies are uniform characteristics of non-survivors.

ABSTRACT

Objectives: To analyze the mortality and characteristics of the deceased patients with COVID-19 during the first year of the pandemic.

Methods: Analysis of all admissions due to COVID-19 disease in a tertiary hospital in Madrid. Three waves were considered: March to June 2020, July to November 2020 and December 2020 to April 2021.

Results: A total of 3,676 patients were identified. Among inpatients, no differences regarding age, sex, admission length or mortality were found between the three waves ($p>0.05$). The overall mortality rate was 12.9%. Among deceased patients, median age was 82 and the median Charlson co-morbidity index was 6. Considering the main predictors for mortality by COVID-19 (age, sex and concomitant comorbidities), only patients with previous lung disease were more prevalent in the third period ($p <0.01$). Finally, among deceased patients, higher intensive care unit admission rates, a lower rate of patients coming from nursing homes, and with dementia were noted in the third period ($p<0.05$).

Conclusion: One year after the onset of the pandemic, the mortality rate of hospitalized patients and the profile of non-survivors has not changed significantly. Advanced age and multiple pathologies are uniform characteristics of non-survivors in the absence of the benefit of the vaccines.

Keywords: COVID-19, mortality rate, Spain, comorbidities.

Abbreviations

ARDS: Acute respiratory distress syndrome.

CCI: Charlson comorbidity index.

ICU: Intensive care unit.

SD: Standard deviation

INTRODUCTION

SARS-CoV-2 infection is currently the largest health problem worldwide leading to more than 3 million deaths all over the globe during the first year of the pandemic (WHO, 2021). From the beginning, population studies regarding mortality and susceptible population highlighted that elderly patients, and those who suffered from chronic conditions such as hypertension, diabetes, heart failure, lung disease or immunosuppression, among others, were at higher risk of death [Wu *et al*., 2020; Chen *et al*., 2020]. Similarly, with a 13.8% mortality rate, we confirmed that mortality was strongly related to age and comorbidities during the first wave of COVID-19 in a referral center in Madrid [Moreno-Torres *et al*., 2021].

Since the pandemic outbreak, different strategies have been implemented in order to reduce the transmission of the virus and its health impact. In the hospital setting, several approaches were tried out to treat the disease in those patients with acute respiratory distress syndrome (ARDS). Among others, dexamethasone, tocilizumab, and anakinra have been shown to be effective in treating severe COVID-19 [Horby et al., 2021; Stone *et al*., 2020; Kyriazopoulou *et al*., 2021]. On top of which, the measures introduced of the wearing of facial masks, social distancing and mass lockdowns resulted in fewer hospital admissions. Finally, the rapid development of vaccines led to wide vaccination programs all over the world at the beginning of the year 2021 [Privor-Dumm *et al*., 2021].

All these factors have resulted in the different waves of the disease that occurred throughout 2020, and have conditioned the variations of the number and the profile of affected individuals. However, there is no data to confirm that the profile of patients with

severe COVID-19 and related mortality has changed during the first year of pandemic. Our study analyzes the mortality from COVID-19 in a hospital of reference in Madrid over a year since the start of the pandemic (March 2020 to April 2021), and before the impact of vaccination programs.

MATERIALS AND METHODS

Study design and patients

This retrospective study included all admitted patients with COVID-19 pneumonia at the Puerta de Hierro-Majadahonda University Hospital from March 1st 2020, to April 30th 2021. This is a large tertiary university hospital with 613 beds, whose catchment population approaches 375,000 people. The hospital review board approved the study (PI134-20), and a waiver for informed consent was granted.

Data collection and outcomes

We reviewed electronic medical records for all hospital admitted patients with confirmed COVID-19 disease. The main demographics (gender and age), admission to ICU, and diagnosis discharge (including death if so) were recorded for each one. For all deceased COVID-19 patients, origin (home, nursing homes or referral from other institutions) and the presence of co-morbidities was recorded, including neurocognitive impairment (Alzheimer, other dementias and/or mental disorders), arterial hypertension, diabetes, dyslipidemia, heart disease, cerebrovascular disease, peripheral arterial disease, chronic kidney disease, liver disease, lung disease, cancer, autoimmune diseases and other immunosuppression conditions. The baseline Charlson co-morbidity Index (CCI) was used to estimate the risk of mortality, taking into consideration that the overall 10-year survival rate is 98.3% in patients

with a CCI = 0, drops to 77.48% in those with a CCI of 3, and drops further to 21.36% in those with a CCI of 5 or more [Charlson et al., 1987].

Study periods

In order to examine the course of the pandemic and the changes in patients' profile during the first year after the onset of the pandemic, three different periods were considered: March to June 7th 2020 (first wave), June 8th to December 5th 2020 (second wave) and December 6th 2020 to April 30th 2021 (third wave). These dates were selected according to the national restrictions as well as the defined waves seen corresponding to spring 2020, after summer 2020 and from Christmas time up to April 2021.

Statistical analysis

Continuous variables were expressed as mean and standard deviations (SD), and medians with percentiles (P25-P75). Categorical variables were summarized as counts and percentages. The Kolmogorov test was used to evaluate data distribution and the Student t-test or Mann-Whitney U-test were performed consequently to assess differences between groups. Levene's test was used for the homogeneity of variance test and the $\chi 2$ test (with the two-sided Fisher's exact test) was used to compare categorical variables. For all analyses, significance was defined as a P value of less than 0.05. Statistical analysis was performed using the SPSS version 26.0 software statistical package (SPSS Inc., IBM, Armonk, NY).

RESULTS

Between March 1st 2020 and April 30th 2021, a total of 3,676 patients were hospitalized with COVID-19 at the Hospital Puerta de Hierro-Majadahonda (**table 1**). Overall, 60.1% of admitted patients were male and the average age was 65 years-old (54-77). Median length of admission was 8 days (4-14). During the whole study period, 474 patients died (12.9% mortality rate).

The hospital admissions during this year were evaluated taking into consideration the three waves (**figure 1, table 1)**. Overall, almost a 50% of individuals were included in the first wave (1,788; 48.6%), followed by the second and third wave, with a similar number of individuals in each (926; 25.2% and 962; 26.2%, respectively). When compared, no differences regarding age, sex, admission-length or mortality rates were found.

In order to evaluate the differences between the 3 waves of the pandemic, as well as the clinical impact of the measures adopted, we compared the characteristics of the deceased patients. As expected, the mean age of the deceased patients was significantly higher than the age of the survivors (62.8 vs 79.4 years-old, p< 0.001), but no significant differences were noted in the median age of deceased individuals when the 3 periods were compared (82 years-old (72-88)). In addition, no differences were seen regarding sex, length of admission or previous comorbidities, measured as CCI (median 6) (**table 2**). For previous conditions, only patients with lung disease were more prevalent in the third wave (17.5% vs 5.1% vs 37.1% in the first, second and third wave respectively, p<0.01). On the other hand, non-survivors in the third period presented higher ICU admission rates (12.6% in the first wave, 14.4% in the second wave and 25.8% in the third wave, p<0.03), a lower rate of patients coming from nursing homes (35%, 31.7% and 12.2%, respectively, p<0.001) and a

lower rate of dementia (35%, 42.3% and 23.4%, respectively, p<0.02). Similarly, dependence rates were higher in the second period (39.4%, 56.7%, 31.5%, respectively, p<0.01). To note, only 2 patients (1.6%) who died in the third period had received the complete vaccination cycle.

DISCUSSION

The aim of the present study was to analyze the course and the different waves during the first year of COVID-19 pandemic in our hospital, focusing in the epidemiology and characteristics of the deceased patients. After the abrupt irruption of COVID-19 disease, many cohorts worldwide analyzed the mortality rates and the severe disease risk factors, uniformly concluding that frail and comorbid patients were at higher risk [Wu *et al*., 2020; Chen *et al.*, 2020; Moreno-Torres *et al*., 2021]. In addition, once the pandemic stabilized during the summer in 2020, several studies demonstrated that mortality rates significantly improved after the first wave probably because of better understanding of the disease, greater availability of infrastructures, staff or equipment, and the wide use of corticosteroids, among other therapies [Vahidy *et al*., 2020; Kurtz *et al*., 2021; Armstrong *et al*., 2021; Roth *et al*., 2020; Jones *et al.*, 2021]. However, there is conflicting evidence about the association between these improvements and the changes in the characteristics of the admitted patients. Finally, there is no data about whether the mortality rate and the profile of deceased patients has changed in the last months.

Our study reflects that admissions dramatically decreased from the first wave because of the national lockdown and population prophylactic measures adopted in Spain. In addition, it is noticably that each wave presented a peak which was attributed to the greater

flexibility and poorer compliance of restrictions during holidays periods such as summer, December national holidays and Christmas [Soriano *et al*., 2021].

Despite these clearly identifiable dynamics regarding the number of new cases and hospital-admitted patients, no differences in the deceased patients, by age or gender, were found between the three waves. Moreover, the mortality rate remained constant through the study period, and the profile of the non-survivor individuals remained unchanged. Thus, these results confirm that age and frailty are still by far the strongest risk factors, despite greater knowledge of the disease and better therapeutic management of patients with severe COVID-19.

These findings and the discrepancies with others studies could be partially explained with the following reasons. First, broad corticosteroid and tocilizumab treatment was promptly considered in our institution's protocols, leaving less room for improvement in subsequent waves [Fernández-Cruz et al., 2021; Ruiz-Antorán et al., 2021]. Second, early planning and the possibility to double the number of beds per room delayed the early overload of wards and ICUs, contributing to attenuate the mortality rate in comparison with other Spanish hospital cohorts [Moreno-Torres et al., 2021, Yehia et al., 2020, Casas-Rojo et al., 2020]. Third, poor knowledge of the natural course of COVID-19 during the first wave resulted in a higher number of hospital admissions for patients who would have benefited from adequate home care. This fact was translated into lower mortality rates at the beginning [Moreno-Torres et al., 2021]. At the same time, the higher number of ICU admissions during the third wave could also be a consequence of the greater severity observed in hospitalized patients during this period. Finally, it should be considered that the majority of the studies carried

out during the first wave were based on the diagnosis made in the hospital context [Soriano et al., 2021]. In the following waves, the greater availability of diagnostic tests led to the inclusion of information related to asymptomatic or less severe diagnoses, thus reducing the overall mortality of the infection. Consequently, overall population reported mortality rates could have probably decreased but because a higher number of non-severe patients have been identified, and not necessarily because the disease prognosis has improved. However, our study is based exclusively on the diagnoses that required hospital admission, and on the mortality observed in this setting during the whole study period. Overall, and despite these possible explanations, the fact that mortality rates and the profile of deaths have not changed in one year in our center, yields two possible interpretations. On the one hand, with the therapeutic tools at our disposal, we have managed the disease in an acceptable way from the beginning. On the other hand, these findings emphasize the need for specific antivirals against SARS-CoV-2 [Beigel et al., 2020; Jayk-Bernal et al., 2021; Owen et al., 2021].

Finally, it should be mentioned that mass vaccination programs, which began in Madrid at the beginning of the year 2021, have clearly modified the course of severe disease [Barandalla *et al*., 2021]. In our study, the vaccine impact was intentionally was not yet fully noted, since in April only a small percentage of the population have been fully vaccinated. However, it is observed that during the third wave, the number of dependent and dementia patients was significantly lower than in the previous waves, which can be explained by the impact of the vaccine in these groups. Besides, it is noteworthy that we only identified two deaths in this period who have received the complete vaccination schedule. Altogether, prior data shows that in the absence of effective treatment, the vaccine is an essential tool

to reduce mortality from COVID-19 and to prevent the current wave of infections (November 2021) as the one that is occurring in some Central European countries among unvaccinated people.

Our analysis has several limitations. First, it is a single-center, observational, retrospective study. Second, we have not analyzed the different therapeutic options and the possible associated benefits. Finally, we have limited ourselves to analyzing the baseline characteristics of the deceased patients, not being able to rule out differences in the total number of hospitalized patients.

In conclusion, one year after the onset of the pandemic, the mortality rate of in-hospital patients and the profile of the non-survivors has not varied, being older age and pluri-pathology constant features of the deceased individuals. Despite the absence of solid improvements in the mortality rates, recent trends might announce the expected benefits of the vaccine.

Potential conflicts of interest. The authors declare no conflicts of interest.

Funding source. This work has been supported by a grant from Instituto de Salud Carlos III (Expedient number CM19/00223).

Ethical Approval Statement. The hospital review board approved the study (PI134-20) and a waiver for informed consent was granted.

Contributions

- Victor Moreno-Torres: Study concept and design, statistical analysis, interpretation of results, drafting of manuscript, critical revision of manuscript, approval the final version of the manuscript.
- Carmen de Mendoza: Study concept and design, interpretation of results, drafting of manuscript, critical revision of manuscript, approval the final version of the manuscript.
- All other authors: Interpretation of results, critical revision of manuscript, approved the final version of the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Figure 1. Weekly hospital admissions and exitus with COVID-19 from March 2020 to April 2021 at Puerta de Hierro-Majadahonda Hospital in Madrid.

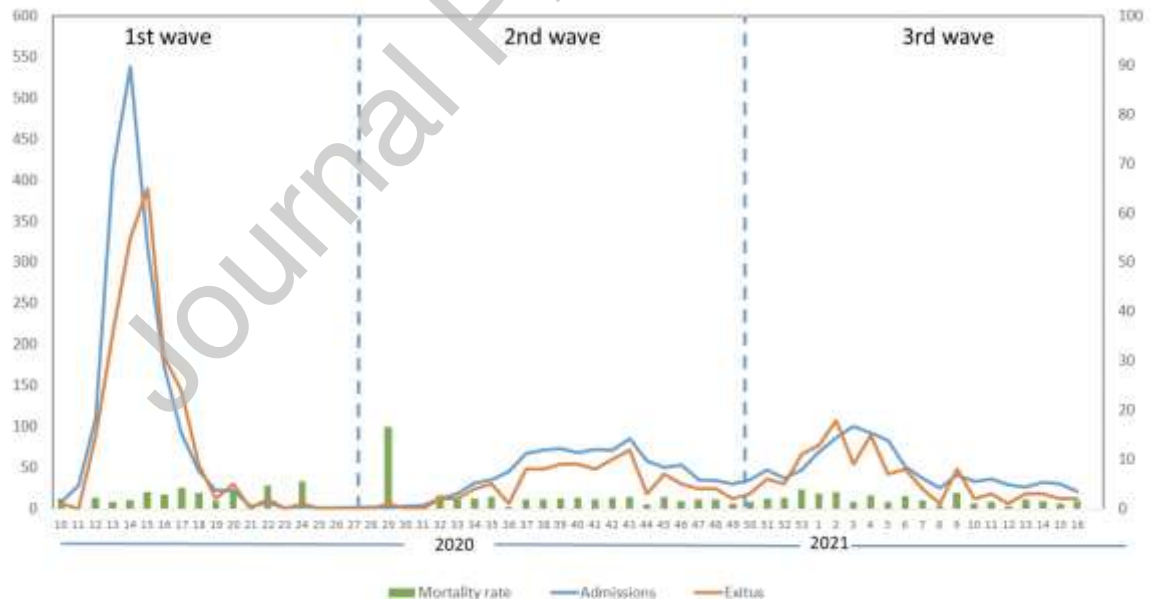

Table 1. Main characteristics of inpatients with COVID-19 according to the study period

	First period (1st March- 7th June 2020)	**Second period** (8th June-5th Dec 2020)	**Third period** (6th Dec 2020-30th April 2021)	**Total**
N (% of admissions)	1,788 (48.6)	926 (25.2)	962 (26.2)	3,676

Age (years) (Median, P25-P75)	65 (55-76)	66 (54-77)	66 (54-77)	65 (54-77)
Male (N,%)	1,109 (62)	537 (58)	564 (58.7)	2,210 (60.1)
Admission length (days) (Median, P25-P75)	7 (4-12,8)	9 (5-15)	9 (5-15)	8 (4-14)
Deaths (N)	246	104	124	474
Mortality rate (%)	13.8	11.2	12.9	12.9

SD: Standard deviation.

Table 2. Main characteristics of deceased patients with COVID-19 according to the study period

	First period	**Second period**	**Third period**
N (mortality rate)	246 (13.8)	104 (11.2)	124 (12.9)
Age (years) (Median, P25-P75)	81 (72-87)	83 (76-89)	82 (72-88)
Male, N (%)	149 (60.6)	62 (59.6)	79 (63.7)
ICU admission, N (%)	31 (12.6)	15 (14.4)	**32 (25.8)***
Admission length (d) (Median, P25-P75)	6 (3-14)	9 (6-18)	11 (6-20)
Prior living at nursing homes, N (%)	86 (35)	33 (31.7)	**15 (12.2)***
Dementia, N (%)	86 (35)	44 (42.3)	**29 (23.4)***
Dependence, N (%)	97 (39.4)	**59 (56.7)***	39 (31.5)
CCI (median, P25-P75)	6 (4-8)	6 (5-8)	6 (4-8)
Hypertension, N (%)	169 (68.7)	80 (76.9)	90 (72.6)
Diabetes, N (%)	76 (30.9)	36 (34.6)	39 (31.5)
Dyslipemia, N (%)	107 (43.5)	49 (47.1)	51 (41.1)
Heart disease, N (%)	84 (34.1)	43 (41.3)	40 (32.3)
Cerebrovascular disease, N (%)	54 (22)	21 (20.2)	25 (20.2)
Peripheral arterial disease, N (%)	13 (5.4)	13 (12.5)	11 (8.9)
Chronic kidney disease, N (%)	44 (17.9)	25 (24)	27 (21.8)
Liver disease, N (%)	11 (4.5)	4 (3.8)	2 (1.6)
Lung disease, N (%)	43 (17.5)	24 (5.1)	**46 (37.1)***
Cancer, N (%)	41 (16.7)	13 (12.5)	20 (16.1)
Autoimmune disease, N (%)	19 (7.7)	11 (10.6)	7 (5.6)

Immunosuppression, N (%)	41 (1.7)	16 (15.4)	26 (21)

ICU: Intensive Care Unit. CCI: Charlson co-morbidity index

*p<0.05 (marked with bold): When compared to the other periods.

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Severe Lymphopenia as a Predictor of COVID-19 Mortality in Immunosuppressed Patients

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Abstract: Background. Coronavirus disease 2019 (COVID-19) has a high mortality in certain group of patients. We analysed the impact of baseline immunosuppression in COVID-19 mortality and the role of severe lymphopenia in immunocompromised subjects. Methods. We analysed all patients admitted with COVID-19 in a tertiary hospital in Madrid between March 1st and April 30th 2020. Epidemiological and clinical data, including severe lymphopenia (<500 lymphocytes/mm^3) during admission, were analysed and compared based on their baseline immunosuppression condition. Results. A total of 1594 patients with COVID-19 pneumonia were hospitalised during the study period. 166 (10.4%) were immunosuppressed. Immunocompromised patients were younger (64 vs. 67 years, $p = 0.02$) but presented higher rates of hypertension, diabetes, heart, neurological, lung, kidney and liver disease ($p < 0.05$). They showed more severe lymphopenia (53% vs 24.1%, $p < 0.001$), lower $SapO_2/FiO_2$ ratios (251 vs 276, $p = 0.02$) during admission and higher mortality rates (27.1% vs 13.5%, $p < 0.001$). After adjustment, immunosuppression remained as an independent factor related to mortality (Odds Ratio (OR): 2.24, $p < 0.001$). In the immunosuppressed group, age (OR = 1.06, $p = 0.01$), acute respiratory distress syndrome (ARDS) (OR = 12.27, $p = 0.017$) and severe lymphopenia (OR = 3.48, $p = 0.04$) were the factors related to high mortality rate. Conclusion. Immunosuppression is an independent mortality risk factor in COVID-19. Severe lymphopenia should be promptly identified in these patients.

Keywords: COVID-19; immunosuppression; severe lymphopenia

Citation: Martínez-Urbistondo, M.; Gutiérrez-Rojas, Á.; Andrés, A.; Gutiérrez, I.; Escudero, G.; García, S.; Gutiérrez, A.; Sánchez, E.; Herráiz, J.; De La Fuente, S.; et al. Severe Lymphopenia as a Predictor of COVID-19 Mortality in Immunosuppressed Patients. *J. Clin. Med.* **2021**, *10*, 3595. https://doi.org/10.3390/jcm10163595

Academic Editor: Giuseppe A. Palumbo

Received: 13 July 2021
Accepted: 12 August 2021
Published: 15 August 2021

1. Introduction

Coronavirus disease 2019 (COVID-19) emerged in China in December 2019 and rapidly spread worldwide, being declared as a pandemic by the World Health Organization (WHO) in March 2020 [1,2]. By mid-year 2021, more than 176 million confirmed cases with over 3.8 million deaths have been attributed to this pathogen. Two different phases have been described in SARS-CoV-2 infection [3]. The initial viral period lasts for 8 to 10 days and has a self-limited course in almost 80–90% of cases. The second one, defined as "the inflammatory stage", correlates with the host immune response and a massive inflammation mediators release known as the cytokine storm. As a consequence, endothelial cells, macrophages, monocytes and lymphocytes are involved with a wide spectrum of clinical manifestations [4].

However, while this hyperinflammation and cytokine storm seem to lead to the acute respiratory distress syndrome (ARDS) and severe COVID-19 disease, the role of baseline immunosuppression in COVID-19 pandemic has not been defined yet despite recent studies

that have assessed this matter [5–8]. On one hand, Giannakoulis et al. [6], reported in a wide meta-analysis that patients with cancer presented more Intensive Care Unit (ICU) admission and death. On the other hand, Minotti et al. [7] identified better outcomes in this population, proposing a possible protective role of a potential weaker immune response. In addition, immunosuppression probably entails a different natural course of the disease, atypical presentations and the higher probability of other complications such as bacterial superinfections [9], leading to a more complex management of this population. Indeed, deep lymphopenia is observed in most immunosuppressed patients as a result of a defective immune response. Multiple models revealed lymphocyte count as a useful prognostic biomarker in SARS-CoV-2 infection [10,11]. In this context, underlying immunosuppression may have synergistic effect in SARS-CoV-2 mortality rates [12].

Therefore, the aim of this study was to evaluate the prognostic role of immunosuppression in COVID-19 patients. In addition, we analysed the mortality risk factors in our immunosuppressed population as well as the influence of severe lymphopenia in this specific cohort.

2. Materials and Methods

2.1. Study Design and Patients

This retrospective observational cohort study was performed at Hospital Puerta de Hierro-Majadahonda, a large tertiary university hospital located in Madrid. The study population consisted of 1594 adult patients who were admitted between 1st March and 30th April because of interstitial pneumonia detected by chest X-ray due to suspected or confirmed SARS-CoV-2. Both real time polymerase chain reaction (RT- PCR) on nasopharyngeal exudate confirmed SARS-CoV-2 infection and suspected SARS-CoV-2 interstitial pneumonia (in the absence of other causes) were included according to the WHO criteria [13]. Follow-up continued to June 30th, 2020. The study was approved by the hospital's Research Ethics Commission and all patients were requested their consent to register their clinical information into a database for epidemiological studies. The subjects were then distributed and compared according to immunosuppression condition considering non-immunosuppressed as controls. Mortality was the main outcome as the chief aim of the investigation is the identification of prognostic determinants in COVID-19 evolution.

2.2. Data Collection

Electronic medical records for all hospital admitted patients with COVID-19 pneumonia were reviewed. Main demographics, baseline comorbidities, including immunosuppression and immunosuppressive treatment, minimum lymphocyte counts during hospitalisation, immunosuppressive treatment received to treat COVID-19, ARDS and outcomes, were collected directly from the electronic medical record. All data were registered by a primary reviewer and subsequently checked by at least two senior physicians.

2.3. Definitions

Immunosuppression was defined either as the presence of hematologic disease (active lymphoproliferative, myeloproliferative disorders or bone marrow transplantation), solid organ transplantation, active and disseminated solid organ neoplasm or any condition, including autoimmune disease (e.g., Systemic Lupus Erythematosus, Sjögren Syndrome, inflammatory bowel disease) that had required immunosuppressive treatment for at least 3 months before admission due to COVID-19. Immunosuppressive treatment was considered when the patient was either receiving active treatment at the moment of admission, including equivalent prednisone doses above 5 mg, or had received chemotherapy or immunotherapy 6 months before disease onset.

Regarding clinical data, severe lymphopenia in the context of COVID-19 was defined as minimum lymphocyte count below $500/mm^3$ during admission [14,15]. On the other hand, ARDS and its severity were defined according to the Berlin definition [16]. In the patients whose partial pressure of oxygen (PaO_2) was unavailable, $SapO_2/FiO_2$ ratio was

used to assess ARDS and severity [17]. Mild ARDS was considered when PaO_2/FiO_2 ratio was >200 mmHg or $SapO_2/FiO_2$ >235 mmHg, moderate when PaO_2/FiO_2 ratio was >100 mmHg or $SapO_2/FiO_2$ > 160 mmHg and severe when PaO_2/FiO_2 ratio was $\leq$100 mmHg.

2.4. Statistical Analysis

Descriptive analyses were performed through the mean (standard deviation, SD) for quantitative variables and absolute (and relative) frequencies for the categorical. A univariate analysis was performed comparing those characteristics for patients who were immunosuppressed versus those who did not, and also, between (immunosuppressed) survivors and non-survivors by means of chi-square test in case of categorical variables and Mann-Whitney's U or Student t-test for numerical variables depending on their distributions and performing the Levène test. Potential confounding variables were entered into different multivariable logistic regression analyses to identify factors related to mortality. For all analyses, significance level was defined as a *p*-value below 0.05. Statistical analysis was performed using SPSS software version 26.0 (IBM, Madrid, Spain).

3. Results

3.1. Patients' Characteristics

A total of 1594 patients admitted because of suspected or confirmed SARS-CoV-2 pneumonia between March and April 2020 were analysed. Mean age was 65 years old, 62.1% were male and 87.2% had a positive RT-PCR for SARS-CoV-2 at the time of admission. Overall, 166 (10.4%) were immunosuppressed (Table 1). Autoimmune disease was the most frequent cause of immunosuppression (51 patients, 39.2%), followed by hematologic disease (35 patients, 21.1%), solid organ neoplasm (32 patients, 19.3%) and solid organ transplantation (30 patients, 18.1%). Overall, 40.4 % of immunosuppressed patients were receiving steroid therapy, 18.1 % biological agents, 15.1% calcineurin inhibitors, 9.6% mycophenolate and active chemotherapy prior to admission.

Table 1. Baseline conditions of the study population *.

	GLOBAL	NON-IS (*n* = 1428)	IS (*n* = 166)	*p*-Value
Baseline Conditions				
Age (*N*, %)	65 (15)	67 (14)	64 (15)	**0.02**
Male sex (*N*, %)	990 (62.1)	887 (62.1)	103 (62.0)	1
HBP (*N*, %)	699 (43.9)	612 (42.9)	87 (52.4)	**0.02**
DM (*N*, %)	281 (17.6)	236 (16.5)	45 (27.1)	**0.001**
Obesity (*N*, %)	424 (35.2)	386 (36.1)	38 (27.9)	**0.001**
Heart disease (*N*, %)	270 (16.9)	229 (16.0)	41 (24.7)	**0.01**
Neurological disease (*N*, %)	225 (14.1)	191 (13.4)	34 (20.5)	**0.02**
Lung disease (*N*, %)	248 (15.6)	212 (14.8)	36 (21.7)	**0.03**
Kidney disease (*N*, %)	112 (7.0)	78 (5.5)	34 (20.5)	**<0.001**
Liver disease (*N*, %)	48 (3)	30 (2.1)	18 (10.8)	**<0.001**

* IS: Immunosuppressed. HBP: High Blood Pressure. DM: Diabetes Mellitus. Qualitative variables are expressed in number and proportion. Quantitative variables are expressed in mean and standard deviation. Statistically significant results are remarked in bold.

Average age was lower in the immunosuppressed group in comparison to not immunocompromised patients (64 vs. 67 years, $p = 0.02$). However, they presented higher rates of baseline comorbidities such as high blood pressure (52.4% vs. 42.9%, $p = 0.02$), diabetes mellitus (27.1% vs. 16.5%, $p = 0.001$), heart disease (24.7% vs. 16.0%, $p = 0.01$), neurological disease (20.5% vs. 13.4%, $p = 0.02$), lung disease (21.7% vs. 14.8%, $p= 0.03$), kidney disease (20.5% vs. 5.5%, $p < 0.001$) and liver disease (10.8% vs. 2.1%, $p < 0.001$) (Table 1).

Concerning disease severity, no differences were observed in ARDS rates during admission (78.3% vs. 75.8%, $p = 0.50$) or minimum mean lymphocyte count (1227/mm^3 vs. 1060/mm^3, $p = 0.551$); but patients with prior immunosuppression presented lower $SapO_2/FiO_2$ ratios (251 vs. 276, $p = 0.02$) and more severe lymphopenia (53%, vs. 24.1%, $p < 0.001$). They received steroid therapy more frequently during admission (70.5% vs. 56.7%, $p = 0.001$). Immunocompromised subjects presented infectious complications more frequently ($p < 0.001$) (Table 2).

Table 2. Clinical characteristics, treatment received and outcomes during admission *.

	GLOBAL	NON-IS (*n* = 1428)	IS (*n* = 166)	*p*-Value
Clinical Characteristics				
Minimum lymphocyte count (cells/mm^3), (Mean, SD)	1078 (3393)	1060 (3244)	1227 (4476)	0.551
Severe Lymphopenia (*N*, %)	432 (27.2)	344 (24.1)	88 (53)	**<0.001**
Sap02/FiO2 ratio (Mean, SD)	273 (124)	276 (123)	251 (131)	**0.02**
ARDS (*N*, %)	1212 (76.1)	1082 (75.8)	130 (78.3)	0.5
Mild (*N*, %)	660 (41.4)	604 (42.3)	56 (33.7)	**0.04**
Moderate (*N*, %)	420 (26.3)	365 (25.6)	55 (33.1)	**0.04**
Severe (*N*, %)	129 (8.1)	110 (7.7)	19 (11.4)	0.1
Infectious complications	135 (8.5)	96 (6.7)	39 (23.5)	**<0.001**
Treatment Received during Admission				
Steroids (*N*, %)	926 (58.1)	809 (56.7)	117 (70.5)	**0.001**
Tocilizumab (*N*, %)	300 (18.8)	260 (18.2)	40 (24.1)	0.07
Anakinra (*N*, %)	42 (2.6)	30 (2.1)	12 (7.2)	**0.001**
Outcomes				
ICU admission (*N*, %)	110 (6.9)	93 (6.5)	17 (10.2)	0.07
In-hospital stay (Mean, SD)	9.3 (10.1)	9 (9)	13 (14.0)	**<0.001**
Readmission (*N*, %)	91 (5.7)	736 (5.1)	18 (10.8)	**0.007**
Death (*N*, %)	241 (15.1)	193 (13.5)	45 (27.1)	**<0.001**

* IS: Immunosuppressed. ARDS: Acute Respiratory Distress Syndrome. ICU: Intensive Care Unit. Qualitative variables are expressed in number and proportion. Quantitative variables are expressed in mean and standard deviation. Statistically significant results are remarked in bold.

Considering outcomes, immunosuppressed patients had a longer hospital average stay (13 vs. 9 days, $p < 0.001$) and higher readmission rates after discharge (10.8% vs. 5.1%, $p = 0.007$). Overall mortality was 15.1%, being significantly higher in patients with baseline immunosuppression (28.9 % vs. 13.5%, $p < 0.001$) (Table 2).

3.2. Immunosuppression as an Independent Factor Related to Mortality

Since a higher proportion of individuals with immunosuppression died during admission, a multivariate analysis to identify the mortality risk factors was performed (Table 3). Mortality was associated with previous immunosuppression (OR: 2.24, 95%CI= 1.37–3.65) as well as other known baseline comorbidities, including age (OR:1.11, 95%CI:1.09–1.13), neurological disease (OR: 2.79, 95%CI: 1.89–4.11), kidney disease (OR: 2.62, 95%CI: 1.58–4.34) and by the presence of ARDS (OR: 14.06, 95% CI 4.80–41.20) and severe lymphopenia (OR: 2.95, 95% CI 2.03–4.30).

Table 3. Multivariate analysis for factors associated with mortality in 1594 COVID-19 hospitalised patients *.

	Odds Ratio	95% Confidence Interval
Age	**1.11**	**1.09–1.13**
HBP	1.1	0.74–1.66
DM	1.33	0.90–1.96
Heart disease	1.26	0.85–1.887
Neurological disease	**2.79**	**1.89–4.11**
Lung disease	0.75	0.47–1.18
Kidney disease	**2.62**	**1.58–4.34**
Liver disease	1.72	0.73–4.03
Immunosuppression	**2.24**	**1.37–3.65**
Severe lymphopenia	**2.95**	**2.03–4.30**
ARDS	**14.06**	**4.80–41.20**
Infectious complications	1.1	0.65–1.87
Steroids	0.68	0.45–1.03

* HBP: High Blood Pressure. DM: Diabetes Mellitus. SON: Solid organ neoplasm. SOT: Solid organ transplantation. ARDS: Acute Respiratory Distress Syndrome. Statistically significant results are remarked in bold.

3.3. Prognostic Risk Factors of Mortality in Immunosuppressed Patients

In order to identify the prognostic risk factors in immunosuppressed patients (166 individuals), as well as the impact of lymphopenia, univariate and multivariate analyses were performed in this population. Immunosuppressed non-survivors were significantly older than survivors (73.9 vs 64.6 years, $p < 0.001$). In addition, in the univariate analysis (Table 4), mortality in immunosuppressed patients was associated with heart disease ($p = 0.003$), kidney disease ($p = 0.04$), severe lymphopenia ($p < 0.001$) as well as the presence of ARDS ($p < 0.001$) and $SapO_2/FiO_2$ ratio ($p < 0.001$).

Table 4. Baseline conditions, clinical characteristics and outcomes of immunosuppressed patients *.

	Survivors (*n* = 118)	Non-Survivors (*n* = 48)	*p*-Value
Baseline Conditions			
Age (*N*, %)	64.6 (12.4)	73.9 (14.5)	**<0.001**
Male sex (*N*, %)	73 (61.9%)	30 (62.5%)	1
HBP (*N*, %)	56 (47.5%)	31 (64.6%)	0.059
DM (*N*, %)	27 (22.9%)	18 (37.5%)	0.082
Obesity (*N*, %)	26 (26.5%)	12 (31.6%)	0.671
Heart disease (*N*, %)	21 (17.8%)	20 (41.7%)	**0.003**
Neurological disease (*N*, %)	24 (20.3%)	10 (20.8%)	1
Lung disease (*N*, %)	27 (22.9%)	9 (18.8%)	0.679
Kidney disease (*N*, %)	19 (16.1%)	15 (31.3%)	**0.035**
Liver disease (*N*, %)	12 (10.2%)	6 (12.5%)	0.783
Immunosuppression causes			
Autoimmune disease	51 (43.2%)	14 (29.2%)	0.115
Hematologic	22 (18.6%)	13 (27.1%)	0.294
Solid organ neoplasm	19 (16.1%)	13 (27.1%)	0.129
Solid organ transplantation	22 (18.6%)	8 (16.7%)	0.828
Others	4 (3.4%)	0 (0%)	0.325
Severe lymphopenia	50 (42.4%)	38 (79.2%)	**<0.001**
Sap02/FiO2 ratio (Mean, SD)	298 (119)	134 (77)	**<0.001**
ARDS (*N*, %)	83 (70.3%)	47 (97.9%)	**<0.001**
Mild (*N*, %)	52 (44.1%)	4 (8.3%)	**<0.001**
Moderate (*N*, %)	24 (20.3%)	31 (64.6%)	**<0.001**
Severe (*N*, %)	7 (5.9%)	12 (25%)	**<0.001**

* HBP: High Blood Pressure. DM: Diabetes Mellitus. ARDS: Acute Respiratory Distress Syndrome. Qualitative variables are expressed in number and proportion. Quantitative variables are expressed in mean and standard deviation. Statistically significant results are remarked in bold.

In the multivariate analysis (Table 5), severe lymphopenia associated a three-fold increased mortality risk in the immunosuppressed subjects (OR = 3.48, 95% CI 1.49–8.12), along with age (OR = 1.06, 95% CI 1.02–1.09) and ARDS (OR = 12.27, 95% CI 1.56–96.41).

Table 5. Multivariate analysis for factors associated with mortality in COVID-19 immunosuppressed hospitalised patients *.

	OR	95% CI
Age	**1.06**	**1.02–1.09**
Heart disease	1.96	0.82–4.73
Kidney disease	1.47	0.58–3.71
Severe lymphopenia	**3.48**	**1.49–8.12**
ARDS	12.27	**1.56–96.41**

* OR: Odds Ratio. CI: Confidence interval. ARDS: Acute Respiratory Distress Syndrome. Statistically significant results are remarked in bold.

4. Discussion

Immunosuppression has a controversial role in COVID-19 pneumonia, because of the key hyperinflammation cascade determining ARDS appearance and the proper defective host immune response in these particular subjects [18]. In this study, we assessed the impact of immunosuppression in COVID-19 mortality and the added value of lymphopenia in immunosuppressed individuals as a prognostic marker in one of the most affected regions by COVID-19 during the first wave [9].

In our cohort, immunosuppressed patients presented higher comorbidities and more severe disease. However, immunosuppression remained as an independent condition for fatal outcomes after adjustment by the factors previously described [19]. Emerging studies also show a worse trend among COVID-19 patients with immunosuppressant conditions [20]. This fact might suggest that immunosuppression intrinsically involves poor prognosis as published by Williamson et al. in a wide transplanted cohort [21] and Giannakoulis et al. in a meta-analysis based in patients diagnosed with cancer [6]. Consequently, ARDS severity and worse outcomes could be related with the dysfunctional immune response, rather than with direct viral damage to the lungs. Similarly, Ritchie et al. considered this disorder as a "double-edged sword" given a delayed SARS-CoV-2 clearance and a subsequent longer disease course [22]. In addition, a systematic review proposed these subjects as possible viral reservoirs in this context given the previously described data [7]. A pro-inflammatory previous status and a deficient counter-regulation to the cytokine delivery might aggravate clinical manifestations. In fact, persistent COVID-19 has been reported in patients receiving immunosuppressive therapies for several causes as cancer, solid organ transplantation or autoimmune diseases [23–25].

Therefore, immunosuppression may be an underlying condition explaining some deaths in younger and more comorbid subjects. However, not every immunosuppressed individual reacts equally to SARS-CoV-2 infection. Almost 80% of fatal cases in our immunocompromised population presented severe lymphopenia, even though there were not statistically significant differences in total lymphocyte counts. Decreased lymphocyte count has been published as a viral infection biomarker [26–28]. In previous literature, 63–85% SARS-CoV-2 infected patients showed lymphopenia, up to 20% deeper in those who died [29]. This fact could be partially explained by impaired myelopoiesis and lymphohematopoiesis in COVID-19 necropsies. Concomitantly, lymphocyte redistribution has been recognised as a physiological response in viral pathologies lowering peripheral levels [30]. In immunocompromised individuals, a prolonged virus exposure might involve T-cell and natural killer cells exhaustion with an even more marked lymphopenia and an uncontrolled hypercytokinemia [31,32]. Therefore, severe lymphopenia may reflect a basal defective immune response to an aggressive viral infection, which unfortunately results in increased mortality rates in this specific population. Considering the diversity of our

immunosuppressed cohort, we could define lymphopenia as a global mortality risk factor in these patients, independently of its trigger. Pharmacological targets or underlaying disease activity have not been tested in a more detailed analysis, but in the light of the conclusions this finding could possibly be a class effect derived of immunosuppression itself. Nevertheless, more investigations are required in this field. Lymphocyte count is an accessible and non-invasive determination that could probably be helpful to implement early and targeted therapeutic measures, in this concrete scenario. In addition, these findings confirm the requirement of specific management protocols for immunosuppressed patients with COVID-19, to avoid the excessive immunosuppression and lymphopenia that entail a fatal outcome.

Possible limitations include that the study is retrospective, cross-sectional and single-center and the sample (166 patients) may not be representative of the whole immunosuppressed population. In addition, it should be considered that immunosuppressed patients were analysed altogether in a heterogeneous population. As a consequence, the intensity and type of immunosuppression was not properly assessed. However, the described results are plausible with the previous published literature. Low statistical power could be responsible for some of the non-significant outcomes. We tried to minimise bias including the potential confounding variables in multivariate models. As a strong point, lymphocyte count has demonstrated to be a reliable marker in SARS-CoV-2 infection in other analyses, but this investigation contributes to identify a new role for this biomarker that has not been described before. Furthermore, sensitivity analysis allowed to discriminate the involvement of specific variables in the outcomes.

In summary, immunosuppression and severe lymphopenia in immunosuppressed patients might have a key role as predictors of fatal events in the COVID-19 pandemic. These approaches implementation will elicit promising outcomes in personalised medicine. In view of these results, the detection of severe lymphopenia in immunocompromised individuals should probably be considered as a red flag in usual clinical practice. Thus, the inclusion of these criteria in hospital protocols for an early intensive therapeutic approach could be a useful measure for reducing mortality in this vulnerable patient profile. As a concluding remark, this research will help to provide guidelines where the immunosuppression condition should be taken into account.

Author Contributions: Conceptualization, M.M.-U. and V.M.-T.; methodology, C.D.M., M.M.-U. and V.M.-T.; formal analysis, S.D.L.F. and V.M.-T.; investigation, M.M.-U., Á.G.-R., A.A., I.G., G.E., S.G., A.G., E.S., J.H., S.D.L.F., A.C., C.D.M. and V.M.-T.; data curation, Á.G.-R., A.A., I.G., G.E., S.G., A.G., E.S. and J.H.; writing—original draft preparation, M.M.-U., Á.G.-R., V.M.-T. and C.D.M.; writing—review and editing, M.M.-U., Á.G.-R., A.A., I.G., G.E., S.G., A.G., E.S., J.H., S.D.L.F., A.C., C.D.M. and V.M.-T.; supervision, S.D.L.F., A.C., C.D.M. and V.M.-T.; funding acquisition, institutional own funds. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This research received a certificate from the Hospital Puerta de Hierro Research Ethics Commission stating that the experimental design and procedures followed the required ethical principles (20 June 2020).

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

Data Availability Statement: The data are filed in the SELENE platform (SELENE System, Cerner Iberia, S.L.U, Madrid, España).

Acknowledgments: The authors wish to acknowledge the staff and personnel in Hospital Puerta de Hierro, as well as the patients that contributed to this investigation.

Conflicts of Interest: The authors declare no conflict of interest.

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International Journal of Infectious Diseases 116 (2022) 51–58

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Influence of chronic use of corticosteroids and calcineurin inhibitors on COVID-19 clinical outcomes: analysis of a nationwide registry

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ARTICLE INFO

Article history:
Received 3 November 2021
Revised 7 December 2021
Accepted 8 December 2021

Keywords:
COVID-19
immunocompromised host
prognosis factors
solid organ transplantation
autoimmune diseases
immune-mediated inflammatory diseases

ABSTRACT

Objectives: The aim of this study was to analyze whether subgroups of immunosuppressive (IS) medications conferred different outcomes in COVID-19.
Methods: The study involved a multicenter retrospective cohort of consecutive immunosuppressed patients (ISPs) hospitalized with COVID-19 from March to July, 2020. The primary outcome was in-hospital mortality. A propensity score-matched (PSM) model comparing ISP and non-ISP was planned, as well as specific PSM models comparing individual IS medications associated with mortality.
Results: Out of 16 647 patients, 868 (5.2%) were on chronic IS therapy prior to admission and were considered ISPs. In the PSM model, ISPs had greater in-hospital mortality (OR 1.25, 95% CI 0.99–1.62), which was related to a worse outcome associated with chronic corticoids (OR 1.89, 95% CI 1.43–2.49). Other IS drugs had no repercussions with regard to mortality risk (including calcineurin inhibitors (CNI); OR 1.19, 95% CI 0.65–2.20). In the pre-planned specific PSM model involving patients on chronic IS treatment

Abbreviations: AHF, acute heart failure; AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; CCI, Charlson comorbidity index; CHF, chronic heart failure; CI, confidence interval; CNI, calcineurin inhibitors; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; CRP, C-reactive protein; DIC, diffuse intravascular coagulopathy; LDH, lactate dehydrogenase; HR, hazard ratio; ICU, intensive care unit; IHD, ischemic heart disease; IQR, interquartile range; IS, immunosuppressive; ISP, immunosuppressed patient; IMID, immune-mediated inflammatory disease; MOF, multiple organ dysfunction syndrome; OR, odds ratio; RT-PCR, real-time polymerase chain reaction; SOT, solid organ transplant.

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https://doi.org/10.1016/j.ijid.2021.12.327

before admission, corticosteroids were associated with an increased risk of mortality (OR 2.34, 95% CI 1.43–3.82).

Conclusions: Chronic IS therapies comprise a heterogeneous group of drugs with different risk profiles for severe COVID-19 and death. Chronic systemic corticosteroid therapy is associated with increased mortality. On the contrary, CNI and other IS treatments prior to admission do not seem to convey different outcomes.

INTRODUCTION

Since the beginning of 2020, the world has faced the coronavirus disease 2019 (COVID-19) pandemic. As of November 11, 2021, more than 250 million people had contracted COVID-19 worldwide, and more than 5 million had died (Dong et al., 2020).

COVID-19 progresses with an initial viral replication phase, followed by a viral clearance phase as a result of the immune response. In some patients, SARS-CoV-2 replication in the lungs may trigger a cytokine storm that leads to the development of uncontrolled inflammation, an acute respiratory distress syndrome (ARDS), and respiratory failure; these are the main causes of death in these patients (Rodríguez-Baño et al., 2020). This uncontrolled inflammation has prompted the use of several anti-inflammatory drugs in severe cases (Horby et al., 2020).

It has been speculated that patients receiving chronic systemic corticosteroids or other immunosuppressive (IS) therapies are likely to have a lower risk of this uncontrolled inflammation (D'Antiga, 2020). In this regard, special attention should be paid to calcineurin inhibitors (CNI), including cyclosporine and tacrolimus (Gálvez-Romero et al., 2021; Solanich et al, 2021), which form the basis of immunosuppression therapy in solid organ transplant (SOT) recipients, and are also used in some patients with immune-mediated inflammatory diseases (IMID). *In vitro* studies have shown that cyclosporine and tacrolimus inhibit viral replication of several coronaviruses through binding to intracellular cyclophilins, inactivating peptidyl-prolyl cis/trans isomerase function (Ma-Lauer et al., 2020). Therefore, chronic treatment with CNI could reduce the severity of SARS-CoV-2 infection (Belli et al., 2021). On the other hand, as in other viral infections, IS therapies may lead to uncontrolled initial viral replication (Urra et al., 2020), viral immune evasion, and higher risk of mortality (Belsky et al., 2021). Data on the natural course of COVID-19 in chronically immunosuppressed patients (ISPs) are scarce and inconsistent when compared with those for the general population. Some studies suggest that ISPs have higher rates of severe COVID-19 and mortality compared with the general population, while others show just the opposite — a lower incidence of severe COVID-19 and lower mortality (Suárez-García et al., 2021; Minotti et al., 2020; Martínez-Urbistondo et al., 2021)

In the light of the above, our study aimed to assess whether patients receiving certain IS treatments — corticosteroids and CNI in particular — may be at different risk of severe COVID-19 and adverse outcomes compared with the non-immunosuppressed population.

PATIENTS AND METHODS

Study population and participants

This was a retrospective cohort study of all adult patients (18 years of age or older) admitted to hospital for the first time due to COVID-19, in 150 hospitals across Spain, from March to July, 2020, and who had reached a hard endpoint (death or hospital discharge). Information on the SEMI-COVID-19 registry and data collection procedures have been described in previously published works (Suárez-García et al., 2021).

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Provincial Research Ethics Committee of Málaga (Spain) pursuant to the recommendation of the Spanish Agency of Medicines and Medical Products (AEMPS). All patients gave their informed consent.

Definitions and variables

SARS-CoV-2 infection was confirmed by a positive real-time polymerase chain reaction (RT-PCR) test of a nasopharyngeal exudate sample, sputum, or bronchoalveolar lavage.

Patients were defined as on IS treatment if they were receiving any immunosuppressive medication, including systemic corticosteroids, CNI (tacrolimus and cyclosporine), antimetabolites (mycophenolate, azathioprine), mTOR inhibitors (sirolimus, everolimus), and/or other immunosuppressive treatments at the time of admission. ISPs were classified either as SOT recipients or IMID patients. Due to limitations in the database, it was not possible to identify the specific IMID disease. Patients with hematological malignancies (involving active lymphoproliferative or myeloproliferative disorders, or bone marrow transplantation) or solid organ malignancies were not included in this study. ARDS and severity were defined according to the Berlin definition (Ranieri et al., 2012). Patients not receiving IS treatments prior to admission (non-IS population) were used as controls.

Study outcomes

The primary endpoint was in-hospital all-cause mortality. Secondary endpoints were 30-day mortality and in-hospital complications, including bacterial pneumonia, sepsis, septic shock, acute kidney injury (AKI), acute heart failure (AHF), myocarditis, stroke, or multiple organ dysfunction syndrome (MOF).

Statistical analysis

Quantitative variables were expressed as median and interquartile range (IQR). Categorical variables were expressed as percentages and absolute frequencies.

Clinical presentations and complications were compared between each ISP group and controls, using the chi-square test for qualitative variables (or Fisher's exact test when appropriate) and Student's *t*-test for quantitative variables (or the Mann–Whitney U-test when appropriate).

The influence of belonging to either ISP group (SOT recipients or IMID patients) as well as specific IS medications on mortality were analyzed by including demographic and comorbidity variables in a single-step multivariate logistic regression model, which also included the aforementioned groups (model 1) or medications

(model 2). The corrected odds ratios (OR) and 95% confidence intervals (CI) were calculated for statistically significant variables.

A survival analysis was also performed, comparing time to death between groups, with data censored at 30 days of clinical progress (30-day mortality). Time to death was modeled using Kaplan–Meier curves, and differences were assessed using stratified Cox regression models. Hazard ratios (HR) and 95% CI were determined.

In order to better estimate the influence of chronic immunosuppressive medication on clinical course and mortality, a 1:1 propensity score analysis was performed comparing ISPs with the non-IS population after matching according to sex, age, and comorbidities. A propensity score-matching analysis was also conducted for ISPs on specific medications found to be associated with mortality in comparison with ISPs who were receiving other medications. All models were required to have only exact matches. The validity of all propensity score-matching models was assessed by comparing demographic and comorbidity variables between the groups. Clinical course and mortality were also compared between groups, using the same analytical method as described above. OR and 95% CI were provided for all variables with a p-value < 0.10.

For all statistical analysis, two-tailed p-values < 0.05 were considered significant. The statistical analyses were performed using the SPSS version 25 software package (IBM SPSS Statistics).

RESULTS

In total, 16 647 consecutive adult patients hospitalized with COVID-19 were included in the registry. 1674 patients with malignancy were excluded from the analysis. Of the remaining 14 973 evaluable patients, 868 (5.79%) were considered ISPs and 14 105 (94.2%) were not. Among the ISPs, 654 patients had a prior history of IMID (4.36% overall) and 214 were SOT recipients (1.42% overall, with 151, 32, 16, and 15 undergoing kidney, liver, lung, and heart transplantation, respectively). There were 1243 prescriptions for immunosuppressive medications among the 868 ISPs. The most common treatments were glucocorticoids (593 patients, 68.3%), followed by antimetabolites such as mycophenolate, azathioprine, and methotrexate (369 patients, 42.5%), CNI (155 patients, 17.9%), and m-TOR inhibitors (65 patients, 7.5%).

The demographic characteristics, general baseline data, comorbidities, clinical presentations, and outcomes for ISPs and controls are summarized in Table 1. Overall, the mean age was 69 years and 8460 patients (56.5%) were male. The hospital mortality rate was 19.1% (2857 deaths). In the multivariate logistic regression analysis, after adjusting for age and comorbidities (Table 2), higher in-hospital mortality was found both in SOT recipients (OR 2.46, 95% CI 1.73–3.49) and IMID patients (OR 1.38, 95% CI 1.10–1.72). Among specific chronic IS treatments, only corticoids use at admission was associated with in-hospital mortality (OR 2.24, 95% CI 1.41–3.55). Interestingly, after adjusting for chronic glucocorticoid use at admission in the survival analysis (Figure 1), SOT recipients remained at higher risk of 30-day mortality (HR 1.69, 95% CI 1.23–2.35), while IMID patients had a similar risk to the general non-IS population (HR 0.86, 95% CI 0.76–1.15). On the other hand, chronic glucocorticoid use was strongly associated with 30-day mortality (HR 2.00, 95% CI 1.43–2.79)

Propensity score-matched analysis

A propensity score-matching analysis was performed for a total of 636 pairs of ISP patients and controls. Differences in clinical courses and complications between the groups are shown in Table 3. Figure 2 shows the time-to-death analysis for the groups. Although their clinical presentation was similar, in-hospital mortality was higher in patients receiving any immunosuppressive medications compared with controls (25% vs 21.1%; HR 1.21, 95% CI 1.01–1.52). In this model, glucocorticoid use was associated with higher in-hospital mortality than that for the general non-IS population (OR 1.89, 95%CI 1.43–2.49), while CNI (OR 1.19, 95% CI 0.65–2.20), antimetabolites (OR 1.09, 95% CI 0.59–2.00), and mTOR inhibitors (OR 0.76, OR 0.23–2.61) were not associated with worse outcomes.

Chronic glucocorticoid treatment

A specific propensity score-matched analysis regarding chronic systemic glucocorticoid therapy confirmed that their use before admission was associated with mortality in the whole study population (OR 1.89, 95% CI 1.43–2.49). Furthermore, patients under corticoid treatment presented more in-hospital complications, such as severe ARDS (OR 1.75, 95% CI 1.05–2.91), sepsis (OR 1.99, 95% CI 1.06–4.38), septic shock (OR 3.67, 95% CI 1.19–11.36, AKI (OR 2.28, 95% CI 1.37–3.80), and MOF (OR 2.43, 95% CI 1.41–4.26). Finally, chronic systemic corticoid treatment was also associated with worse outcomes among SOT recipients (OR 1.82, 95% CI 1.01–3.30).

As planned, a separate propensity score-matching analysis for a total of 212 ISPs treated with systemic glucocorticoids, paired with ISPs without glucocorticoids, was performed. Differences in clinical courses and complications between patients with and without systemic glucocorticoids are summarized in Table 4. Figure 3 shows the time-to-death analysis for the groups. In-hospital mortality was higher in IS patients with glucocorticoids (27.8% vs 14.2%; HR 2.08, 95% CI 1.30–3.31). Interestingly, in this model, patients without glucocorticoids but with other immunosuppressive treatments had similar in-hospital mortality rates to the general non-IS population (14.2% vs 18.6%, respectively), although the groups were not statistically comparable.

Chronic calcineurin inhibitors

In the propensity score analysis, chronic CNI therapy before hospital admission was not associated with worse outcomes (OR 1.19, 95%CI 0.65–2.20). Notably, the majority of patients on CNI were SOT recipients (85.2%, 132/155). Consequently, a sub-analysis was performed to analyze the role of CNI treatment before admission in SOT patients. When chronic CNI treatment was considered, no differences regarding mortality were found (31.7% vs 32.6%, $p = 1.000$).

DISCUSSION

A recently published study involving the Spanish cohort showed that immunosuppression and immunosuppressant drugs conferred a higher death risk associated with COVID-19 (Suárez-García et al., 2021). In light of these findings, our study sought to evaluate which immunosuppressant drugs in particular were associated with this greater risk, using a propensity-score analysis. Consequently, the main finding of our study was that chronic systemic glucocorticoid therapy at admission was the strongest risk factor for death in immunosuppressed COVID-19 patients. Our study also found that immunosuppression with CNI was not associated with better outcomes.

Our results indicated that not all chronic immunosuppressive treatments may be comparable with regard to COVID-19 severity risk, as previously postulated by other authors (Pablos et al., 2020; FAI2R/SFR/SNFMI/SOFREMIP/CRI/IMIDIATE consortium and contributors, 2021). Of special relevance was the deleterious effect of chronic glucocorticoid treatment at admission on immunosuppressed patients with COVID-19, confirming that, in our previous study, the impact of immunosuppressant drugs on mortality was

Table 1
Demographic factors, comorbidities, clinical presentations, and outcomes according to patient group

Variable	Total (n = 14973)	Non–IS (n = 14105)	SOT (n = 214)	$p1$	IMID (n = 654)	$p2$
Demographic factors and comorbidities						
Age (years)	69 (56–79)	68 (55–79)	65 (54–73)	0.014	71 (60–81)	< 0.001
Sex (male)	56.5% (8460)	56.8% (8008)	62.8% (134)	0.109	48.6% (318)	< 0.001
Obesity	20.4% (3059)	22.2% (2876)	21.0% (44)	0.677	23.5% (139)	0.479
CCI	1 (0–2)	0 (0–1)	2 (1–4)	< 0.001	1 (1–2)	< 0.001
Age-adjusted CCI	3 (2–5)	3 (1–5)	4 (3–7)	< 0.001	4 (3–6)	< 0.001
Alcohol	4.2% (630)	4.4% (596)	5.1% (11)	0.710	3.7% (23)	0.425
Active smoking	4.7% (701)	5.0% (669)	3.4% (7)	0.008	4.0% (25)	< 0.001
Hypertension	50.1% (7497)	49.4% (6959)	74.8% (160)	< 0.001	57.8% (378)	< 0.001
Dyslipidemia	39.0% (5847)	38.7% (5450)	57.0% (122)	< 0.001	42.0% (275)	0.092
Diabetes mellitus	14.1% (2112)	14.0% (1972)	20.6% (44)	0.008	14.7% (96)	0.645
Cardiac failure	6.8% (1021)	6.5% (918)	10.8% (23)	0.017	12.2% (80)	< 0.001
Atrial fibrillation	10.4% (1558)	10.2% (1434)	16.9% (36)	0.002	13.5% (88)	0.007
Acute IHD	5.6% (832)	5.4% (768)	10.4% (22)	0.003	6.4% (42)	0.291
Chronic IHD	3.5% (521)	3.4% (479)	5.6% (12)	0.085	4.6% (30)	0.123
Peripheral vascular disease	4.4% (662)	4.2% (589)	10.3% (22)	< 0.001	8.0% (51)	< 0.001
COPD	6.3% (936)	6.0% (847)	4.7% (10)	0.422	12.1% (79)	< 0.001
Asthma	7.3% (1097)	7.1% (1006)	4.7% (10)	0.181	12.4% (81)	< 0.001
Stroke	2.7% (411)	2.8% (388)	2.3% (5)	0.836	2.8% (18)	1.000
Cognitive decline	9.7% (1458)	9.9% (1397)	3.3% (7)	0.002	8.3% (54)	0.179
Depression	10.3% (1545)	10.2% (1436)	8.4% (18)	0.427	14.0% (91)	0.003
CRF	5.6% (845)	4.9% (696)	46.3% (99)	< 0.001	7.7% (50)	0.003
Liver cirrhosis	0.9% (135)	0.7% (102)	7.9% (17)	< 0.001	2.5% (16)	< 0.001
Anticoagulation	10.8% (1618)	10.5% (1475)	18.7% (40)	< 0.001	15.7% (103)	< 0.001
Antiaggregation	15.2% (2273)	15.0% (2109)	23.5% (50)	0.001	17.5% (114)	0.093
Clinical presentations						
Cough	73.5% (10998)	73.8% (10375)	66.3% (142)	0.018	73.5% (481)	0.973
Arthromyalgia	30.8% (4614)	31.3% (4374)	24.6% (52)	0.043	29.0% (188)	0.225
Asthenia	42.4% (6343)	42.8% (5967)	36.5% (77)	0.068	46.8% (299)	0.073
Fever	63.3% (9472)	63.8% (8967)	59.3% (127)	0.202	58.0% (378)	0.009
Dyspnea	57.6% (8620)	57.8% (8118)	46.3% (99)	< 0.001	61.6% (403)	0.052
Diarrhea	24.4% (3659)	24.5% (3425)	34.9% (74)	0.001	24.6% (160)	0.963
Rx infiltrate	64.5% (9653)	64.5% (9102)	61.1% (149)	0.310	62.1% (402)	0.195
Lymphocytes	0.94 (0.68–1.30)	0.96 (0.70–1.30)	0.80 (0.50–1.18)	< 0.001	0.84 (0.55–1.20)	< 0.001
CRP	61 (20–130)	60 (20–128)	60 (22–106)	0.417	65 (21–141)	0.202
LDH	322 (247–434)	322 (249–432)	290 (223–338)	< 0.001	325 (253–439)	0.607
Ferritin	613 (287–1231)	612 (287–1242)	662 (333–1455)	0.324	543 (277–1043)	0.100
D–dimer	0.67 (0.37–1.26)	0.64 (0.36–1.20)	0.64 (0.37–1.24)	0.385	0.74 (0.39–1.54)	0.001
Complications and outcomes						
Severe distress	17.4% (2602)	17.3% (2428)	24.1% (51)	0.029	18.8% (123)	0.292
Bacterial pneumonia	10.6% (1590)	10.6% (1490)	9.9% (21)	0.823	12.1% (79)	0.242
Sepsis	6.4% (954)	6.3% (882)	6.6% (14)	0.886	8.9% (58)	0.009
Septic shock	4.6% (686)	4.6% (640)	4.2% (9)	0.872	5.7% (37)	0.213
ARI	13.5% (2027)	13.1% (1842)	36.3% (77)	< 0.001	16.5% (108)	0.013
ACF	5.5% (819)	5.3% (739)	8.5% (18)	0.044	9.5% (62)	< 0.001
Myopericarditis	0.9% (130)	0.8% (109)	2.4% (5)	0.010	2.5% (16)	< 0.001
AIHD	0.8% (120)	0.8% (113)	0	0.271	1.1% (7)	0.499
Stroke	0.7% (110)	0.8% (105)	0.5% (1)	0.872	0.6% (4)	0.793
DIC	1.0% (152)	1.0% (141)	0	0.179	1.7% (11)	0.077
MOF	5.7% (854)	5.5% (773)	11.8% (25)	< 0.001	8.6% (56)	< 0.001
ICU admission	9.3% (1388)	9.3% (1314)	7.5% (16)	0.407	8.9% (58)	0.731
Hospital mortality	19.1% (2857)	18.6% (2618)	32.2% (69)	< 0.001	26.0% (170)	< 0.001
COVID-related mortality	94.2% (2691/2857)	94.2% (2465/2618)	89.9% (62/69)	0.136	96.4% (164/170)	0.207

Qualitative variables are expressed as percentage (absolute number). Quantitative variables are expressed as median (interquartile range). $p1$: univariant analysis between SOT and non-IS. $p2$: univariant analysis between IMID and non-IS. IS: immunosuppressed. SOT: solid organ transplant. IMID: immune-mediated inflammatory disease. CCI: Charlson comorbidity index. IHD: ischemic heart disease. COPD: chronic obstructive pulmonary disease. CRF: chronic renal failure. Rx infiltrate: radiological infiltrate. CRP: c-reactive protein. LDH: lactate dehydrogenase. ARI: acute renal injury. ACF: acute cardiac failure. AIHD: acute ischemic heart disease. DIC: disseminated intravascular coagulation. MOF: multiorgan failure. ICU: intensive care unit

probably attributable to chronic corticoids (Suárez-García et al., 2021). In our population, patients receiving chronic glucocorticoid therapy prior to hospital admission had similar clinical presentations, but they developed more complications, including severe ARDS, sepsis, AKI, and MOF. In addition, mortality rates were clearly higher in patients with glucocorticoids after adjusting for comorbidities and in propensity score-matched analysis. Moreover, when analyzing the different patient subgroups, chronic glucocorticoid treatment was found to be at least partly responsible for the higher mortality seen in ISPs, since it was the strongest risk factor for death. Furthermore, in our study, patients with IMID who were not on chronic systemic glucocorticoids had a comparable in-hospital mortality to non-immunocompromised patients. Additionally, among SOT recipients (who had higher in-hospital mortality compared with the general non-IS population after adjusting for chronic corticosteroid therapy), chronic corticoid treatment was also associated with increased risk of mortality and complications.

These results may seem shocking, considering that glucocorticoids are, to date, the most effective treatment for this disease (Rodríguez-Baño et al., 2021; Horby et al., 2021). However, some smaller studies analyzing patients treated with chronic immunosuppressive medications have shown that patients receiving glucocorticoids seem to be at higher risk of death than those not receiving them (Ayala-Gutiérrez et al., 2021; Anikhindi et al., 2020;

Table 2
Multivariant analysis, by logistic multivariant regression, of association with mortality of demographic factors and comorbidities

Variable	OR	95% CI
Demographic factors and comorbidities		
Age	1.08	1.07–1.09
Sex (female)	0.58	0.52–0.65
Obesity	1.35	1.20–1.53
Charlson index	1.15	1.09–1.23
Alcoholism	1.10	0.86–1.39
Active smoking	1.05	0.95–1.16
Hypertension	1.10	0.98–1.24
Dyslipidemia	1.04	0.93–1.16
Diabetes mellitus	1.02	0.89–1.17
Cardiac failure	1.06	0.88–1.27
Atrial fibrillation	0.84	0.69–1.04
Acute IHD	0.89	0.73–1.10
Chronic IHD	1.11	0.87–1.41
Peri. vasc. disease	1.04	0.83–1.29
COPD	1.15	0.95–1.38
Asthma	0.75	0.60–0.94
Stroke	1.25	0.97–1.61
Cognitive decline	1.32	1.13–1.55
Depression	1.24	1.07–1.45
CRF	1.18	0.93–1.48
Liver cirrhosis	1.03	0.62–1.68
Anticoagulation	1.30	1.12–1.50
Antiaggregation	1.21	1.06–1.39
Model 1		
SOT	2.46	1.73–3.49
IMID	1.38	1.10–1.72
Model 2		
Corticoids	2.24	1.41–3.55
CNI	1.46	0.84–2.54
Methotrexate	0.86	0.45–1.60
Antimetabolite	1.44	0.89–2.34
mTOR	0.78	0.30–1.97

Model 1: demographic factors, comorbidities, and patient groups. Model 2: demographic factors, comorbidities, and immunosuppressive treatment drugs. All demographic and comorbidity variables are included in both models. Adjusted odds ratio and their 95% confidence intervals (CI) are included.
IHD: ischemic heart disease. COPD: chronic obstructive pulmonary disease. CRF: chronic renal failure. SOT: solid organ transplantation. IMID: immune-mediated inflammatory disease. CNI: calcineurin inhibitor

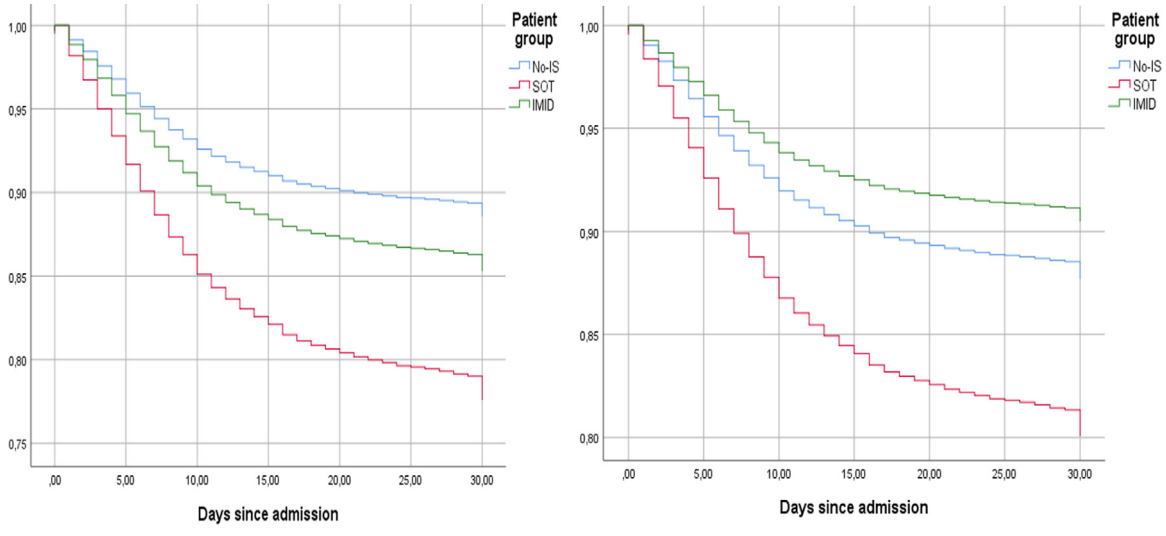

Figure 1. Time to death according to patient group (no-IS, SOT, and IMID).
Kaplan-Meier curves were used to show survival trends, while stratified Cox regression was used to estimate hazard ratios and their 95% confident intervals. (A) Cox regression models were adjusted for sex, age, obesity, cognitive decline, anticoagulation, chronic renal failure, liver cirrhosis, cardiac failure, COPD. HR IMID 1.31 (95% CI 1.11–1.55, $p = 0.002$). HR SOT 2.10 (95% CI 1.63–2.70, $p < 0.001$). (B) Model A plus corticoids. HR IMID 0.86 (95% CI 0.76–1.15, $p = 0.306$). HR SOT 1.69 (95% CI 1.23–2.35, $p = 0.001$). HR corticoid 2.00 (95% CI 1.43–2.79, $p < 0.001$).
IS: immunosuppressed; SOT: solid organ transplant; IMID: immune-mediated inflammatory disease; HR: hazard ratio; COPD: chronic obstructive pulmonary disease

Table 3
Analysis of patients with chronic immunosuppressive treatment at admission, matched by propensity score to non–IS patients

Variable	IS (n = 636)	Non-IS (n = 636)	p	OR	95% CI
Demographic factors and comorbidities					
Age	70 (59–78)	70 (59–78)	1.000		
Sex (male)	47.6% (303)	47.6% (303)	1.000		
Obesity	21.2% (135)	21.2% (135)	1.000		
CCI	1 (0–2)	1 (0–2)	0.102		
Age-adjusted CCI	4 (2–5)	3 (2–5)	0.123		
Alcoholism	3.1% (20)	4.4% (28)	0.190		
Smoking	4.1% (26)	5.0% (32)	0.180		
Hypertension	61.6% (392)	61.6% (392)	1.000		
Dyslipidemia	50.0% (318)	43.6% (277)	0.251		
Diabetes mellitus	13.8% (88)	13.8% (88)	1.000		
CHF	8.8% (56)	8.8% (56)	1.000		
Atrial fibrillation	12.9% (82)	12.3% (78)	0.736		
Acute IHD	8.2% (52)	7.7% (49)	0.836		
Chronic IHD	4.1% (26)	3.8% (24)	0.885		
Peri. Vasc. Dis.	8.0% (51)	7.4% (47)	0.753		
COPD	7.9% (50)	7.9% (50)	1.000		
Asthma	8.2% (52)	8.2% (52)	1.000		
Stroke	4.6% (29)	3.0% (19)	0.185		
Cognitive decline	6.6% (42)	6.6% (42)	1.000		
Depression	12.1% (77)	12.5% (79)	0.865		
CRF	10.1% (64)	10.1% (64)	1.000		
Liver cirrhosis	0.8% (5)	0.8% (5)	1.000		
Antiaggregation	21.1% (134)	20.0% (127)	0.627		
Anticoagulation	13.1% (83)	13.1% (83)	1.000		
Clinical presentations					
Cough	70.9% (457)	68.2% (432)	0.210		
Arthromyalgia	27.3% (172)	30.1% (190)	0.290		
Asthenia	43.1% (271)	42.2% (267)	0.776		
Fever	59.6% (378)	57.2% (362)	0.599		
Dyspnea	56.8% (361)	60.4% (382)	0.190		
Diarrhea	26.4% (167)	23.6% (149)	0.270		
Rx infiltrate	63.9% (403)	67.7% (423)	0.342		
Lymphocytes	0.8 (0.5–1.2)	1.0 (6.9–1.4)	<0.001	1.00	1.00–1.01
CRP	62 (22–129)	68 (18–134)	0.687		
LDH	319 (241–433)	327 (240–442)	0.627		
Ferritin	568 (284–1054)	569 (260–1156)	0.912		
D-dimer	688 (370–1362)	737 (376–1310)	0.487		
Complications and outcomes					
Severe distress	18.7% (119)	20.8% (131)	0.247		
Bact. pneumonia	10.7% (68)	12.6% (80)	0.336		
Sepsis	8.5% (54)	9.0% (57)	0.767		
Septic shock	4.6% (29)	6.8% (43)	0.091	0.83	0.68–1.01
ARI	19.0% (121)	17.6% (112)	0.562		
ACF	7.7% (49)	6.5% (41)	0.444		
Myocarditis	2.2% (14)	1.3% (8)	0.142		
Stroke	0	0.2% (1)	–		
MOF	9.0% (57)	7.2% (46)	0.304		
DIC	1.1% (7)	1.1% (7)	1.000		
ICU admission	7.9% (50)	11.2% (71)	0.045	0.83	0.71–0.98
Hospital mortality	25.0% (159)	21.1% (134)	0.055	1.25	0.99–1.62
COVID-related mortality	93.7% (149/159)	93.2% (123/134)	1.000		

Variables included in propensity score: sex, age, hypertension, obesity, CHF, COPD, asthma, liver cirrhosis, CRF, diabetes mellitus, cognitive decline, and anticoagulation. Only exact matches were allowed. Qualitative variables are expressed as percentage (absolute number). Quantitative variables are expressed as median (interquartile range). Qualitative variables were compared using the chi-squared test. Quantitative variables were compared by the Mann–Whitney U-test. Odds ratios and their 95% confident intervals are provided for variables with p-values less than 0.10.

Pablos et al., 2020; Schulze-Koops et al., 2021). Furthermore, higher mortality rates have been found even in patients taking chronic inhaled glucocorticoids (Schultze et al., 2020). It has been suggested that patients on chronic glucocorticoids have a longer incubation period and present with atypical symptoms (Han et al., 2020), probably due to a decrease in SARS-CoV-2 RNA clearance (Ma et al., 2020). In addition, some authors have found a harmful effect of glucocorticoid treatments in COVID-19 patients when they are administered too soon in the disease's clinical course (Li et al., 2020). This has led to some experts suggesting that glucocorticoids should indeed be administered, but only at the right time (Fernández-Cruz et al., 2021). Our results support the theory that glucocorticoids should only be prescribed in the inflammatory phase of COVID-19 (Griffin et al., 2021; Ngo et al, 2021), as it has been demonstrated that patients treated with chronic glucocorticoids during the initial stages of infection are at high risk of severe COVID-19, complications, and death.

Another point of interest is the hypothesized protective role of calcineurin inhibitors via the suppression of SARS-CoV-2 viral replication (Poulsen et al., 2020). This effect may provide benefits during both the inflammatory and first phases of COVID-19, where there is a predominance of viral replication (Griffin at al., 2021; Ngo et al., 2021). Some authors have reported favorable results for COVID-19 patients treated with cyclosporine (Guisado-Vasco et al., 2020; Gálvez-Romero et al., 2021). It has also been reported that chronic CNI treatment prior to COVID-19 may en-

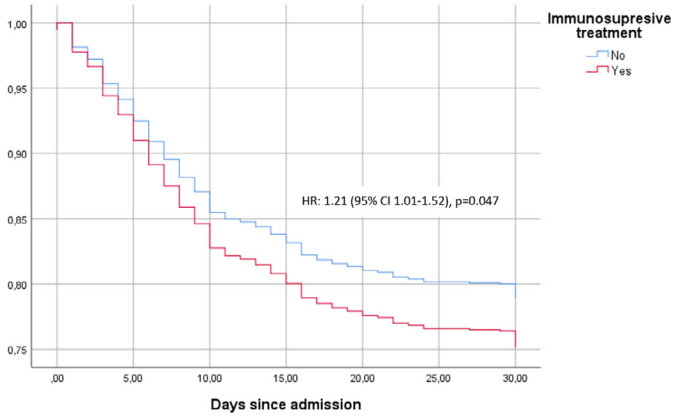

Figure 2. Time to death according to immunosuppresive treatment.
Kapplan-Meier curves were used to show survival trends, while stratified Cox regression was used to estimate hazard ratios and their 95% confident intervals.
HR: hazard ratio; CI: confidence interval

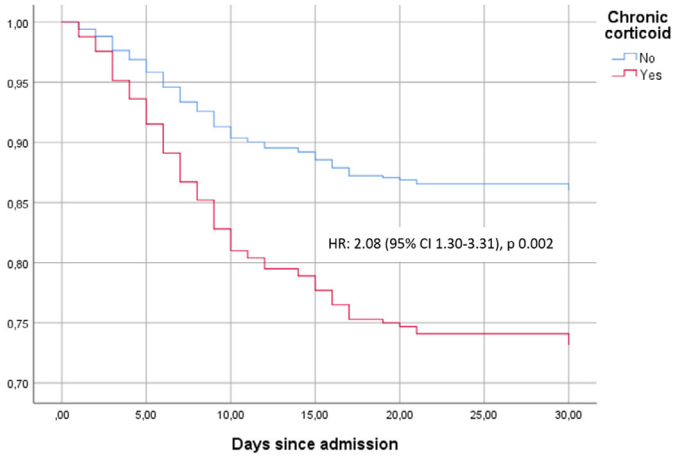

Figure 3. Time to death according to chronic corticoid treatment.
Kapplan-Meier curves were used to show survival trends, while stratified Cox regression was used to estimate hazard ratios and their 95% confident intervals. Cox regression models were adjusted for other IS treatments (including CNI, mTOR inhibitors, and antimetabolites), none of which showed a significant association with time to death.
HR: hazard ratio; CI: confidence interval; CNI: calcineurin inhibitor

tail a better prognosis (Belli et al. 2021). However, other studies have failed to corroborate this finding (Yin et al., 2021). Our data also suggest that CNI treatment is not associated with favorable outcomes. Indeed, SOT recipients on chronic immunosuppressive treatment with CNI at admission presented similar in-hospital mortality to those without CNI. The lack of benefit found could relate to the fact that clinically targeted concentrations of CNI are much lower than those required to inhibit viral replication (Poulsen et al., 2020; Solanich et al., 2021). Therefore, our findings support the idea that immunosuppression with CNI during the early stages of COVID-19 is not associated with favorable outcomes.

Our study did not find higher in-hospital mortality in patients with other immunosuppressive medications, including antimetabolites, methotrexate, mTOR inhibitors, tyrosine-kinase inhibitors, and anti-TNF-alpha monoclonal antibodies. After adjusting for confounding factors, none of these medications was associated with worse outcomes in hospitalized COVID-19 patients. Other authors have also noted that some immunosuppressive medications may not result in more severe COVID-19 disease (Pablos et al., 2021; Schultze et al., 2021; Han et al., 2020). This may be a result of the different biological effects of these medications and/or the different baseline characteristics of the patients receiving the different treatments (Suárez-García et al., 2021; Calderón-Parra et al., 2021; Ward et al., 2021).

Our study showed various strengths associated with a large, multicenter cohort, but it also had several limitations. Firstly, the database was not specifically designed to analyze COVID-19 prognosis in ISPs. Therefore, some relevant variables, such as immunosuppressive medication management during hospital admission, specific IMID condition, and date of transplant in SOT patients, were not available. Secondly, not knowing the cumulative doses of steroids or the dose before the admission was another potential pitfall, since the risk of death might have depended on these (Ward et al., 2021). Thirdly, the low number of non-SOT patients treated with CNI limited the external validity of our conclusion regarding this therapy beyond SOT recipients. Finally, the paucity of patients treated with some drugs, including mTOR inhibitors, tyrosine-kinase inhibitors, anti-TNF-alpha monoclonal antibodies, and anti-CD20 monoclonal antibodies, prevented us from drawing any robust conclusions about the influence of these therapies on COVID-19 clinical outcomes. However, our results emphasized that we should identify and carefully monitor ISPs at special risk of severe COVID-19, which may include SOT recipients and those on chronic glucocorticoid therapy.

CONCLUSION

Immunosuppressant therapies form a heterogeneous group of drugs with different risk profiles for severe COVID-19 and death. While corticosteroids present a well-established benefit during the inflammatory phase in COVID-19, chronic treatment with glucocorticoids at the time of admission entails a special risk of severe COVID-19, complications, and death. On the contrary, chronic CNI treatment at the time of admission does not seem to have any effect on mortality. More studies are needed to clarify the profile of COVID-19 in different immunosuppressed patients, and the influence of specific immunosuppressive drugs on their outcomes.

Potential conflicts of interest

The authors declare no conflicts of interest.

Funding sources

No funding was received for this article.

Ethical approval statement

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Provincial Research Ethics Committee of Málaga (Spain) pursuant to the recommendation of the Spanish Agency of Medicines and Medical Products (AEMPS). All patients gave their informed consent.

Acknowledgements

The authors gratefully acknowledge all the investigators who participated in the SEMI-COVID-19 Registry (supplementary appendix).

Contributions

Jorge Calderon: Study concept and design, statistical analysis, interpretation of results, drafting of manuscript, critical revision of manuscript, approval of final version of manuscript.

Valentin Cuervas-Mons: Study concept and design, interpretation of results, drafting of manuscript, critical revision of manuscript, approval of final version of manuscript.

Victor Moreno-Torres: data acquisition, drafting of manuscript, critical revision of manuscript, approved of final version of manuscript.

All other authors: data acquisition, critical revision of manuscript, approved of final version of manuscript.

Supplementary materials

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.ijid.2021.12.327.

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ANNALS OF TRANSPLANTATION

ORIGINAL PAPER

e-ISSN 2329-0358
© Ann Transplant, 2021; 26: e933152
DOI: 10.12659/AOT.933152

Received: 2021.05.15
Accepted: 2021.08.25
Available online: 2021.10.18
Published: 2021.11.12

Coronavirus Disease 2019 (COVID-19) in Solid Organ Transplant Recipients: A Case-Control Study

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Financial support: None declared
Conflict of interest: None declared

Background: It is unclear whether solid organ transplant (SOT) patients have more severe coronavirus disease 2019 (COVID-19) and worse outcome than the general population.

Material/Methods: We conducted a case-control study on 32 SOT recipients and 84 non-SOT controls matched for age and sex admitted for confirmed COVID-19. The primary endpoint was in-hospital all-cause mortality rate. Secondary endpoints included severe acute respiratory distress syndrome (ARDS), use of high-flow oxygen therapy, and length of hospital stay.

Results: The median (IQR) Charlson comorbidity index (CCI) at admission was significantly higher in SOT recipients (6 (3-8) vs 3 (2-4); *P*<0.01). Fever was less frequent in SOT recipients (78% vs 94%, *P*=0.01). SOT recipients had a higher median SaO2/FiO2 at admission (452 [443-462] vs 443 [419-452], *P*<0.01) and reached the worst SaO2/FiO2 value later during hospitalization 15 (10-21) vs 11 (9-14) days, *P*=0.01). Both groups had a similar severe ARDS rate during hospitalization (33% vs 28%) (p=0.59). There were no significant differences during hospitalization in terms of highest level of respiratory support needed, or length of hospital stay: 8.5 (5.5-21) vs 11.5 (6.5-16.5) days; *P*=0.34) in SOT recipients when compared to controls. In-hospital all-cause mortality rates were significantly higher in SOT recipients (21.9% vs 4.7%, *P*<0.01; OR 1.08; 95% CI 0.10-10.98), but among patients who died, median CCI was similar between groups (8 [6-8] vs 7 [6-8]).

Conclusions: In our experience, hospitalized SOT recipients for COVID-19 had higher in-hospital mortality compared to non-SOT patients, probably due to the greater number of underlying comorbidities, and not directly related to chronic immunosuppression.

Keywords: **Liver Transplantation • Heart Transplantation • Kidney Transplantation • COVID-19 • Lung Transplantation • Severe Acute Respiratory Syndrome Coronavirus 2**

Abbreviations: **COVID-19** – coronavirus disease 2019; **SARS-CoV-2** – severe acute respiratory syndrome coronavirus 2; **ARDS** – acute respiratory distress syndrome; **SOT recipients – solid organ transplant recipients; non-SOT patients** – non-solid organ transplant patients; **rt-PCR** – real-time reverse transcriptase polymerase chain reaction assay; **LDH** – lactate dehydrogenase; **CRP** – C-reactive protein; **IL-6** – interlekin-6; **SaO2/FiO2** – arterial oxygen saturation and inspiratory oxygen fraction ratio; **PaO2** – partial pressure of arterial oxygen; **MMF/MPA** – mofetil/mycophenolic acid; **IQR** – interquartile ranges; **ORs – odds ratio**

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Background

Coronavirus disease 2019 (COVID-19) was first reported in December 2019 when a new strain of coronavirus was isolated and named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2]. Due to the alarming levels of spread and severity of the outbreak, the World Health Organization characterized COVID-19 as a pandemic on March 11th, 2020 [3]. To date, it is reported that 202 608 000 people have been infected with SARS-CoV-2 worldwide and 4 290 000 people have died due to COVID-19 [4]. Studies on the general population suggest that factors linked to severe disease include advanced age, male sex, and underlying comorbidities such as hypertension, obesity, diabetes, chronic kidney disease, chronic lung disease, and coronary heart disease [1,5-9].

Solid organ transplant (SOT) recipients are also at risk of SARS-CoV-2 infection. Whether SOT recipients are at particularly high risk for severe COVID-19 and worse outcome compared with non-transplant (non-SOT) patients is unclear, as the impact of post-transplant chronic immunosuppression on the natural history of COVID-19 is uncertain. On one hand, chronic immunosuppression can increase the viral load, leading to more severe disease, but on the other hand, these drugs can attenuate the inflammatory response linked to cytokine release syndrome [10,11]. Many of the comorbid conditions linked to severe COVID-19 frequently occur among SOT recipients and it is unclear whether these or other potential confounding features, rather than chronic immunosuppression itself, contribute to the risk.

We report the clinical characteristics and outcomes of a cohort of SOT recipients admitted to hospital with COVID-19 compared with a concomitant cohort of non-SOT COVID-19 patients.

Material and Methods

Design of the Study

This was a single-center retrospective study of consecutive SOT recipients admitted to hospital for confirmed COVID-19 from March 1 to May 31, 2020. Non-SOT patients admitted to hospital due to COVID-19 during the same study period, matched according to sex and age, were used as controls. According to the World Health Organization (WHO) COVID severity scale, patients to be included in the study must have ≥3 points in the scale [12].

SARS-CoV-2 infection was confirmed by real-time reverse transcription polymerase chain reaction assay (rt-PCR) in nasopharyngeal swabs specimens. The study was approved by the hospital review board (PI134-20) and informed consent was obtained from all patients to include their clinical information within a database for epidemiological and clinical studies.

Data Collection

Data were retrospectively collected from electronic medical records, and included demographic variables, past medical history, comorbidities, clinical symptoms, physical examination findings, laboratory and diagnostic imaging tests at admission, treatments, in-hospital complications, length of hospital stay, and outcomes (hospital discharge or death).

Age was divided into 3 groups: ≤60 years, 61-70 years, and ≥71 years. Maximum body temperature was stratified as follows: ≤37.3ºC, 37.4-38ºC, 38.1-39ºC, and ≥39.1ºC. Highest level of respiratory support was categorized attending to the maximum request during admission: room air, oxygen supplementation, use of high-flow oxygen therapy or BiPAP, need for mechanical ventilation, and extracorporeal membrane oxygenation (ECMO). In SOT recipients, time from transplant to COVID-19 diagnosis was expressed in years and categorized into 3 groups: <1 year, 1-5 years, and >5 years.

Arterial oxygen saturation and inspiratory oxygen fraction ratio (SaO2/FiO2) was calculated by pulse oximetry. SaO2/FiO2 has a good correlation with the partial pressure of arterial oxygen ratio (PaO2/FiO2) [13]. The worst registry of respiratory situation on medical records during hospitalization was defined by the highest level of respiratory support that the patient needed. Time from symptom onset to the highest respiratory support date was measured in days.

Outcomes and Study Definitions

The primary endpoint was in-hospital all-cause mortality. Secondary endpoints included severe acute respiratory distress syndrome (ARDS) defined as SaO2/FiO2 ratio of 148 (PaO2/FiO2 ratio of 100) [14,15], and length of hospitalization. The outcomes from SOT recipients were compared with those of non-SOT patients.

Immunosuppressive Treatment Approach

According to the general approach in our center, blood levels of tacrolimus, cyclosporine, everolimus, and sirolimus levels were reduced or mantained, and mycophenolate mofetil/mycophenolic acid was reduced or temporally discontinued according to the criteria of the treating physician. The baseline dose of steroids was not modified.

Statistical Analysis

Quantitative variables are expressed as medians with interquartile ranges (IQR) and qualitative variables as counts and percentages. The Mann-Whitney U test and chi-square test were used to compare differences between the SOT recipient group

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and the non-SOT recipient group, as appropriate. The association of transplantation and the primary endpoint was assessed through conditional logistic regression to compute odds ratios (ORs) and their 95% confidence intervals (CI). The statistical significance level was set at two-sided *P* value of <0.05. Statistical analyses were performed using the STATA system.

Results

We identified 1494 consecutive adult patients hospitalized with confirmed COVID-19 in our center during the study period. Thirty-two (2.1%) of the 1494 patients were SOT recipients (78.1% males, median age 66.5 years [IQR 63.5-72]). Of the remaining 1462 patients, 84 non-SOT patients that were not under immunosuppressive therapy, matched according to sex and age, were used as controls (79.8% males, median age 65.5 years [IQR 60.5-70.5], *P*=0.40). Characteristics of patients are shown in **Table 1**.

The median time from symptoms onset to hospital admission was 6 days (IQR 4-9), without differences between groups. The median Charlson comorbidity index (CCI) for the overall study population was 3 (IQR 2-5.5). Median CCI was significantly higher in the SOT recipients (6 [IQR 3-8] vs 3 [IQR 2-4]; *P*<0.01).

The most common presenting symptoms were fever, cough, and dyspnea. Fever was less frequent in SOT recipients (78% vs 94%, *P*=0.01). No significant differences between SOT recipients and controls were found for cough (62.5% in SOT recipients vs 76%; *P*=0.14) and dyspnea (44% in SOT recipients vs 54%, *P*=0.34). All patients presented pneumonia at admission. SOT recipients had a higher median SaO2/FiO2 at admission (452 [IQR 443-462] vs 443 [IQR 419-452], *P*<0.01) and reached the worst SaO2/FiO2 value later during hospitalization (15 days [IQR10-21] vs 11 days [IQR 9-14], *P*=0.01).

Biochemical findings upon admission are shown in **Table 2**. Significant differences between SOT recipients and controls were found for serum creatinine (1.7 mg/dl [1.1-3.0 mg/dl]) vs 0.8mg/dl [0.6-1.0 mg/dl], *P*<0.001). Blood cell count and inflammatory biochemical parameters (CRP, serum ferritin, and IL-6) were comparable.

Severe ARDS was developed in 37 (31.9%) patients overall and was similar between the 2 groups (SOT recipients 28.1% vs 33.3%, *P*=0.59). There were no significant differences during hospitalization in terms of highest level or respiratory support needed or length of hospital stay in SOT recipients when compared to controls (**Table 3**).

The in-hospital all-cause mortality rate was significantly higher in SOT recipients than in the control group (21.9% vs 4.7%, *P*<0.01). It is noteworthy that among patients who died, the median CCI score was similar between groups (SOT recipients 8 [IQR 6-8] vs 7 [IQR 6-8]. Four out of the 7 SOT recipients who died were older than 70 years compared with 2 out of the 4 controls (*P*=0.819). COVID-19-related lung disease was the main cause of death in both groups. The relatively small sample size of SOT recipients did not allow further risk stratification analysis. Conditional logistic regression was performed, but due to the low number of events, it cannot be inferred that immunosuppression and/or being a SOT recipient are conditioning factors of higher risk of mortality (OR 1.08; 95% CI 0.10-10.98).

Among SOT recipients, 11 (34%) received a liver, 9 (28%) received a kidney, 7 (22%) received a heart, and 5 (16%) received a lung. The median time from transplant to hospital admission for COVID-19 was shorter in lung transplant recipients (3.13 years [IQR 0.68-9.85]) and longer in liver transplant recipients (13.25 [IQR 3.71-15.94] years). Baseline immunosuppression regimen and management of immunosuppressive drugs after COVID-19 diagnosis are described in **Table 4**. Tacrolimus (n=21; 65.6%), mycophenolate mofetil (n=21; 65.6%), and steroids (n=21; 65.6%) were the predominant immunosuppressants. Among COVID-19 diagnosis, immunosuppressive regimens were modified in all patients. Tacrolimus dose was reduced in 10 of the 21 patients, discontinued in 4 patients, and remained unchanged in 7 patients. Cyclosporine A dose was reduced in 1 out of 5 patients, discontinued in 1 patient, and unchanged in 3 patients. Mycophenolate mofetil dose was reduced in 6 patients and discontinued in 15 of the 21 patients (71%). There were no graft rejection episodes during the study period.

Discussion

In the present study, days elapsed from symptoms onset to hospital admission were comparable between groups, which allowed us to compare COVID-19 disease evolution over time in SOT recipients and in controls. SOT recipients were less likely to have fever at admission (78% vs 94%) and had lower body temperature values during hospitalization. This fact is well known in SOT recipients, and it is related to the anti-inflammatory effect of immunosuppressive drugs. Dyspnea, cough, inflammatory biochemical parameters values at admission, and risk of developing severe ARDS in SOT recipients were similar to controls. Elapsed time from admission to worst SaO2/FiO2 value during hospitalization was greater in SOT recipients (15 days [IQR10-21] vs 11 days [IQR 9-14]; *P*=0.01). These data suggest that the clinical course among patients hospitalized due to COVID-19 is similar and evolves slower compared with a general population matched according to sex and age, contrary to the results of other series [16].

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Table 1. Epidemiological and clinical characteristics.

	All patients (N=116)	SOT-recipients (N=32)	No-SOT patients (N=84)	p
Epidemiological characteristics				
Age				
Median (IQR)	66 [61.5-71]	66.5 [63.5-72]	65.5 [60-70.5]	0.40
Distribution (%)				
<60 years	28 (24.1)	6 (18.7)	22 (26.2)	
61-70 years	58 (50)	17 (53.1)	41 (48.8)	
>71 years	30 (25.9)	9 (28.1)	21 (25)	
Female gender (%)	24 (20.7)	7 (21.9)	17 (20.2)	0.84
Hypertension (%)	65 (56)	21 (65.6)	44 (52.4)	0.2
Diabetes (%)	34 (29.3)	14 (43.7)	20 (23.8)	0.03
Chronic heart failure (%)	15 (12.9)	10 (31.2)	5 (5.9)	<0.01
Coronary heart disease (%)	11 (9.5)	5 (15.6)	6 (7.1)	0.16
Chronic obstructive pulmonary disease (%)	16 (13.8)	8 (25)	8 (9.5)	0.03
Chronic renal disease (%)	23 (19.8)	20 (62.5)	3 (3.6)	<0.01
Median Charlson Index (IQR)	3 [2-5.5]	6 [3-8]	3 [2-4]	<0.01
Clinical characteristics				
Days from clinical onset to admission (IQR)	6 (4-9)	6.5 [3-9.5]	6 [4-8]	0.79
Fever on admision (%)	104 (89.7)	25 (78.1)	79 (94.1)	0.01
Maximum temperature during hospitalization				0.15
<37.3ºC (%)	15 (12.9)	7 (21.9)	8 (9.5)	
37.4-38ºC (%)	37 (31.9)	12 (37.5)	25 (29.8)	
38.1-39ºC (%)	55 (47.4)	12 (37.5)	43 (51.2)	
>39ºC (%)	19 (7.7)	1 (3.1)	8 (9.5)	
Dry cough	84 (72.4)	20 (62.5)	67 (76.2)	0.14
Dyspnea	59 (50.9)	14 (43.7)	45 (53.6)	0.34
Diarrhea	24 (20.7)	10 (31.2)	14 (16.7)	0.08
Myalgia or arthralgia	42 (36.2)	11 (34.4)	31 (36.9)	0.8
SaO2/FiO2 on admision	447.62 [428.6-457.1]	452.38 [442.9-461.9]	442.86 [419.1-452.4]	<0.01
Severe ARDS during admision (%)	37 (31.9)	9 (28.1)	28 (33.3)	0.59
Highest level of respiratory support (%)				
Room air	28 (24.1)	12 (37.5)	16 (19.1)	0.07
Nasal cannula	45 (38.8)	11 (34.4)	34 (40.5)	
Non-rebreather-mask	20 (17.2)	3 (9.4)	17 (20.2)	
High flow or BiPAP	9 (7.7)	5 (15.6)	4 (4.8)	0.12
Intubation	14 (12.1)	1 (3.1)	13 (15.5)	0.13
Days to worst respiratory parameters since clinical onset	11 [9-15]	15 [10-21]	11 [9-14]	0.01

Results are expressed as mean±standard deviation and number (percentage). SOT – solid organ transplant; IQR – interquartile ranges; SaO2/FiO2 – arterial oxygen saturation and inspiratory oxygen fraction ratio; BiPAP – bilevel positive airway pressure.

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Table 2. Laboratory findings.

	All patients (N=116)	SOT-recipients (N=32)	No-SOT patients (N=84)	p
Laboratory findings on admision (IQR)				
Creatinine – mg/dl	0.9 [0.7-1.2]	1.7 [1.1-3.0]	0.8 [0.6-1.0]	<0.01
Bilirrubin – mg/dl	0.5 [0.4-0.8]	0.5 [0.4-0.6]	0.5 [0.3-0.8]	0.64
AST – U/L	35.5 [27-44]	31.5 [22.5-42]	36.5 [29-51]	0.03
ALT – U/L	24.5 [18-36]	24 [17-33]	25 [19-39]	0.28
GGT – U/L	45.5 [32-87]	54 [36-66.5]	44.5 [31-93]	0.62
ALP – U/L	65 [56-88]	72 [55-93]	64 [56.5-78.5]	0.21
LDH – U/L	277 [225-343.5]	263 [226-336]	279 [224-347]	0.58
Serum ferritin ng/ml	797 [458-1156]	700.5 [404-1146.5]	813 [480-1261]	0.47
CRP – mg/L	73.4 [34-156.7]	68.1 [31.3-139.3]	85 [34.3-160]	0.38
IL6 – pg/ml	44.5 [17.9-93.4]	38.85 [16.8-100.9]	46.86 [17.4-93.4]	0.86
Leukocytes 10^3/µL	6.3 [4.4-7.9]	6.56 [4.3-7.6]	6.06 [4.5-8.3]	0.73
Lymphocytes 10^3/µL	0.9 [0.6-1.2]	0.9 [0.6-1.6]	0.96 [0.7-1.2]	0.86
Platelets 10^3/µL	169 [136-220]	153.5 [131-206]	171 [142-223]	0.19
D-dimer – µg/ml	0.7 [0.5-1.3]	0.7 [0.4-1.1]	0.7 [0.5-1.3]	0.61
INR	1.1 [1.0-1.2]	1.0 [1.0-1.3]	1.1 [1.0-1.1]	0.03
Laboratory findings on 7th day of hospitalization (IQR)				
Creatinine (mg/dl)	0.8 [0.7-1.1]	1.3 [1-1.9]	0.8 [0.6-1]	<0.01
Bilirrubin (mg/dl)	0.7 [0.4-0.9]	0.4 [0.3-0.7]	0.7 [0.5-0.9]	<0.01
AST – U/L	35 [26.5-49]	33 [27-47]	35.5 [26-49]	0.80
ALT – U/L	35 [22.5-58.5]	34.5 [16-46]	36 [24-66]	0.23
GGT – U/L	65 [42-125]	68 [46-111]	65 [42-125]	0.88
ALP – U/L	60 [53-92]	60 [56-93]	60 [49-91]	0.58
LDH – U/L	300 [239-431]	264 [235-438]	306 [242-417]	0.83
Serum ferritin ng/ml	915 [486-1453]	962 [417-1608]	915 [491-1439]	0.95
CRP – mg/L	34.5 [15.4-107]	29.15 [13.1-80.7]	35.3 [16.2-117.1]	0.35
IL6 – pg/ml	119.4 [10-570.3]	226 [4.7-660]	91.75 [10-511.6]	0.32
Leukocytes 10^3/µL	7.0 [4.7-10.3]	4.6 [3.5-7.1]	7.9 [5.3-10.8]	<0.01
Lymphocytes 10^3/µL	0.9 [0.6-1.3]	0.7 [0.4-1.3]	1 [0.7-1.4]	0.07
Platelets 10^3/µL	267 [198-339.5]	184 [143-291]	278 [228-347]	<0.01
D-dimer – µg/ml	1.0 [0.5-1.8]	0.8 [0.5-1.5]	1 [0.5-2]	0.44
INR	1.1 [1-1.2]	1.1 [1-1.2]	1.1 [1-1.1]	0.35

Results are expressed as mean±standard deviation. AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT – gamma-glutamyl transferase; ALP – alkaline phosphatase; LDH – lactate dehydrogenase; CRP – C-reactive protein; IL-6 – interleukin 6; INR – international normalized ratio.

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Table 3. Primary and secondary outcomes.

	All patients (N=116)	SOT-recipients (N=32)	No-SOT patients (N=84)	p
Mortality (%)	11 (9.5)	7 (21.9)	4 (4.8)	<0.01
Severe ARDS (%)	37 (31.9)	9 (28.1)	28 (33.3)	0.59
Days of hospitalization	10.5 [6-17]	8.5 [5.5-21]	11.5 [6.5-16.5]	0.34

Results are expressed as mean±standard deviation and number (percentage). ARDS – acute respiratory distress syndrome.

Table 4. Baseline characteristics of solid organ transplant recipients.

	All transplant recipients N=32	Liver recipients N=11 (34.37)	Kidney recipients N=9 (28.12)	Heart recipients N=7 (21.87)	Lung recipients N=5 (15.62)
Median age (IQR)	66.5 [63.5-72]	68 [63-69]	68 [64-74]	67 [56-72]	64 [64-66]
Years from transplant to diagnosis					
<1 year (%)	4 (12.5)	1 (9.1)	1 (11.1)	0	2 (40)
1-5 years (%)	9 (28.1)	2 (18.9)	3 (33.3)	3 (42.8)	1 (20)
>5 years (%)	19 (59.4)	8 (72.7)	5 (55.6)	4 (57.1)	2 (40)
Baseline Immunosuppresant (%)					
Tacrolimus	21 (65.6)	6 (54.5)	7 (77.8)	3 (42.8)	5 (100)
Ciclosporine A6	5 (15.6)	1 (9.1)	1 (11.1)	3 (42.8)	0
Mycophenolate	21 (65.6)	5 (45.45)	8 (88.9)	5 (71.4)	3 (60)
Everolimus	5 (15.6)	3 (27.3)	0	1 (14.3)	1 (20)
Steroids	21 (65.6)	0	9 (100)	7 (100)	5 (100)
Changes in immunosuppression (%)					
Decrease or hold CNI	17/26 (65.4)	4/7 (57.1)	8/8 (100)	2/6 (30)	3/5 (60)
Decrease or hold mycophenolate	15/21 (71.4)	0	7/8 (87.5)	5/5 (100)	2/3 (66.7)
Decrease or hold steroids	2/21 (9.5)	0	0	0	2/5 (40)
Primary outcome					
Mortality (%)	7 (21.9)	2 (18.2)	1 (11.1)	3 (42.8)	1 (20)

Results are expressed as mean±standard deviation and number (percentage). IQR – interquartile ranges.

The in-hospital mortality rate in SOT recipients in our series was 21.9%, similar to the rates of 4.8-37% described by others [16-36]. To date, several articles related with COVID-19 mortality in transplant patients have been published, but their results are variable and heterogeneous. Discarding the studies that analyzed a single organ type of transplant and hematopoietic transplant, most of them demonstrate high mortality rates [17-24]. However, it is noteworthy that some of these studies did not compare their results with the non-transplanted population [17-20], while others did not find such high mortality rates or any differences compared with the non-transplanted population [16,25-30]. Mortality rates in these series were affected by age, number of underlying comorbidities, and by the population analyzed (hospitalized patients only [16,21,24,30] or also outpatients [17-20,22-23,25-28]). In our opinion, inclusion of outpatients can bias the comparison between both groups, since transplant patients are usually closely monitored, which makes it more likely to diagnose mild or asymptomatic cases. The study and follow-up periods were also heterogeneous among publications, which makes interpretation of

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results difficult, especially considering how fast the protocols were changing for this group of patients in recent months. Some studies adjusted the comparison of groups according to their comorbidities but not to the Charlson index [23]. In our series, mortality during admission was almost 4 times higher in SOT recipients (21.9% vs 4.7%). The lower mortality rate observed in our study in non-SOT patients compared to other series could be explained by the low number of patients between 71 and 79 years (25%) and older than 80 years (4.16%) in our cohort. Mortality for these age groups is closer to that reflected in other series, at 12% and 40%, respectively [31].

To date, some studies suggested that elapsed time from transplantation is a risk factor linked to higher mortality in patients hospitalized for COVID-19 [32-34], with higher mortality in patients transplanted more than 10 years ago, with reduced immunosuppression, higher comorbidities, and age over 65 years [34]. In our series, of the 7 dead SOT patients, 29% had been transplanted less than 5 years ago, 14% 5-10 years ago, and 57% more than 10 years ago. This higher mortality in long-term survival SOT recipients could be explained by the high rate of comorbidities in this population, conditioned by chronic immunosuppressive treatment and other pathologies, some of which were the direct cause of the need for transplant. Some studies have shown a worse course of infection as the CCI increases [35]. In our series, among the 7 SOT recipients who died, the median CCI was 8 points (6-8) and 4 were older than 70 years. Non-SOT patients who died had a median of 7 points (6-8) in CCI, and 2 out of 4 were older than 70 years. All of them had severe ARDS.

The strengths of this study are that the same criteria were used for all the patients, for hospitalization and similar treatment strategy, and modifications in immunosuppressant treatment. Nevertheless, we are aware that our study suffers from some limitations, mainly due to the retrospective design and the small number of SOT recipients, which prevent forming strong conclusions on the efficacy of the optimal management of immunosuppression in SOT recipients with COVID-19.

Conclusions

Despite the published literature, there are still many gaps in knowledge about the relationship between COVID-19 and solid organ transplantation. There is no doubt that these are high-risk patients due to their close contact with the hospital environment, but the role of immunosuppression and the best management options are still unknown. The results in different publications regarding mortality due to COVID-19 in transplant patients are heterogeneous, probably due to the difficulty of knowing the degree of immunosuppression of each patient and the different treatment regimens available. We contribute with a new series of admitted transplant patients compared with non-transplanted patients of the same age and sex during a period of time in which the admission criteria and the management protocol were the same for both groups.

In our experience, SOT recipients hospitalized for COVID-19 had higher in-hospital mortality compared to non-SOT patients, probably due to greater underlying comorbidities and not directly related to chronic immunosuppression. No differences were found between groups in clinical course, severe ARDS, length of hospital stay, and highest level or respiratory support needed. However, the patients who died in both groups had an elevated and similar CCI, suggesting the high mortality risk of comorbidity. In our series, the CCI was higher in transplant patients, which could explain the higher mortality in this group.

Patient Permission/Consent

The study was approved by the hospital review board (PI134-20) and informed consent was obtained from all patients to include their clinical information within a database for epidemiological and clinical studies.

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Indexed in: [Science Citation Index Expanded] [Index Medicus/MEDLINE] [Chemical Abstracts] [Scopus]

Vascular Pharmacology 143 (2022) 106955

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Interaction of ACEI antihypertensive agent's administration with the inflammatory status at admission concerning COVID-19 clinical stay outcomes

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A B S T R A C T

Interactions between anti-hypertensive agents (ACEI), comorbidities, inflammation, and stress status may impact hospital stay duration in COVID-19 patients. This retrospective study analyzed epidemiological data, comorbidities, metabolic/inflammatory markers, and clinical information from 165 SARS-CoV-2 positive patients. In a multiple linear regression model, an IL-6 higher than 100 mg/L, glucose at admission (baseline levels at the hospital entry), and the interaction between ACEI administration and LDH predicted the days of hospital admission ($P < 0.001$). In conclusion, hypertensive patients suffering more severe inflammatory condition assessed by LDH levels clinically benefited more and reduced the hospital stay when prescribed ACEI agents than those with lower systemic baseline inflammation at admission.

1. Introduction

The coronavirus disease 2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), outreached in China at the end of 2019, with a later rapid worldwide extension, and a later surge in the case lethality ratio, pathogenesis and transmissibility, in most countries [1]. The viral infectious incidence and severity depend on the presence of associated comorbidities or metabolic and inflammatory-related diseases [2,3]. In this context, obesity and hypertension (HT) are associated with unfavorable evolution of the COVID-19 disease, with a high probability to develop severe pneumonia and impaired inflammatory reactions, in addition to organ and tissue damage. These outcomes were also related to increases in stay length, intensive care unit (ICU) stay, and mortality, as described elsewhere [4,5]. Covid-19 patients display inflammatory complications that have been related to a "cytokine storm" involving exacerbated blood interleukin 6 (IL-6), C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), neutrophil/lymphocyte index (NLI), D-Dimer, and red cell distribution width (RDW) levels. Furthermore, consistent associations between hospital stay days and ICU admission implications concerning prescribed therapies and clinical outcomes were found as described elsewhere [6]. During the COVID-19 pandemic, several therapeutic approaches have been tested to fight against this viral disease [7], including the frequent administration of drugs belonging to Angiotensin-converting enzyme inhibitors (ACEI) group. Given the high prevalence of hypertension in severe cases of COVID-19 and the known clinical associations of pathogenic and inflammatory mechanisms accompanying SARS-CoV-2 infection, which may interfere with the administration of anti-hypertensive pharmacological agents (i.e. ACEI), the current research targeted in analyzing interactions of stay duration days at the hospital (dependent variable) with obesity, hypertension, the inflammatory status at admission based on IL-6 and LDH measurements and the metabolic stress status based on glycemia values (independent variables).

2. Materials and methods

This retrospective study was based on a series of 165 subjects

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https://doi.org/10.1016/j.vph.2022.106955
Received 12 July 2021; Received in revised form 8 December 2021; Accepted 16 January 2022
Available online 20 January 2022

consecutively admitted at the Puerta de Hierro Majadahonda University Hospital (Madrid, Spain) between March 15 and April 15, 2020. The following inclusion criteria were considered: patients >18 years old with positive PCR for SARS-CoV-2, moderate/severe pneumonia according to WHO guidelines, oxygen saturation rate < 94%, and respiratory rate > 22 breaths per minute. Patients with COVID-19 at 90 days before or those with incomplete clinical/metabolic information were excluded from the analyses. The variables recorded included administrative and epidemiological data, comorbidities, diagnosis examinations and information about the evolution of the disease following accepted standards [8]. Comorbidities and complications during hospital stay were defined as diagnoses included in the clinical history at admission and at discharge. Systolic and diastolic blood pressures (SBP and DBP, respectively) were recorded by the center's triage system upon arrival at the emergency room. HT was established as: SBP >140 mmHg and DBP >90 mmHg. Baseline blood cholesterol, glucose, inflammatory markers (IL-6, CRP, LDH), and liver enzymes (AST: aspartate aminotransferase; ALT: alanine transaminase) were collected from the first analysis performed during hospital admission. Height and weight were self-reported in all cases, with subsequent calculation of the Body Mass Index (BMI: kg/m^2). Obesity was setup as BMI ≥30 kg/m^2. The data record was developed through information collected in the electronic medical record (SELENE System, Cerner Iberia, S.L.U, Madrid, Spain) filling in a template form previously established by the main investigators of this study. The laboratory analyzes were carried out by the Biochemistry Service of the center, according to validated analytical protocols. Triage was performed according to the Manchester scale [9], as a standard procedure in our center. Implementation was reviewed by two expert investigators to ensure the validity of the protocol. The Research Ethics Committee of the Puerta de Hierro Majadahonda University Hospital approved the study (PI 94/20). All the participating subjects gave their informed consent prior to its inclusion. This work respects the guidelines of Spanish and European Laws, as well as the Declaration of Helsinki, declaring no conflicts of interests or external sources of funding concerning this investigation. Quantitative variables are expressed as means and standard deviations, while qualitative variables are expressed as frequencies and proportions. Clinical and phenotypical characteristics of COVID-19 patients were compared by categorical variables or by the median values of continuous variables. For this last purpose, the reference values of IL-6 and LDH considered in our design are compatible with previous reports exploring laboratory predictors and prognostic factors of severe COVID-19 disease concerning respiratory functions [10,11] as well as pharmacological prescription making decision criteria [12]. Moreover, Chi-square and unpaired Student's *t*-tests were applied for the univariate analysis. Multivariate regression analyses were used to predict stay duration days at the hospital, which were adjusted for baseline characteristics of the population and the day of extraction and including appropriate product terms. Eventual statistical interactions between clinical, inflammatory, and pharmacological markers were evaluated by multivariate regression analyses using product-term variables in the model. Statistical analyses were performed in the STATA software (version 12.1 for Windows, Texas, USA). P values (two-tailed) below 0.05 were considered statistically significant. P values (two-tailed) below 0.10 were considered as marginal significance.

3. Results

The clinical and phenotypical characteristics of COVID-19 patients based on the presence or absence of obesity, HT, and ACEI administration are reported (Table 1). As expected, patients with obesity underwent higher frequencies of ICU stay/mortality as well as greater blood levels of glucose at admission than their lean counterparts (Table 1). Moreover, statistical tendencies (P < 0.10) to display elevated levels of LDH, CRP and higher frequencies of increased IL-6 were found in subjects with obesity, although these were at a marginal significance (P < 0.10). Meanwhile, patients with diagnosed HT were statistically older and had a higher AST:ALT ratio as well as marginally greater serum levels of total cholesterol, CRP, DBP, ALT, and elevated IL-6 than normotensive individuals (Table 1). Instead, no statistical differences were found in liver markers neither inflammatory features when compared HT patients with ACEI prescription that those who were not under this treatment.

The demographic, clinical and biochemical characteristics of the total COVID-19 sample based on cutoffs of IL-6 (clinical criteria) and median values of LDH (statistical criteria) are reported (Table 2). Patients with blood levels of IL-6 greater than 100 mg/L had significantly shorter days of admission, but higher frequencies of ICU stay/mortality than those with IL-6 concentrations equal or lower than 100 mg/L (Table 2). In addition, increased levels of glucose at admission, CRP and ALT were identified in subjects with elevated IL-6 (Table 2). Similarly, older age and greater AST:ALT ratio were found in this same group, although at marginal (P < 0.10) significance (Table 2). Patients with LDH values above the median (332.5 IU/L) presented higher levels of LDH, AST, AST:ALT ratio as well as a tendency (P < 0.10) to have

Table 1
Clinical and phenotypical characteristics of COVID-19 patients based on obesity, hypertension and ACEI administration.

	Obesity			HT			HT + ACEI		
Variable	No (n = 103)	Yes (n = 51)	P	No (n = 99)	Yes (n = 66)	P	No (n = 17)	Yes (n = 49)	P
Age	63.7 ± 11.5	61.4 ± 11.9	0.259	59.1 ± 11.2	68.4 ± 9.9	**<0.001**	69.4 ± 9.0	68.0 ± 10.3	0.612
Sex (F/M)	36/67	18/33	0.967	39/60	17/49	0.070	5/12	12/37	0.689
Days of admission	3.90 ± 2.76	3.42 ± 2.53	0.307	3.76 ± 2.60	3.98 ± 2.87	0.616	4.31 ± 3.16	3.87 ± 2.79	0.599
No ICU/No mortality	88 (85.4)	35 (68.6)	**0.014**	80 (80.8)	50 (75.8)	0.437	13 (76.5)	37 (75.5)	0.937
ICU/mortality	15 (14.6)	16 (31.4)		19 (19.2)	16 (24.2)		4 (23.5)	12 (24.5)	
Glucose at admission (mg/dL)	118.7 ± 48.6	145.2 ± 64.7	**0.007**	124.9 ± 56.8	137.5 ± 61.7	0.200	125.1 ± 65.7	141.7 ± 60.5	0.355
Total cholesterol (mg/dL)	138.1 ± 31.4	145.4 ± 34.2	0.270	144.5 ± 34.7	132.9 ± 25.8	0.053	133.4 ± 34.9	132.7 ± 23.2	0.942
LDH (IU/L)	346.1 ± 134.0	378.1 ± 148.8	0.097	352.3 ± 153.4	366.3 ± 117.6	0.539	387.0 ± 128.1	358.8 ± 114.1	0.402
CRP (mg/L)	120.4 ± 109.4	158.2 ± 205.6	0.073	118.1 ± 106.2	155.4 ± 186.2	0.055	159.5 ± 69.6	154.1 ± 210.5	0.923
IL-6 (0–100 mg/L)	74 (71.8)	31 (60.8)	0.083	70 (70.7)	40 (60.6)	0.089	10 (58.8)	30 (61.2)	0.861
IL-6 (>100 mg/L)	29 (28.2)	20 (39.2)		29 (29.3)	26 (39.4)		7 (41.2)	19 (38.8)	
SBP (mmHg)	138.9 ± 21.3	137.7 ± 22.4	0.748	136.7 ± 20.4	139.6 ± 23.9	0.402	145.7 ± 19.3	137.5 ± 25.2	0.224
DBP (mmHg)	79.3 ± 14.2	79.0 ± 13.9	0.895	80.3 ± 13.4	77.3 ± 15.2	0.094	79.0 ± 10.9	76.7 ± 16.4	0.593
D-dimer (μg/mL)	1.86 ± 6.85	1.55 ± 2.01	0.757	1.93 ± 7.00	1.43 ± 1.78	0.580	1.28 ± 0.77	1.48 ± 2.03	0.683
AST (IU/L)	53.4 ± 35.2	46.9 ± 19.4	0.223	52.5 ± 33.0	48.9 ± 25.8	0.456	53.0 ± 28.1	47.5 ± 25.2	0.467
ALT (IU/L)	42.9 ± 34.4	37.7 ± 21.1	0.333	43.6 ± 32.3	35.9 ± 25.4	0.055	36.8 ± 21.9	35.6 ± 26.6	0.879
AST:ALT ratio	1.46 ± 0.84	1.40 ± 0.56	0.640	1.35 ± 0.45	1.61 ± 1.02	**0.029**	1.49 ± 0.31	1.64 ± 1.17	0.609

Values are expressed as means ± standard deviations. P values were calculated by Student's T-tests. Bold numbers indicate P value lower than 0.05. F: female; M: male; HT: hypertension; ACEI: Angiotensin Converting Enzyme Inhibitors; LDH: lactate dehydrogenase; IL-6: interleukin 6; CRP: C-reactive protein; SBP: systolic blood pressure; DBP: diastolic blood pressure; AST: aspartate aminotransferase; ALT: alanine transaminase.

Table 2
Clinical and phenotypical characteristics of COVID-19 patients based on cut-offs of IL-6 and median values of LDH.

	All	IL-6			LDH		
Variable	n = 165	0–100 mg/L (n = 110)	>100 mg/L (n = 55)	P	<332.5 IU/L (n = 77)	≥332.5 IU/L (n = 77)	P
Age	62.8 ± 11.6	61.5 ± 11.3	65.3 ± 11.9	0.051	63.1 ± 12.3	63.1 ± 10.9	0.983
Sex (F/M)	56/109	38/72	18/37	0.816	29/48	23/54	0.307
Days of admission	3.85 ± 2.70	4.27 ± 2.76	2.96 ± 2.35	**0.004**	3.74 ± 2.57	3.88 ± 2.88	0.758
No ICU/No mortality	130 (78.8)	101 (91.8)	29 (52.7)	**<0.001**	61 (79.2)	59 (76.6)	0.698
ICU/mortality	35 (21.2)	9 (8.2)	26 (47.3)		16 (20.8)	18 (23.4)	
Glucose at admission (mg/dL)	130.2 ± 59.1	119.9 ± 42.7	148.1 ± 77.0	**0.004**	121.2 ± 53.7	138.4 ± 64.1	0.087
Total cholesterol (mg/dL)	140.1 ± 32.1	142.6 ± 31.3	135.3 ± 33.2	0.246	141.5 ± 30.5	135.2 ± 32.0	0.298
LDH (IU/L)	358.1 ± 139.4	362.4 ± 150.2	350.1 ± 117.6	0.603	253.2 ± 57.3	463.1 ± 116.2	**<0.001**
CRP (mg/L)	132.8 ± 143.7	107.7 ± 73.9	182.1 ± 217.8	**0.002**	127.2 ± 193.9	150.0 ± 70.7	0.342
SBP (mmHg)	137.8 ± 21.9	137.2 ± 20.7	139.2 ± 24.2	0.578	136.4 ± 22.7	139.1 ± 21.2	0.439
DBP (mmHg)	79.1 ± 14.1	79.3 ± 14.0	78.6 ± 14.5	0.749	77.6 ± 14.4	80.6 ± 12.4	0.080
D-dimer (μg/mL)	1.71 ± 5.46	1.78 ± 6.58	1.60 ± 2.11	0.846	0.96 ± 0.98	2.53 ± 7.68	0.084
AST (IU/L)	51.1 ± 30.3	53.1 ± 34.4	47.2 ± 20.1	0.240	41.1 ± 23.3	59.7 ± 30.7	**<0.001**
ALT (IU/L)	40.6 ± 29.9	45.0 ± 34.5	32.0 ± 15.0	**0.009**	35.0 ± 25.6	43.6 ± 30.3	0.059
AST:ALT ratio	1.45 ± 0.74	1.38 ± 0.83	1.58 ± 0.53	0.057	1.31 ± 0.39	1.63 ± 0.97	**0.009**

Values are expressed as means ± standard deviations. P values were calculated by Student's T-tests. Bold numbers indicate P value lower than 0.05. F: female; M: male; LDH: lactate dehydrogenase; IL-6: interleukin 6; CRP: C-reactive protein; SBP: systolic blood pressure; DBP: diastolic blood pressure; AST: aspartate aminotransferase; ALT: alanine transaminase.

elevated glucose at admission, DBP, D-dimer, and ALT than individuals below the median value. About 75% of patients had been treated with corticoids at hospital admission, with an increasing proportional trend in those with higher IL-6 concentrations (about 63% in patients with 0–100 mg/dL and 96% in those with >100 mg/dL). Interestingly, regression analyses including corticoid therapy demonstrated the same statistical trends. The multiple linear regression model that predicted the days of admission in COVID-19 patients using clinical, inflammatory, and pharmacological variables is reported (Table 3). Noteworthy, increased IL-6 (>100 mg/L), glucose at admission, obesity (at a marginal significance), and the interaction between ACEI and LDH predicted the days of admission in approximately 27%. The LDH * ACEI interaction is depicted (Fig. 1). Patients who were under ACEI treatment and presented elevated LDH stayed lower days of admission, which was not observed in those without ACEI treatment (Fig. 1).

4. Discussion

The time of hospitalization in COVID patients diagnosed with hypertension and treated with ACE2 antagonists was dependent on the baseline inflammatory condition as assessed via IL-6 levels, and circulating glucose concentrations at admission. The most relevant finding of this study was that a negative interaction between LDH and ACEI prescription was statistically demonstrated concerning hospital stay, which means a protective role of these antagonists/blockers on those patients with high LDH values as a marker of tissue damage and inflammation. This information is compatible with the differential responses found in some patients and allow to understand and predict personalized outcomes concerning clinical stays of COVID-19 patients within precision medicine endeavors.

COVID-19 not only affects the pulmonary tissue, but also induces harms in multiple organs, particularly the cardiovascular system [13]. This infectious condition may lead to myocardial injuries, endothelial dysfunctions and microvascular spasms, usually requiring preventive or pharmacological measures including ACE2 antagonist's administration [14]. These findings warrant specific analyses considering factors with potential impact on the clinical and stay outcomes. In this context, available mortality figures suggest that COVID-19 is more lethal in aged patients with comorbid conditions including hypertension [15]. The SARS-CoV-2 pathogenesis is initiated by the binding of viral spike protein with the target receptor ACE2 facilitating virus internalization within host cells [16]. Mechanistically, SARS-CoV-2, following serine protease cleavage of the S protein, binds to the transmembrane ACE2 to penetrate into type 2 pneumocytes, macrophages, perivascular pericytes, and cardiomyocytes, whose entry may be blocked by ACE2 antagonists/blockers [17]. Although there were a debate about whether the use of ACEI are useful or hazardous in patients with COVID-19, several reviews and analyses now suggest that ACEI and angiotensin receptor blockers (ARBs) administration should not be discontinued in COVID-19 patients with hypertension since that there was no evidence that both pharmacological agents affected the risk COVID-19 incidence and mortality [18–22]. However, it appears that phenotypical interactions and confounding factors such as the baseline inflammatory status, concurrent metabolic diseases and complications (obesity, diabetes dyslipidemia), hyperglycemia associated to morbid stress, age or sex need to be individually accounted for precision management. These features may explain clinical discrepancies concerning clinical outcomes and duration stays among COVID 19 patients administered ACE2 antagonists as found in current analyses, where inflammation markers (LDH/IL-6), circulating glucose and ACEI were involved concerning clinical outcomes.

Given that accumulating evidence suggests that SARS-CoV2 is associated with a hyperinflammation condition characterized by excessive release of pro-inflammatory cytokines, anti-viral agents alone will not provide the much required therapeutic effect [23]. Hence, the need to combine anti-inflammatory agents such as interferons, ACE2 inhibitors, IL-6, and Janus kinase (JAK) family inhibitors, anticoagulants and other agents involved in inflammation resolution needs to conjointly examine the inflammatory status in these patients by measuring inflammatory markers such as IL-6 or LDH [24]. Using meta-regression tests, neutrophils, lymphocytes, IL-6, ferritin, C-reactive protein, D-dimer and LDH demonstrated that hyperinflammation, blunted adaptive immune response and intravascular coagulation, playing key roles in the pathogenesis of COVID-19 [25]. Indeed, LDH and IL-6 are valid inflammation markers and reliable surrogates for inflammation categorization in

Table 3
Multiple linear regression models deeming clinical, inflammatory, and pharmacological variables as important predictors of stay duration days at the hospital as the main outcome.

Variable	β coefficients (CI 95%)	P
IL6 (>100 mg/L)	5.1995 (2.8072, 7.5917)	**<0.001**
Obesity (yes)	0.4117 (0.0168, 0.8402)	0.060
Glucose at admission	0.0353 (0.0002, 0.0703)	**0.049**
HT (yes)	2.2986 (3.7035, 8.3006)	0.450
ACEI * LDH	0.0361 (0.0695, 0.0025)	**0.035**
Adjusted R^2	0.2683	**<0.001**

Bold numbers indicate P < 0.05.

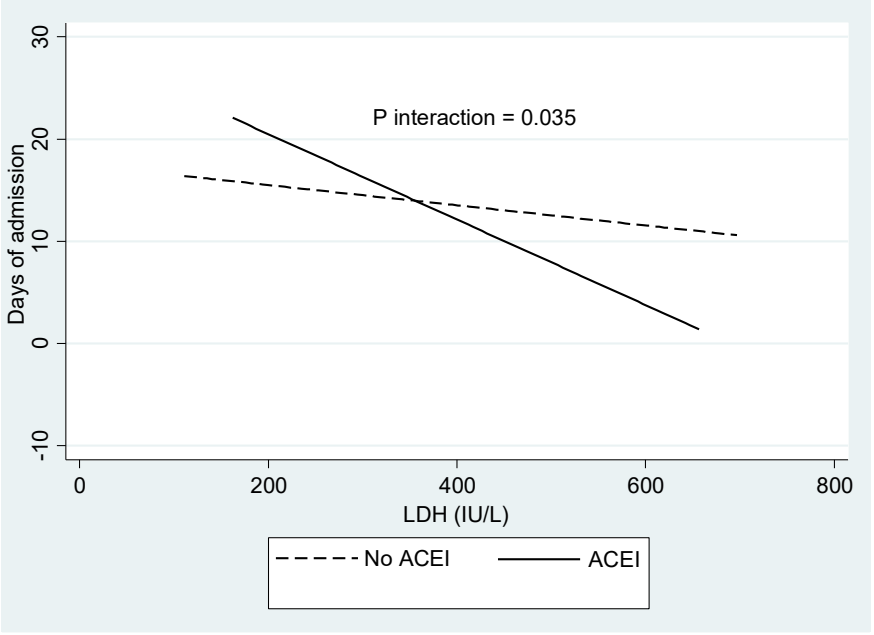

Fig. 1. Interaction between ACEI and LDH concerning days of admission in COVID-19 patients.

COVID-19 patients regardless of age or sex [24], and support the inclusion of these inflammation proxies in our regression analyses to explain the hospital stay duration implications. In fact, IL-6, LDH, C-reactive protein, and lymphocytes are key inflammatory markers in COVID-19 patients with hypertension receiving ACEI therapy, who had a lower rate of severe diseases and a trend toward a lower level of IL-6 in peripheral blood, which supports the benefit of using ACEIs to potentially contribute to the improvement of clinical outcomes of COVID-19 patients with inflammation features and hypertension [26].

A limitation of this analysis is the retrospective nature of the design, but the results were plausible according to available evidences and the hypothesis of this research. Although the analyzed sample was relatively small, it is comparable to larger series such as the COVID-DATA-SAFE-LIFES cohort [27]. Also, initial stages of COVID-19 hampered to collect some clinical information. This research should be considered as a proof of principle with valuable results and actual clinical applications, although type I and type II errors cannot be discarded given the relative small sample size. As positive strengths are that an interaction of ACE2 antagonist with the baseline LDH status has been demonstrated after adjusted by relevant inflammation variables and confounding factors. In this regard, alternative linear regression models with corticoids administration as covariable revealed that the use of corticoids did not affected the ACEI*LDH interaction (data not shown). Interestingly, those with higher IL-6 values were more prone to be treated with corticoids, which provide additional support to the need to appraise the inflammatory status in hypertensive patients.

5. Conclusion

In conclusion, the interaction between ACEI administration and the inflammatory marker LDH influenced the stay duration (days) at the hospital, which could contribute to improve the clinical/pharmacological management of COVID-19 disease under a personalized medicine approach, where patients with a more severe inflammatory status may probably benefit more specifically by ACEI treatment. This investigation allowed to define some factors that discriminate some divergent personalized clinical outcomes with emphasis on factors explaining clinical interindividual differences for a precision vascular pharmacology in COVID-19 patients.

Declaration of Competing Interest

We guarantee that there is no conflict of interest in our paper.

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