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Optimierung und Individualisierung des Therapiemanagements bei COVID-19

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Der Inhalt dieser Habilitationsschrift basiert auf den folgenden Publikationen (siehe Anhang #1 bis #6)

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Abkürzungen

ABS	Antibiotic Stewardship
ABT	Antibiotika-Therapie
ACE2	Angiotensin-konvertierendes Enzym 2
BMI	Body-Mass-Index
CI	Konfidenzintervall
COVID-19	Coronavirus Erkrankung 2019
COVRIIN	Fachgruppe Intensivmedizin, Infektiologie und Notfallmedizin
CRP	C-reaktives Protein
DRKS	Deutsches Register für Klinische Studien
eGFR	geschätzte glomeruläre Filtrationsrate
EMA	Europäische Arzneimittelagentur
FDA	Food and Drug Administration
GGT	Gamma-Glutamyltransferase
HbA1c	Glykiertes Hämoglobin
HIV	Humanes Immundefizienz Virus
SCT	Stammzell-Transplantation
Il-6	Interleukin-6
JAK	Janus-Kinase
LEOSS	Lean European Open Survey on SARS-CoV-2 Infected Patients
LY-CoV555	Bamlanivimab
mAb	monoklonale Antikörper
MERS	Mittlerer-Osten-Atemwegssyndrom
OR	Odds Ratio
PCR	Polymerase-Kettenreaktion
PCT	Procalcitonin
RBD	Rezeptor-Binde-Domäne
RKI	Robert-Koch-Institut
CP	Rekonvaleszentenplasma
RNA	Ribonukleinsäure
SARS-CoV-1	schweres akutes respiratorisches Syndrom-Coronavirus-1
SARS-CoV-2	schweres akutes respiratorisches Syndrom-Coronavirus-2

SOT	Organtransplantation
ssRNA	einzelsträngige Ribonukleinsäure
UKD	Universitätsklinikum Düsseldorf
VL	Viruslast
VOC	besorgniserregende Variante
WHO	Weltgesundheitsorganisation

Inhaltsverzeichnis

1. Einführung

1.1. Die SARS-CoV-2 Pandemie 2019-2022

Die COVID-19-Pandemie ist eine anhaltende globale Pandemie durch das schwere akute respiratorische Syndrom Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 wurde erstmals im Rahmen eines Ausbruchs schwer und akut verlaufender Lungenentzündungen in Wuhan, China, im Dezember 2019 identifiziert (1). Trotz aller Versuche, es durch strenge Isolationsmaßnahmen und Reisebeschränkungen einzudämmen, konnte sich das Virus in wenigen Monaten weltweit ausbreiten. Die Weltgesundheitsorganisation (WHO) erklärte den Ausbruch am 30. Januar 2020 zu einem internationalen Gesundheitsnotfall und gab der im Rahmen der Virusinfektion auftretenden Erkrankung am 11. Februar 2020 den Namen „**Co**rona**vi**rus **d**isease 20**19** (COVID-19)“ (2). Kurz danach stufte die WHO die Ausbreitung von SARS-CoV-2 als Pandemie ein (3). Bis zum 03. Dezember 2022 hatte die Pandemie mehr als 640 Millionen Krankheitsfälle und 6,62 Millionen bestätigte Todesfälle verursacht, was sie zu einer der tödlichsten Pandemien der Geschichte macht (4).

SARS-CoV-2 wird typischerweise übertragen, wenn Menschen Luft einatmen, die durch SARS-CoV-2 enthaltende kleinste Tröpfchen (Aerosole), kontaminiert ist (5). Infizierte Personen sind in der Regel 5-10 Tage lang ansteckend und können das Virus auch in der präsymptomatischen und asymptomatischen Phase weitergeben (6).

Strategien zur epidemiologischen Kontrolle der Pandemie umfassten unter anderem die Reduktion von menschlichen Kontakten durch soziale Distanzierung bis hin zu gesellschaftlichen „lock-downs“ unterschiedlichen Ausmaßes, Reisebeschränkungen, die Quarantäne von Personen, die dem Virus ausgesetzt waren oder Symptome zeigen, das Tragen von Masken sowie die bessere Belüftung von Innenräumen bzw. der Einbau von raumlufttechnischen Anlagen mit Luftfilterung.

Diese Maßnahmen konnten die globale Ausbreitung von SARS-CoV-2 zwar nicht verhindern, waren jedoch von entscheidender Bedeutung, um während der regional unterschiedlich starken Wellen der SARS-CoV-2 Pandemie eine Überlastung der Gesundheitssysteme zu verhindern oder diese zumindest zu limitieren.

Aufgrund intensivster und in der Geschichte der Medizin einmaliger wissenschaftlicher Bemühungen waren bereits ab Dezember 2020, also nach weniger als einem Jahr nach Entdeckung von SARS-CoV-2 verschiedene Impfstoffe gegen SARS-CoV-2 verfügbar und haben nach Modellrechnungen Millionen Todesfälle durch COVID-19 verhindert (7).

Durch anhaltende Virusevolution und insbesondere unter dem Selektionsdruck der, sich durch Impfung und Genesung entwickelnden, Immunität in der menschlichen Population kam es zur Entwicklung zahlreicher SARS-CoV-2-Varianten mit unterschiedlichem Grad an Infektiosität, Virulenz und Immunevasion (8, 9).

1.2. Eigenschaften von SARS-CoV-2

SARS-CoV-2 ist ein einzelsträngiges RNA-Virus mit positiver Polarität (+ssRNA) und eng mit dem Corona-Virus verwandt, das den ersten Ausbruch eines Coronavirus-induzierten schweren akuten respiratorischen Syndroms (SARS) in den Jahren 2002-2004 verursachte und inzwischen als SARS-CoV-1 bezeichnet wird (10).

Sowohl SARS-CoV-1 als auch SARS-CoV-2 gehören innerhalb der Familie der Coronaviren zur Unterfamilie der Orthocoronaviren und zum Genus β-Coronaviren. Im Genus β-Coronaviren stellen sie die humanpathogenen Vertreter des Subgenus der Sarbecoviren dar (11).

Humanpathogene Coronaviren können Krankheiten verursachen, die von einer gewöhnlichen Erkältung bis hin zu schwereren Erkrankungen wie dem Middle East Respiratory Syndrome (MERS, Sterblichkeitsrate ~34 %) reichen. SARS-CoV-2 ist das siebte bekannte Coronavirus, das Menschen infizieren kann, nach 229E, NL63, OC43, HKU1, MERS-CoV und SARS-CoV-1 (11, 12).

Coronaviren infizieren neben Menschen auch zahlreiche andere Säugetiere sowie manche Vogelarten (14), so dass nach dem Ausbruch im Umfeld eines Wildtiermarktes in Wuhan COVID-19 bereits früh als Zoonose eingeschätzt wurde. SARS-CoV-2 weist dabei eine große genetische Ähnlichkeit mit Fledermaus-Coronaviren auf und wurde nach aktuellem Stand der Wissenschaft sehr wahrscheinlich entweder direkt von Fledermäusen oder indirekt über tierische Zwischenwirte auf den Menschen übertragen (13).

Jedes SARS-CoV-2-Virion hat einen Durchmesser von 60-140 Nanometern (1). Seine RNA-Sequenz ist etwa 30.000 Basen lang und damit repräsentativ für Coronaviren, welche die größten Genome aller RNA-Viren enthalten (10).

Von besonderer Bedeutung für das Sars-CoV-2 Virus ist das Spike-Protein auf seiner Oberfläche, das den Eintritt des Virus in die Zelle durch das Angiotensin-konvertierende Enzym-2 (ACE2) vermittelt (10). Ein charakteristisches Merkmal von SARS-CoV-2 ist das Vorhandensein einer polybasischen Furin-Spaltstelle im Spike-Protein. Es ist davon auszugehen, dass der Erwerb der Furin-Spaltstelle für die zoonotische Übertragung von im SARS-CoV-2 auf den Menschen wesentlich war (15).

SARS-CoV-2 entwickelt sich evolutionär weiter und bringt neue Varianten hervor, die sich aufgrund seiner Bedeutung für Übertragbarkeit und Immunität besonders durch Spike-Protein-Mutationen auszeichnen. Obwohl die im Rahmen der Replikation zufällig entstehenden Mutationen im SARS-CoV-2-Genom für das Virus in der Regel neutral oder nachteilig sind, können bestimmte Mutationen dem Virus Vorteile verschaffen (16).

Bis Ende 2022 sind fünf sich schnell ausbreitende Stämme von SARS-CoV-2 aufgetaucht, die als besorgniserregende Varianten (Variants of Concern, VOC) bezeichnet werden, darunter die Alpha-Variante (B.1.1.7), die Beta-Variante (B.1.351), die Gamma-Variante (P.1), die Delta-Variante (B.1.617.2) und die Omikron-Variante (B.1.1.529).

Die Alpha-Variante ist in der zweiten Hälfte des Jahres 2020 im Vereinigten Königreich beschrieben worden und hat sich schnell weltweit verbreitet. Die Beta- und Gamma-Varianten wurden zuerst in Südafrika bzw. Brasilien beobachtet und waren durch zusätzliche Mutationen an den Positionen E484 und K417 in der Rezeptor-Bindedomäne (RBD) charakterisiert. Diese beiden SARS-CoV-2-Varianten, die die Kombination der Mutationen N501Y, E484K und K417N/T enthalten, weisen eine geringere Empfindlichkeit gegenüber neutralisierenden Antikörpern auf, die als Antworten auf die erste SARS-CoV-2 Impfstoffgeneration oder Infektionen mit früheren Virusvarianten aus dem ersten Pandemiejahr generiert wurden. Die Delta-Variante tauchte dann im Dezember 2020 in Indien auf und weist bereits insgesamt 8 Mutationen im Spike-Protein auf. Sie zeichnete sich zusätzlich durch eine höhere Pathogenität aus. Anfang November 2021 wurde dann die Omikron-Variante (B.1.1.529) erstmals in Botswana und Südafrika entdeckt, Vorläufer der Omikron-Variante BA.1 konnten aber retrospektiv bereits ab August 2021 in mehreren

afrikanischen Ländern nachgewiesen werden (17). Die ursprüngliche Omikron-Variante weist bereits mehr als 30 Mutationen im Spike-Protein auf, von denen sich viele innerhalb der RBD befinden und die insbesondere zu einer deutlich verbesserten Immunevasion gegenüber Immunantworten auf nicht-Omikron-Varianten führen. Inzwischen haben sich aus der Omikron-Variante zahlreiche Unterlinien entwickelt, die sich hinsichtlich der Übertragbarkeit, Pathogenität und Resistenz gegen die durch Impfung oder Genesung ausgelöste Immunität unterscheiden (18).

1.3. Charakteristika der verschiedenen Krankheitsphasen von COVID-19

COVID-19 kann sich klinisch mit einem breiten Spektrum von Symptomen und in unterschiedlichen Schweregraden präsentieren, die sowohl asymptomatische Krankheitsverläufe als auch leichte bis sehr schwere und tödlich verlaufende Krankheitsbilder mit verschiedenen Stadien umfassen (19, 20). Alter, männliches Geschlecht sowie Begleiterkrankungen, insbesondere Diabetes, Übergewicht, Niereninsuffizienz und koronare Herzkrankheiten, wurden bereits früh als Hauptrisikofaktoren für die Entwicklung eines schweren Krankheitsverlaufs identifiziert (19, 21).

Nach der Inkubationszeit kommt es bei symptomatischen Verläufen in der Phase der Virusvermehrung häufig zu Fieber, Myalgien, Kopfschmerzen, Anosmie, Ageusie (oder Dysgeusie), Rhinitis und gastrointestinalen Symptomen (22). Im Verlauf kann sich dann eine Pneumonie, sowie ein einem Teil der Patienten eine systemische Hyperinflammation mit komplikativem Verlauf entwickeln (19). Während Alter und Begleiterkrankungen vor allem für die Mortalität im Kontext schwerer Verläufe relevant sind, spielen Faktoren wie Adipositas sowie zahlreiche genetische Faktoren eine zentrale Rolle in der Pathogenese der Hyperinflammation im Rahmen von COVID-19 (23). COVID-19 kann im Rahmen schwerer Verläufe dabei auch langanhaltende und irreversible Organschädigungen nach sich ziehen. So kann es im Rahmen einer schweren pulmonalen Inflammation auch nach Überleben der akuten Krankheitsphase häufig zu Folgeerscheinungen wie Lungenfibrose kommen (24). Bereits früh wurde im Zusammenhang mit COVID-19 ebenfalls über ein erhöhtes Auftreten von akuten Lungenembolien, anderen thromboembolischen Komplikationen sowie intravaskulärer Koagulopathie berichtet (25), die in einem engen Zusammenhang mit der Dysregulation der Entzündungsantwort steht.

Kennzeichnend für COVID-19 war zu Beginn der Pandemie, dass bei 10-20 % der Patienten 7-10 Tage nach dem Auftreten der Symptome eine plötzliche Verschlechterung eintritt, in deren Folge es rasch zu einem akuten Atemversagen, weiteren Organversagen und letztlich zu einem tödlichen Ausgang kommen kann (19, 22). Die dieser Verschlechterung zugrunde liegenden Mechanismen sind im Kontext der immunologisch vermittelten Hyperinflammation zu erklären und nicht direkt durch das Virus bedingt, das in vielen Fällen in diesem Stadium nicht mehr, oder nur noch in geringer Menge, nachweisbar ist (22, 26). Der prozentuale Anteil dieser schweren Verläufe hat sich dabei je nach Ausmaß der in der Bevölkerung durch Impfung und/oder Genesung vorhandenen Immunität sowie aufgrund unterschiedlicher Krankheitsschwere der verschiedenen VOC mehrfach im Verlauf der Pandemie verändert.

Zusammenfassend können für den moderaten und schweren Verlauf von COVID-19 eine frühe Phase, in der die Virusvermehrung im Vordergrund steht und in der typischerweise die Symptome einer viralen Infektion der oberen Atemwege vorliegen, eine pulmonale Phase, in der die Lungenentzündung sich klinisch manifestiert, und eine späte Phase mit Hyperinflammation und entsprechenden Komplikationen unterschieden werden (27). Bei der pulmonalen Phase handelt es sich somit um eine Übergangsphase oder die „frühe Spätphase". Bei asymptomatischen oder milden Verläufen von COVID-19 endet die Erkrankung nach der frühen, „viralen" Phase (27). Zentral für die späte Phase ist eine komplexe Dysregulation des Immunsystems mit überschießender Entzündungsantwort, die als Fehlantwort auf die zuvor stattfindende Virusvermehrung zu interpretieren ist (27, 28). Bereits in der frühen Spätphase von COVID-19, die meist im Laufe der 2. Krankheitswoche vorliegt, ist in der Regel SARS-CoV-2 im Nasenrachenraum nicht mehr oder nur noch in geringer Menge nachweisbar. Im weiteren Verlauf ist dann auch in den tieferen Atemwegen bei gleichzeitig anhaltend hoher Inflammation und Immundysregulation meist kein SARS-CoV-2 mehr nachweisbar (29).

Als weitere Krankheitsphase kann zusätzlich Long-COVID bzw. Post-COVID-Syndrom abgegrenzt werden. Es handelt sich um einen Syndromkomplex, der durch langfristige Gesundheitsprobleme gekennzeichnet ist, die nach der typischen Erholungsphase von COVID-19 anhalten oder auftreten (30-32). Obwohl zahlreiche Studien über das Long-COVID- bzw. Post-COVID-Syndrom durchgeführt werden, gibt es international bisher keinen breiten Konsens über die Definition der Begriffe, für Deutschland wurde jedoch ein Fortbestehen von COVID-19 assoziierten Folgebeschwerden für mehr als 4 bis 12 Wochen

als Long COVID und von mehr als 12 Wochen als Post-COVID-19 Syndrom definiert (32). Long-COVID bzw. das Post-COVID-Syndrom kann nahezu jedes Organsystem beeinträchtigen und weitere Erkrankungen (Folgeerkrankungen) verursachen, darunter Störungen des Atmungssystems, des Nervensystems, psychische Störungen, Stoffwechselstörungen, Herz-Kreislauf-Erkrankungen, Magen-Darm-Erkrankungen oder Schmerzen des Bewegungsapparats. Die am häufigsten berichteten Symptome im Rahmen des Long-COVID- bzw. Post-COVID-Syndroms sind Müdigkeit, Gedächtnisprobleme, Konzentrationsstörungen, fehlende körperliche Leistungsfähigkeit mit Luftnot und Herzrasen unter geringer Belastung sowie Geruchs- und Geschmackstörungen (30-32).

1.4. Therapiemanagement bei COVID-19

In Anbetracht der pathophysiologisch grundlegend verschiedenen Krankheitsphasen von COVID-19 muss sich entsprechend das Therapiemanagement nach der aktuellen Krankheitsphase richten. Schon bevor sich das Konzept der verschiedenen Krankheitsphasen von COVID-19 wissenschaftlich etabliert hatte wurden in Anbetracht der hohen Erkrankungszahlen und Mortalität bereits früh nach dem Beginn der COVID-19 Pandemie zahlreiche antivirale Therapiestrategien evaluiert.

Zu den ersten antiviralen Medikamenten, für die ein Nutzen in bestimmten klinischen Situationen gezeigt werden konnte, gehörte Remdesivir (33-35). Allerdings zeigte sich auch früh die Abhängigkeit des klinischen Nutzens von der Krankheitsphase. So konnte in der ACTT-1 Studie gezeigt werden, dass Patienten mit einer COVID-19-Pneumonie und Sauerstoffbedarf ohne Notwendigkeit der Beatmung von der Remdesivir-Gabe profitieren, während für bereits kritische kranke, beatmete Patienten kein Nutzen gezeigt werden konnte (34, 35). Da eine antivirale Therapiestrategie nur bei noch relevantem Vorhandensein von SARS-CoV-2 zielführend ist und eine bereits angestoßene Dysregulation des Immunsystems mit Hyperinflammation davon nicht beeinflusst werden kann, erklären sich so auch die insbesondere im ersten Pandemiejahr teils widersprüchlichen Studiendaten zum klinischen Nutzen bestimmter Therapiestrategien.

Für andere, aufgrund präklinischer Daten früh in der Pandemie untersuchte und antiviral intendierte Substanzen wie Hydroxychloroquin und Lopinavir/Ritonavir hingegen konnte in

großen, randomisierten Studien übereinstimmend kein Vorteil hinsichtlich der klinischen Endpunkte gezeigt werden (35-37).

Ebenfalls früh und intensiv diskutiert wurde der Einsatz von Rekonvaleszentenplasmen als therapeutische Option bei COVID-19 (38, 39). Auch hier zeigte sich in randomisierten Studien, dass neben der Konzentration der neutralisierenden Antikörper in den verwendeten Plasmen der Zeitpunkt der Rekonvaleszentenplasma-Gabe entscheidend ist (40-42).

Das Konzept einer Therapie der SARS-CoV-2 Infektion mit neutralisierenden Antikörpern wurde dabei nicht nur mit polyklonalen Antikörper-Therapien wie Rekonvaleszentenplasma verfolgt, sondern auch schon ab Frühjahr 2020 mit gegen das Spike-Protein von SARS-CoV-2 gerichteten monoklonalen Antikörpern (mAb) (43-45).

In Deutschland waren die ersten spezifisch gegen SARS-CoV-2 gerichteten monoklonalen Antikörper über eine Beschaffung des Bundesministeriums für Gesundheit ab Anfang 2021 verfügbar. Schwerpunkt der später auch zur formalen Zulassung der mAb-Präparate führenden klinischen Studien war, aufgrund der zuvor angeführten Überlegungen zur Pathophysiologie von COVID-19, die Therapie der frühen Krankheitsphase von COVID-19, die man auf die ersten 5-7 Tage verorten kann (46-49).

Später wurde gezeigt, dass bei Patienten, die mit COVID-19 ins Krankenhaus eingeliefert wurden und die zu Beginn der Studie bezüglich SARS-CoV-2 seronegativ, nicht aber bei Patienten, die zu Beginn der Studie seropositiv waren, die 28-Tage-Sterblichkeit durch die Gabe einer monoklonalen Antikörperkombination aus Casirivimab und Imdevimab verringert werden konnte (50).

Gerade für Menschen mit erhöhtem Risiko für einen schweren Verlauf von COVID-19, die z.B. aufgrund von Immundefizienz oder Kontraindikationen hinsichtlich einer SARS-CoV-2 Impfung nicht erfolgreich durch Impfstoffe geschützt werden konnten, bestand ein hoher Bedarf an über allgemeine Maßnahmen wie Kontaktreduktion und filtrierende Masken hinausgehenden Werkzeugen zur Prävention einer COVID-19-Infektion. Für die subkutane Gabe von Casirivimab/Imdevimab bzw. die intramuskuläre Gabe von Cilgavimab/Tixagevimab konnte eine signifikante Reduktion von Erkrankungen durch COVID-19 in den Therapiegruppen gezeigt werden (51,52).

Aufgrund der anhaltenden Evolution von SARS-CoV-2 und seiner Fähigkeit zur Immunevasion durch Mutationen im Spike-Protein, gegen das sich alle klinisch eingesetzten

mAb richten, wurden im Verlauf der Pandemie viele der zuvor erfolgreichen mAb-basierten Therapiestrategien mit dem Aufkommen der VOC Delta und Omikron unwirksam (54-57).

Da der Selektionsdruck auf diese Virusvarianten vor allem durch die, durch Impfung und Genesung erzeugte, Immunantwort auf das Spike-Protein beruht, bleibt die Wirkung der klassischen antiviral wirksamen Substanzen wie Remdesivir auf die VOC erhalten (58). Neben dem derzeit ausschließlich intravenös applizierbaren Inhibitor der viralen RNA-Polymerase Remdesivir wurde in Deutschland inzwischen auch der oral einnehmbare Wirkstoff Molnupiravir zugelassen, der auch an der viralen RNA-Polymerase ansetzt und hier Hypermutationen im Genom des Virus auslöst (59). Ein weiterer oral antiviral einnehmbarer Wirkstoff, der in Deutschland zur Behandlung von SARS-CoV-2 zugelassen wurde, ist der Proteaseinhibitor Nirmatrelvir, der zum Erreichen ausreichender Wirkspiegel zusammen mit dem pharmakologischen Booster Ritonavir eingenommen werden muss (60).

Für die antivirale Therapie der SARS-CoV-2 Infektion bei Personen mit einem erhöhten Risiko für einen schweren Verlauf in der Frühphase von COVID-19, also in den ersten 5 Tagen nach Symptombeginn, konnte in großen randomisierten Studien eine deutliche Risikoreduktion hinsichtlich erforderlicher Krankenhauseinweisungen und der Mortalität gezeigt werden (60, 61).

Ein Überlebensvorteil durch eine antivirale Therapie mit Remdesivir konnte in den, zu Beginn der Pandemie, erfolgten Studien bei Einsatz in der inflammatorisch geprägten Spätphase von COVID-19 zuerst nicht gezeigt werden, beziehungsweise nur für eine Subgruppe von Patienten mit COVID-19 Pneumonie und Sauerstoffbedarf, aber ohne Notwendigkeit der Beatmung (also noch in der frühen Spätphase). Hingegen führte in einer später durchgeführten klinischen Studie an nicht hospitalisierten Risiko-Patienten in der Frühphase von COVID-19 eine dreitägige Behandlung mit Remdesivir zu einem um 87 % niedrigeren Risiko für Krankenhausaufenthalte oder Tod als eine Behandlung mit Placebo (62).

Zusammenfassend kann aufgrund dieser Daten für Menschen mit einem erhöhten Risiko eines schweren Verlaufs der SARS-CoV-2 Infektion eine klare Therapieempfehlung für eine antivirale Therapie in der frühen Phase der SARS-CoV-2 Infektion gegeben werden. Dies gilt zumindest für ältere Menschen auch noch unter den Bedingungen einer inzwischen durch Impfung und/oder Genesung erreichten Immunität in der Bevölkerung, wie in einer

großen Studie aus Israel für eine frühe Therapie mit Nirmatrelvir/Ritonavir während der Omikron-Variante gezeigt wurde (63).

Parallel zu den klinischen Studien mit antiviral wirksamen Substanzen wurde aufgrund der klinischen Verläufe und experimenteller Ergebnisse zur Immundysregulation und Hyperinflammation bei COVID-19 bereits sehr früh die Hypothese aufgestellt, dass in der Spätphase von COVID-19 der Einsatz immunsuppressiver oder immunmodulierender Substanzen einen günstigen Einfluss auf den Krankheitsverlauf haben könnte (64-66). Eine wesentliche Veränderung der Therapiestrategien erfolgte mit der Veröffentlichung der Daten aus der RECOVERY-Plattform-Studie, die einen Überlebensvorteil für eine Dexamethason-Therapie bei Patienten mit COVID-19 Pneumonie zeigen konnte, die eine zusätzliche Sauerstoffzufuhr benötigten. Für invasiv beatmete Patienten war diese Reduktion in der Mortalität sogar noch ausgeprägter (67). Allerdings können bei der immunmodulierenden Therapie des moderaten und schweren Verlaufs in der Spätphase von COVID-19 im Vergleich zu den antiviralen Therapien in der Frühphase von COVID-19 nur deutlich geringere Risikoreduktionen erreicht werden.

Tocilizumab, ein sonst überwiegend in der Rheumatologie eingesetzter monoklonaler Antikörper, der den Interleukin-6 (Il-6) Rezeptor blockiert, wurde bereits kurz nach Beginn der Pandemie in der Spätphase von COVID-19 bei schweren Verläufen mit Hyperinflammation eingesetzt (64, 65). Auch hier zeigte sich, dass bei der Planung klinischer Studien zu COVID-19 in besonderem Maße sowohl die Auswahl der richtigen Patientengruppe als auch der Zeitpunkt der Therapie für den Erfolg entscheidend war. Während erste Studien trotz umfangreicher, positiver anekdotischer Berichte keinen klinischen Nutzen für den Einsatz von Tocilizumab zeigen konnten (68, 69), konnte die RECOVERY Studiengruppe in einer großen randomisierten Studie einen klaren Vorteil für einen Einsatz von Tocilizumab in der Phase der progredienten Verschlechterung einer COVID-19 Pneumonie mit stark steigendem Sauerstoffbedarf zeigen (70). Dies galt in den Subgruppenanalysen speziell in Kombination mit Dexamethason, so dass Tocilizumab immer in Kombination mit Dexamethason eingesetzt werden sollte. Die Daten zum Überlebensvorteil einer Therapie mit Il-6-Rezeptorblockern wurden durch parallel erfolgte Untersuchungen der REMAP-CAP Studiengruppe bestätigt (71).

Eine weitere, ebenfalls in der Rheumatologie eingesetzte Substanz, die umfangreich im Kontext der Immunmodulation bei schweren Verläufen von COVID-19 untersucht wurde,

ist der Janus-Kinase (JAK)-Inhibitor Baricitinib. Sowohl Modelle als auch kleine Pilotstudien ließen die Blockade der Januskinasen JAK 1 und JAK 2 als ein interessantes Therapiekonzept erscheinen (72, 73). Inzwischen konnten die COV-BARRIER- und die ACTT-2 Studie nachweisen, dass Baricitinib in Kombination mit Dexamethason und auch in Kombination mit Remdesivir jeweils einen signifikanten klinischen Vorteil gegenüber den jeweiligen Vergleichsarmen erbrachte (74, 75).

Neben den Forschungsfragestellungen hinsichtlich der oben aufgeführten spezifischen Therapiestrategien für das Krankheitsbild COVID-19 ergaben sich zahlreiche weitere Fragen zur optimalen supportiven Therapie bei moderatem und schwerem Verlauf von COVID-19. Ungeklärte Fragen umfassten zu Beginn der Pandemie unter anderem den Wert einer Antikoagulation in prophylaktischer oder therapeutischer Dosis, die optimale Beatmungsstrategie oder auch den Nutzen einer ergänzenden, empirischen Therapie mit Antibiotika im Rahmen einer COVID-19 Pneumonie.

2. Fragestellung und Zielsetzung

Mit dem Auftreten des neuen Coronavirus SARS-CoV-2 und seiner pandemischen Ausbreitung ergaben sich vielfältige Fragestellungen zum individuellen Therapiemanagement des durch SARS-CoV-2 ausgelösten Krankheitsbildes COVID-19.

Aufgrund der Komplexität der Erkrankung COVID-19 und der Dynamik der SARS-CoV-2-Pandemie bestand eine dringende Herausforderung darin, besonders gefährdete Patientengruppen zu identifizieren und die für sie geeigneten Behandlungsmethoden zu erarbeiten.

Neben den Risikofaktoren für einen schweren Verlauf von COVID-19 sollten auch die Prädiktoren für die Entwicklung des sich im Verlauf ebenfalls manifestierenden, neuen Krankheitsbildes Long-COVID, das in der Folge einer SARS-CoV-2-Infektion auftreten kann, genauer charakterisiert werden.

Des Weiteren hatte die Anpassung der in großen randomisierten Studien untersuchten Therapiekonzepte an spezifische Patientengruppen, die in diesen Studien nicht adäquat vertreten waren, wie z.B. Patienten mit Immundefizienz, in der klinisch-translationalen Forschung zu COVID-19 eine hohe Priorität.

So zeigten sich mit der Verfügbarkeit der gegen SARS-CoV-2 gerichteten monoklonalen Antikörper und ihrem Einsatz bei komplexen Risikopatienten früh erste Hinweise auf eine Selektion von viralen Resistenzmutationen bei Patienten mit verlängerter Virusausscheidung, wie sie insbesondere bei relevanter Immundefizienz zu beobachten ist. Dieses sowohl für den individuellen Therapieerfolg als auch in Folge der Übertragbarkeit mutierter viraler SARS-CoV-2 Varianten für die Populationsebene relevante Thema entwickelte sich ebenfalls rasch zu einem weiteren Schwerpunkt der wissenschaftlichen Aktivitäten während der COVID-19 Pandemie.

Zusätzlich war auch die Evaluierung von Therapieansätzen, die auf der Grundlage theoretischer Überlegungen, Modellvorstellungen und empirischer Daten für das neue Krankheitsbild COVID-19 diskutiert wurden, ein Ziel der vorliegenden Arbeit.

Zusammenfassend sollten die hier dargestellten wissenschaftlichen Arbeiten zur „Optimierung und Individualisierung des Therapiemanagements von COVID-19“ einen Beitrag zur Bewältigung der SARS-CoV-2 Pandemie leisten.

3. Kurz gefasste Wiedergabe der Ergebnisse aus ausgewählten eigenen Veröffentlichungen und Diskussion

3.1. Risikofaktoren für einen schweren Verlauf von COVID-19 und die Entwicklung von Long-COVID

#1 Stefan N, Sippel K, Heni M, Fritsche A, Wagner R, Jakob CEM, Preißl H, von Werder A, Khodamoradi Y, Borgmann S, Rüthrich MM, Hanses F, Haselberger M, Piepel C, Hower M, Vom Dahl J, Wille K, Römmele C, Vehreschild J, Stecher M, Solimena M, Roden M, Schürmann A, Gallwitz B, Hrabe de Angelis M, Ludwig DS, Schulze MB, **Jensen BEO**, Birkenfeld AL. Obesity and Impaired Metabolic Health Increase Risk of COVID-19-Related Mortality in Young and Middle-Aged Adults to the Level Observed in Older People: The LEOSS Registry. **Front Med (Lausanne)**. 2022 May 11;9:875430. doi: 10.3389/fmed.2022.875430. PMID: 35646955; PMCID: PMC9131026.

#2 Loosen SH, **Jensen BO**, Tanislav C, Luedde T, Roderburg C, Kostev K. Obesity and lipid metabolism disorders determine the risk for development of long COVID syndrome: a cross-sectional study from 50,402 COVID-19 patients. **Infection**. 2022 Oct;50(5):1165-1170. doi: 10.1007/s15010-022-01784-0. Epub 2022 Mar 30. PMID: 35355237; PMCID: PMC8966865.

Das Projekt „Lean European Open Survey on SARS-CoV-2-Infected Patients“ (LEOSS) wurde als europäische, nicht-interventionelle, multizentrische Kohortenstudie im März 2020 ins Leben gerufen, um die Epidemiologie und den klinischen Verlauf der SARS-CoV-2-Infektion zu untersuchen (76). Im Juli 2020 waren bereits mehr als 125 Standorte aus 7 verschiedenen Ländern bei LEOSS registriert. In die Studie, die den Einfluss von Übergewicht und gestörter metabolischer Gesundheit auf den Verlauf von COVID-19 beleuchten sollte, wurden Daten von hospitalisierten und ambulant behandelten Patienten mit positivem SARS-CoV-2-Test einbezogen. Die Genehmigung für LEOSS wurde von den zuständigen lokalen Ethikkommissionen aller teilnehmenden Zentren eingeholt und beim Deutschen Register für klinische Studien (DRKS, Nr. S00021145) registriert.

Von den initial 6457 evaluierten Patienten kamen nur erwachsene Patienten (Alter ≥18 Jahre) mit vollständigen Angaben zu Geschlecht, Alter, BMI und den Begleiterkrankungen

Diabetes, Bluthochdruck, koronare Herzkrankheit, chronische Nierenerkrankung und chronische Lebererkrankung (N = 3517) für die Analysen in Betracht. Von ihnen wurden insgesamt 354 Patienten mit fehlenden Angaben zum Überleben ausgeschlossen, so dass sich für die Hauptanalysen eine Stichprobe von 3163 Patienten ergab. Von diesen 3163 untersuchten Patienten erholten sich 2661 Patienten von COVID-19, während 502 Patienten verstarben.

In einem multivariablen Regressionsmodell, das alle untersuchten Parameter einschloss, waren höheres Alter, männliches Geschlecht, BMI ≥35 kg/m^2, Diabetes, HbA1c >8,1%, CRP ≥30 mg/L und GGT >10-fach über der oberen Grenze des Normalwerts unabhängig voneinander mit einem erhöhten Sterberisiko verbunden.

Um die Zusammenhänge zwischen Adipositas und beeinträchtigter Stoffwechselgesundheit und dem Mortalitätsrisiko in verschiedenen Altersgruppen zu untersuchen, wurden die Patienten in drei Altersgruppen eingeteilt und im Weiteren hinsichtlich des Vorliegens von Adipositas, Diabetes und Bluthochdruck unterschieden.

Es wurde ein additiver Effekt von Adipositas, Diabetes und Bluthochdruck auf das Sterberisiko beobachtet, der bei jungen und mittelalten Patienten besonders ausgeprägt war. Im Vergleich zu jungen und mittelalten (18-55 Jahre) Patienten ohne Fettleibigkeit, Diabetes und Bluthochdruck (nicht adipös und stoffwechselgesund; n = 593) hatten junge und mittelalte erwachsene Patienten mit allen drei Risikoparametern (fettleibig und stoffwechselkrank; n = 31) ein ähnlich hohes bereinigtes Sterberisiko [OR 7,42 (95% CI 1,55-27,3)] wie ältere (56-75 Jahre) nicht fettleibige und stoffwechselgesunde Patienten [n = 339; OR 8,21 (95% CI 4,10-18,3)].

Zusammenfassend lässt sich aus den Daten ableiten, dass die veränderbaren Risikofaktoren Adipositas, Diabetes und Bluthochdruck das Risiko einer COVID-19-bedingten Sterblichkeit bei jungen und mittelalten Patienten auf das im höheren Alter beobachtete Risikoniveau erhöhen.

Diese Ergebnisse stützen die Empfehlungen internationaler medizinischer Fachgesellschaften, wonach Fettleibigkeit, Diabetes und Bluthochdruck wichtige Risikofaktoren sind, die kritisch berücksichtigt werden sollten, wenn bei einem Patienten COVID-19 diagnostiziert wird. Eine intensive klinische Überwachung dieser Patienten, insbesondere in den frühen Stadien der Krankheit, sollte daher sichergestellt werden.

Von besonderer Bedeutung ist jedoch die Erkenntnis, dass man trotz des bereits früh in der Pandemie identifizierten Hauptrisikofaktors Lebensalter keineswegs davon ausgehen kann, dass jüngere Menschen generell ein geringeres Risiko für eine schwere COVID-19-Erkrankung haben. Dies hatte auch Implikationen für die Priorisierung jüngerer Menschen mit diesen Risikofaktoren für nationale Impfstrategien.

Es wurde bereits wenige Monate nach Beginn der SARS-CoV-2 Pandemie berichtet, dass bei COVID-19-Überlebenden über die akute Erkrankung hinaus erhebliche gesundheitliche Beeinträchtigungen zu beobachten sind (30, 31, 77). Die Fragestellung, inwieweit das Vorhandensein von Fettleibigkeit und Stoffwechselstörungen vor der SARS-CoV-2-Infektion das Risiko für die Entwicklung von Long COVID erhöhen könnte, war naheliegend. Insbesondere aufgrund der Bedeutung der Inflammation für COVID-19 und ebenso für das darauffolgende Krankheitsbild Long COVID und der durch Adipositas ausgelösten chronischen Inflammation (78, 79) erschien eine weitere Untersuchung dieser Zusammenhänge als wissenschaftlich geboten.

Eine Gelegenheit zur Untersuchung der Bedeutung von Adipositas und Fettstoffwechselstörungen für das Risiko einer Entwicklung von Long COVID ergab sich aus der Auswertung von Querschnittsdaten aus der Disease-Analyzer-Datenbank (IQVIA), die Diagnosen sowie allgemeinmedizinische und demografische Daten zusammenstellt, die anonym aus Computersystemen von Allgemein- und Fachärzten in Deutschland stammen (80). Die Datenbank deckt ~ 3% aller ambulanten Praxen in Deutschland ab.

Die Analyse umfasste 50.402 Patienten mit einer bestätigten Diagnose von COVID-19 zwischen dem 1. März 2020 und dem 31. März 2021 aus einer von 1056 Hausarztpraxen, die routinemäßig Daten an die Disease-Analyzer-Datenbank senden. Das primäre Ergebnis der Studie war der Anteil der Patienten mit einer Dokumentation von Long COVID oder einer Diagnose, die auf Long COVID hindeutet.

Von den 50 402 Patienten mit einer bestätigten SARS-CoV-2-Infektion wurden 1708 (3,4 %) mit Long COVID oder einer der damit verbundenen Diagnosen diagnostiziert. Zwischen der Diagnose von COVID-19 und der Diagnose von Long COVID lagen durchschnittlich 82 Tage (SD 28 Tage). Das Durchschnittsalter aller COVID-19-Patienten betrug 48,8 Jahre (SD: 19,3 Jahre). 27.512 (54,5 %) der Patienten waren weiblich. Arterielle Hypertonie (n = 12.898, 25,6 %) war die häufigste Komorbidität, gefolgt von Fettstoffwechselstörungen

(n = 8580, 17,0 %), Depressionen (n = 8529, 16,9 %), Diabetes mellitus Typ 2 (n = 5060, 10,0 %), Adipositas (n = 4995, 9. 90 %) und chronische Bronchitis oder chronisch obstruktive Lungenerkrankung (n = 4399, 8,7 %).

In einer multivariaten Regressionsanalyse identifizierten wir Fettstoffwechselstörungen (OR 1,46, 95% CI 1,28-1,65, p < 0,001) und Fettleibigkeit (OR 1,25, 95% CI 1,08-1,44, p = 0,003) als Risikofaktoren für die Entwicklung von Long COVID.

Neben diesen Parametern erwiesen sich ein Alter der Patienten zwischen 46 und 60 Jahren (im Vergleich zu einem Alter ≤ 30 Jahren, (OR 1,81 95% CI 1,54-2,13, p < 0,001), das weibliche Geschlecht (OR 1,33, 95% CI 1,20-1,47, p < 0,001) sowie vorbestehendes Asthma (OR 1. 67, 95% CI 1.39-2.00, p < 0.001), Depression bei Frauen (OR 1.27, 95% CI 1.09-1.47, p = < 0.002) und Krebs bei Männern (OR 1.4, 95% CI 1.09-1.95, p = < 0.012) als Risikofaktoren für die Entwicklung von Long COVID.

Diese Daten legen nahe, dass Fettstoffwechselstörungen und Fettleibigkeit altersunabhängige Risikofaktoren für LCS darstellen. Dies würde die Hypothese stützen, dass fettleibigkeitsbedingte chronische Entzündung und immun-metabolische Prozesse nicht nur schwere klinische Verläufe der akuten SARS-CoV-2-Infektion, sondern auch die Entwicklung von Long COVID fördern. Inzwischen weisen auch mehrere andere Publikationen auf den Zusammenhang zwischen Adipositas und dem Risiko für die Entwicklung von Long COVID hin (81-83)

Zusammenfassend lässt sich sagen, dass Fettleibigkeit und Fettstoffwechselstörungen beeinflussbare Risikofaktoren darstellen und unsere Daten darauf hindeuten, dass Maßnahmen zur Verbesserung des Lebensstils Teil künftiger Strategien zur Pandemievorsorge sein könnten. Patienten mit Adipositas und Krankheiten des metabolischen Syndroms sollten aufgrund dieser Ergebnisse in allen Phasen von COVID-19 als Risikopatienten betrachtet werden.

3.2. Immunevasion von SARS-CoV-2 gegenüber monoklonalen Antikörpern

#3 **Jensen B**, Luebke N, Feldt T, Keitel V, Brandenburger T, Kindgen-Milles D, Lutterbeck M, Freise NF, Schoeler D, Haas R, Dilthey A, Adams O, Walker A, Timm J, Luedde T. Emergence of the E484K mutation in SARS-COV-2-infected immunocompromised patients treated with bamlanivimab in Germany. **Lancet Reg Health Eur**. 2021 Sep;8:100164. doi: 10.1016/j.lanepe.2021.100164. Epub 2021 Jul 14. PMID: 34278371; PMCID: PMC8278033.

#4 Gliga S, Luebke N, Killer A, Gruell H, Walker A, Dilthey AT, Lohr C, Flaßhove C, Orth HM, Feldt T, Bode JG, Klein F, Timm J, Luedde T, **Jensen BO**. Rapid selection of sotrovimab escape variants in SARS-CoV-2 Omicron infected immunocompromised patients. **Clin Infect Dis**. 2022 Oct 3:ciac802. doi: 10.1093/cid/ciac802. Epub ahead of print. PMID: 36189631; PMCID: PMC9619606.

Monoklonale Antikörper (mAb) wie Bamlanivimab (LY-CoV555) stellten, als sie Anfang 2021 in Deutschland verfügbar wurden, eine vielversprechende neue Behandlungsoption für die frühe Phase der SARS-CoV-2-Infektion dar. Im Vergleich zu Rekonvaleszentenplasma haben monoklonale Antikörper mehrere Vorteile, z. B. ihre hohe Bindungsspezifität, Homogenität und das fehlende Risiko einer möglichen Übertragung von Infektionserregern. In anderen Krankheitskontexten, wie z. B. bei der Behandlung von HIV, sind jedoch auch die potenziellen Nachteile monoklonaler Antikörper deutlich geworden, wie z.B. eine mögliche Immunevasion durch Selektion viraler Mutationen.

Bei immungeschwächten Patienten mit COVID-19 besteht ein erhöhtes Risiko, dass bei Ihnen SARS-CoV-2 über einen deutlich längeren Zeitraum replizieren kann, wodurch die Selektion von viralen Mutationen begünstigt wird (84-86). Da diese Patientengruppe in klinischen Studien mit monoklonalen Antikörpern unterrepräsentiert ist, haben wir die virale Evolution bei immungeschwächten Patienten mit verzögerter viraler Clearance nach Behandlung mit dem monoklonalen Antikörper Bamlanivimab charakterisiert.

Von sechs schwer immunsupprimierten SARS-CoV-2-infizierten Patienten, die mit Bamlanivimab behandelt wurden, konnten wir bei fünf Patienten eine virale Immunflucht beobachten. Die Kombination aus längeren Intervallen viraler Replikation unter einem eng

begrenzten Selektionsdruck durch einen einzelnen mAb - hier Bamlanivimab - ist die von uns favorisierte Hypothese für die hohe Rate an Resistenzentstehung in dieser Patientengruppe.

Alle untersuchten Patienten waren aufgrund verschiedener Krankheitsbilder oder der damit verbundenen immunsuppressiven Therapie humoral und/oder zellulär schwer immundefizient.

Die bei fünf von sechs Patienten nachgewiesene E484K-Mutation kommt auch in verschiedenen VOCs von SARS-CoV-2 vor, die mit einer Immunevasion assoziiert sind, darunter die Variante B.1.351 (WHO-Kennzeichnung Beta) und die in Brasilien nachgewiesenen Varianten P1 (WHO-Kennzeichnung Gamma) und P2 (WHO-Kennzeichnung Zeta).

Um die lokale Basisprävalenz der E484K-Mutation zu untersuchen, wurden alle zu diesem Zeitpunkt verfügbaren SARS-CoV-2 Ganzgenomsequenzen aus der Region Düsseldorf gescreent. Bemerkenswerterweise wiesen nur 3 der 1270 verfügbaren Sequenzen die E484K-Mutation auf und wurden als B.1.351/Beta-Isolate charakterisiert. Diese Beobachtung und die Tatsache, dass alle unsere Patienten bei Studienbeginn Varianten mit dem Wildtyp E484E aufwiesen, stützen unsere Hypothese, dass die E484K-Mutation tatsächlich unter dem spezifischen Immundruck von Bamlanivimab bei fünf von sechs Patienten mit beeinträchtigter humoraler und zellvermittelter Immunität neu selektiert wurde.

Unsere klinische Beobachtung, dass diese Mutationen unter der Bamlanivimab-Therapie neu auftraten und den klinischen Verlauf der Patienten beeinträchtigten, haben nicht nur bedeutende Auswirkungen auf das klinische Management einzelner Patienten, sondern auch auf epidemiologische Maßnahmen zur Pandemiebekämpfung.

Ein umsichtiges Management und die strikte Einhaltung von Praktiken zur Infektionsprävention und -kontrolle sind aufgrund dieser Ergebnisse für die Betreuung von mit mAb behandelten SARS-CoV-2-positiven, immunsupprimierten Personen von besonderer Bedeutung.

Unsere Ergebnisse zeigten, dass bei der Verwendung einzelner monoklonaler Antikörper bei immungeschwächten Patienten, die mit SARS-CoV-2 infiziert sind, aufgrund der Fähigkeit des Virus zur raschen Anpassung und damit Resistenzentwicklung Vorsicht geboten ist.

In einer weiteren prospektiven Beobachtungsstudie zur Therapie der SARS-CoV-2 Infektion im Kontext von Immundefizienz, die kurz nach der Ausbreitung der Omikron-Variante in

Deutschland startete, wurden insgesamt 57 Patienten (21 Frauen, 36 Männer) nachverfolgt. Davon waren 47 (82,5 %) mit der Omikron-Variante BA.1 und 10 (17,5 %) mit der Omikron-Variante BA.2 infiziert.

Die Ausbreitung der Omikron VOC ging einher mit einem Verlust der In-vitro-Aktivität der bis dahin gebräuchlichen mAb-Kombination Casirivimab/Imdevimab, weil die Zielregionen im Spike-Protein durch mehrere Mutationen verändert waren (87). Im Januar 2022 wurde Sotrovimab in Deutschland verfügbar. Sotrovimab war zu diesem Zeitpunkt einer der wenigen mAbs, die sich in vitro als wirksam gegen die Omikron-Variante erwiesen und stellte somit eine vielversprechende Behandlungsoption für eine frühe SARS-CoV-2-Infektion dar (53, 88).

In unserer Studie vergingen im Median vom Auftreten der Symptome bis zur Verabreichung von Sotrovimab 3 Tage. Alle behandelten Patienten befanden sich in der frühen Phase von COVID-19, als Sotrovimab verabreicht wurde. Aufgrund unserer Erfahrungen bei der Therapie SARS-CoV-2 infizierter, immundefizienter Patienten mit Bamlanivimab wurde Patienten mit Immundefizienz eine zusätzliche Therapie mit Remdesivir angeboten. Neunundzwanzig von 43 (67,4 %) immundefizienten Patienten entschieden sich nach Beratung zu einer Kombinationstherapie von Sotrovimab und Remdesivir.

Die Patienten wurden unterteilt in immunkompetent (n=14) und immundefizient (n=43). Immundefizienz umfasste meist Organtransplantation (SOT), Stammzelltransplantation (SCT), aktive hämatologische Malignome und Autoimmunkrankheiten. 39 von 43 Patienten (90 %), die als immundefizient eingestuft wurden, erhielten eine immunsuppressive Medikation.

Wir analysierten die Kinetik der viralen Clearance nach dem ersten positiven SARS-CoV-2 PCR-Test und nach Verabreichung von Sotrovimab bei immunkompetenten und immundefizienten Patienten. Alle bis auf einen immunkompetenten Patienten hatten eine Viruslast (VL) unter 10^6 Kopien/ml an Tag 14, während 21 von 43 (48,8 %) immundefizienten Patienten zu diesem Zeitpunkt eine anhaltende Virusausscheidung zeigten (P=0,011). Darüber hinaus wurde auch an Tag 21 bei 12 von 43 (27,9 %) Patienten mit Immundefizienz noch keine VL $<10^6$ Kopien/ml als Surrogat für Nichtinfektiosität erreicht.

Zusammengenommen zeigen diese Ergebnisse, dass bei immundefizienten Patienten auch nach der Verabreichung von Sotrovimab die Virusreplikation in einem hohen Prozentsatz

über einen deutlich verlängerten Zeitraum persistiert und somit die Selektion resistenter Virusvarianten wahrscheinlicher wird. Bedeutsam ist in diesem Zusammenhang, dass für den Fall eines Auftretens resistenter Virusvarianten bei einem anhaltenden Nachweis von $>10^6$ Kopien/ml eben auch von einer anhaltenden Infektiosität dieser Patienten auszugehen wäre.

In Anbetracht der verlängerten viralen Replikation bei immungeschwächten Patienten nach der Verabreichung von Sotrovimab, führten wir dann Ganzgenom-Sequenzierungen aller verfügbaren viralen Proben mit einer VL $>10^6$ Kopien/ml durch. Proben mit nachgewiesenen Resistenz-Mutationen wurden in der Folge mit quantitativer Illumina-Sequenzierung weiter analysiert. Diese Analyse ergab, dass Mutationen im Spike-Protein, die mit einer Resistenz gegen Sotrovimab verbunden sind, bei 14 von 57 Patienten (24,6 %) auftraten.

Während bei immunkompetenten Patienten keine Selektion von Escape-Mutationen beobachtet wurde, zeigte sich bei immundefizienten Patienten in 14 von 43 Patienten (32,6 %) eine Resistenzentwicklung. Zu dieser Gruppe gehörten sechs Patienten mit SOT, zwei allogene SZT-Empfänger, zwei Patienten mit aktiver maligner hämatologischer Bluterkrankung, die eine Chemotherapie erhielten, je ein Patient mit kryoglobulinämischer Vaskulitis, systemischem Lupus erythematodes und Leberzirrhose (Child-Pugh-Klasse A), die jeweils zusätzlich immunmodulatorische Therapien erhielten, sowie ein Patient mit variablem Immundefektsyndrom (CVID).

Die Resistenzmutationen, die zuerst auftraten, wurden ausschließlich an den Positionen 337 oder 340 im Spike-Protein nachgewiesen, überwiegend mit den Mutationen P337S (n=8), E340K (n=9), und E340D (n=5). Darüber hinaus wurden die Aminosäuresubstitutionen P337H/L/R und E340A/V während unseres Beobachtungszeitraums von bis zu 28 Tagen nachgewiesen. Während des Beobachtungszeitraums zeigte sich nicht nur eine Zunahme von Escape-Varianten, sondern auch eine Veränderung in der Häufigkeit der mutierten Varianten. Dies weist auf eine anhaltende virale Evolution in diesen Patienten hin.

Die Sotrovimab-spezifischen Escape-Mutationen (P337S, E340D/K/V), die bereits an Tag 7 entdeckt wurden, wurden im BA.1 und BA.2 Omikron-Hintergrund mit Hilfe eines Pseudovirus-Neutralisationstests charakterisiert. In diesen in vitro-Analysen bestätigte der Pseudovirus-Neutralisierungsassay, dass die beiden Sotrovimab-Mutationen E340K und E340V, die auch schon in Delta beschrieben wurden (89), sowie die Mutationen P337S und E340D, die im Omikron-Kontext neu beschrieben wurden (90-91), die

Neutralisierungsaktivität von Sotrovimab vollständig aufheben. Im B.1-Hintergrund (einer häufigen SARS-CoV-2 Variante aus Anfang des Jahres 2020) hingegen konnte eine stark reduzierte Neutralisierungsaktivität nur für E340K beobachtet werden, während die E340D-Mutation die Neutralisierungsaktivität von Sotrovimab in einem viel geringeren Ausmaß reduzierte. Diese Daten zeigen deutlich, dass sich nicht nur die Escape-Mutation selbst, sondern auch der breitere genetische Hintergrund des Spike-Proteins auf das Ausmaß des Einflusses einer spezifischen Escape-Mutation auf die Wirksamkeit von mAbs auswirkt.

Um die Risikofaktoren für die Selektion von Escape-Mutationen zu charakterisieren, wurde eine Korrelationsanalyse durchgeführt. Diese Analyse ergab, dass 2 Faktoren mit dem Auftreten von Resistenzmutationen korrelierten: Immundefizienz (r=0,305, p=0,021) und Tage bis zum Erreichen einer VL $<10^6$ SARS-CoV-2-RNA-Kopien/ml nach Verabreichung von Sotrovimab (r=0,322, P=0,019).

Darüber hinaus waren bei Patienten mit Tacrolimus-Therapie höhere Tacrolimus-Spiegel bei Studienbeginn positiv mit dem Auftreten von Escape-Mutationen korreliert (r=0,523, p=0,015). Bei immungeschwächten Patienten korrelierte die Verabreichung von Remdesivir negativ mit dem Auftreten von Resistenzmutationen gegen Sotrovimab (r=-0,392, p=0,009).

Zusammengenommen zeigen diese Ergebnisse, dass eine Sotrovimab-Monotherapie bei immungeschwächten Patienten mit einem hohen Risiko einer de novo-Entwicklung Sotrovimab-spezifischer Mutationen verbunden ist.

Es gibt somit immer mehr Belege für die Hypothese, dass neue SARS-CoV-2-Varianten bevorzugt bei immungeschwächten Patienten mit persistierender SARS-CoV-2-Infektion auftreten. Da einige dieser Varianten besser übertragbar sind und/oder insbesondere den durch Impfung oder Genesung induzierten humoralen Immunantworten gegen das Spike-Protein besser entkommen können, hat dies nicht nur erhebliche Auswirkungen auf die individuelle medizinische Versorgung, sondern auch auf die öffentliche Gesundheit.

Bei immungeschwächten Patienten muss daher in Anbetracht unserer Daten und der Literatur eine langanhaltende Virusreplikation und -ausscheidung im Hinblick auf die Infektionskontrolle berücksichtigt werden.

Die Verabreichung eines einzelnen mAb oder eines einzelnen antiviralen Medikaments sollte aufgrund dieser Erkenntnisse bei immungeschwächten Patienten wegen des Risikos der Entstehung von resistenten Virusvarianten vermieden werden. In unserer Studie wurde

erstmalig gezeigt, dass bei immungeschwächten Patienten eine Kombinationstherapie von Sotrovimab und dem antiviral wirksamen Polymerasehemmer Remdesivir zu einem geringeren Auftreten von Escape-Mutationen gegenüber Sotrovimab führt als bei einer Monotherapie mit Sotrovimab. Darüber hinaus führte eine zweite Remdesivir-Gabe über einen längeren Zeitraum von 10 Tagen bzw. die Kombination mit anderen antiviralen Substanzen (Molnupiravir, Nirmatrelvir/Ritonavir) zu einem anhaltenden Rückgang beziehungsweise zur Negativierung der Viruslast bei der überwiegenden Mehrheit der Patienten mit anhaltend hoher nasopharyngealer VL trotz der erfolgten initialen Therapie mit Sotrovimab +/- Remdesivir.

Zusammenfassend deuten unsere Daten darauf hin, dass Kombinationstherapien mit mindestens 2 mAbs oder anderen Virostatika, wie Remdesivir, Molnupiravir und Nirmatrelvir/Ritonavir, bei der Behandlung relevant immundefizienter Patienten mit SARS-CoV-2-Infektion bevorzugt werden sollten. Unsere Ergebnisse zeigen auch die Notwendigkeit, spezielle klinische Studien für Patienten mit ausgeprägter Immundefizienz und SARS-CoV-2 Infektion durchzuführen, um die optimale Behandlungsstrategie für diese Patientengruppe zu ermitteln.

3.3. Evaluation des Therapiemanagements bei COVID-19

#5 Freise NF, Gliga S, Fischer J, Lübke N, Lutterbeck M, Schöler M, Bölke E, Orth HM, Feldt T, Roemmele C, Wilke D, Schneider J, Wille K, Hohmann C, Strauss R, Hower M, Ruf A, Schubert J, Isberner N, Stecher M, Pilgram L, Vehreschild JJ; LEOSS Study Group, Hanses F, Luedde T, **Jensen B**. Convalescent plasma treatment for SARS-CoV-2 infected high-risk patients: a matched pair analysis to the LEOSS cohort. **Sci Rep.** 2022 Nov 9;12(1):19035. doi: 10.1038/s41598-022-23200-1. PMID: 36351986.

#6 Schons MJ, Caliebe A, Spinner CD, Classen AY, Pilgram L, Ruethrich MM, Rupp J, Nunes de Miranda SM, Römmele C, Vehreschild J, **Jensen BE**, Vehreschild M, Degenhardt C, Borgmann S, Hower M, Hanses F, Haselberger M, Friedrichs AK; LEOSS-study group. All-cause mortality and disease progression in SARS-CoV-2-infected patients with or without antibiotic therapy: an analysis of the LEOSS cohort. **Infection** 2022 Apr;50(2):423-436. doi: 10.1007/s15010-021-01699-2. Epub 2021 Oct 8. Erratum in: **Infection**. 2021 Dec 15;: PMID: 34625912; PMCID: PMC8500268.

Zu Beginn der Pandemie gab es keine evidenzbasierten therapeutischen Optionen für COVID-19. Als erste antiviral wirksame Therapieoption wurde Remdesivir im Juli 2020 in Deutschland bedingt zugelassen. Daten zu monoklonalen Antikörpern wie Bamlanivimab oder der Kombination Casirivimab/Imdevimab wurden erst gegen Ende 2020 verfügbar. Rekonvaleszenzplasma als weitere viel diskutierte Therapieoption wurde bereits bei der schweren H1N1-Influenzapandemie zu Beginn des 20. Jahrhunderts (1918), beim schweren akuten respiratorischen Syndrom im Zusammenhang mit dem Corona-Virus SARS-CoV-1 (2003) und bei der Influenza H1N1pdm09 (2009) verwendet und soll dabei zu einer geringeren Sterblichkeit geführt haben. Vor diesem Hintergrund und in Anbetracht der limitierten Therapieoptionen hatte z.B. die US-amerikanische Food and Drug Administration (FDA) im August 2020 eine Empfehlung für die Verwendung von Rekonvaleszenzplasma (CP) für die Behandlung von COVID-19 veröffentlicht (92) und in zahlreichen Ländern wurden Blutspende-Programme zur Gewinnung von Rekonvaleszentenplasma etabliert. Das zugrunde liegende Konzept der Gabe von neutralisierenden Antikörpern, die bei von der Krankheit genesenen Menschen gewonnenen wurden, erschien besonders vielversprechend für immungeschwächte Patienten, die eine verzögerte oder stark reduzierte Immunreaktion haben. Hierzu hatten

wir ebenfalls bereits einen besonders komplexen Einzelfall einer erfolgreichen Therapie einer anhaltenden Virusreplikation bei einer jungen Patientin mit einem schweren kombinierten zellulären und humoralen Immundefekt aus dem Frühjahr 2020 publiziert (93).

Wir haben daraufhin eine retrospektive Analyse von 55 mit Rekonvaleszentenplasma behandelten, hospitalisierten COVID-19-Patienten aus dem Universitätsklinikum Düsseldorf (UKD) durchgeführt, die ein hohes Risiko für einen schweren Verlauf von COVID-19 hatten. Dabei handelte es sich vorwiegend um Patienten mit einer Immunsuppression durch Krebs, Organtransplantation, Autoimmunerkrankungen oder Dialyse. Eine Matched-Pairs-Analyse (1:4) wurde mit 220 Patienten aus der LEOSS-Kohorte durchgeführt. Beide Kohorten hatten aufgrund des untersuchten Risikoprofils eine sehr hohe Sterblichkeit (UKD 41,8 %, LEOSS 34,1 %). Diese Analyse zeigte hinsichtlich der Gesamtkohorte keinen signifikanten Einfluss der Gabe von Rekonvaleszentenplasma auf die Mortalität. Die Verabreichung von Rekonvaleszentenplasma vor der Bildung von Lungeninfiltraten - also noch in der Frühphase von COVID-19 - zeigte allerdings in beiden Kohorten eine niedrige Mortalität (10 %), während die Mortalität bei Gabe in der komplizierten Phase – z.B. bei Patienten, die eine Lungenentzündung mit Sauerstoffbedarf hatten – bereits 27,8 % betrug. Die Verabreichung von Rekonvaleszentenplasma während der kritischen Phase – weitgehend entsprechend bereits Patienten im kritischen Zustand auf der Intensivstation - ergab erwartungsgemäß die höchste Sterblichkeit, die im UKD 60,9 % und in der LEOSS-Kohorte 48,3 % betrug.

Da Rekonvaleszentenplasma gerade im ersten Jahr der Pandemie in Anbetracht weitgehend fehlender therapeutischer Alternativen oft bei Hochrisiko-Patienten mit ungünstigem klinischem Verlauf und erst spät im Laufe der Erkrankung im Sinne einer ultima ratio eingesetzt wurde, unterstützen unsere Analysen das Konzept eines kritischen Zeitfensters für den Einsatz antiviral intendierter Therapiestrategien. Dazu gehöhren sowohl die antiviralen Polymerasehemmer (Remdesivir, Molnupiravir) und Proteaseinhibitoren (Nirmatrelvir/Ritonavir) als auch die monoklonalen Antikörper gegen SARS-CoV-2 und das Rekonvaleszentenplasma mit seinem Gehalt an polyklonalen Antikörpern gegen SARS-CoV-2.

Unsere Beobachtung einer erniedrigten Mortalität bei Behandlung von Risikopatienten mit Rekonvaleszentenplasma noch in der Frühphase von COVID-19 zeigte sich auch in einer randomisierten Studie, die bei früher Gabe von Rekonvaleszentenplasma eine Risikoreduktion hinsichtlich des Auftretens einer schweren respiratorischen Erkrankung von 48% zeigen konnte (42).

Eine weitere zu Beginn der SARS-CoV-2 Pandemie ungelöste Frage war der Nutzen einer frühen empirischen Antibiotika-Therapie von Patienten mit COVID-19 Pneumonie. Gerade aufgrund der in diesem Kontext in der Regel vorhandenen deutlich erhöhten Entzündungsparameter wie CRP und der oft ausgeprägten radiologischen Veränderungen ist die klinische Einschätzung bezüglich einer gleichzeitig bestehenden bakteriellen Superinfektion herausfordernd. Bei viralen Atemwegsinfektionen wie der Influenza können bakterielle Superinfektionen zu einer höheren Morbidität und Mortalität führen und erfordern dann eine rechtzeitige Diagnose und Einleitung einer Antibiotikatherapie. Die durch Influenzaviren verursachte Sterblichkeit ist dabei eng mit sekundären bakteriellen Erregern assoziiert, ein besonders eindrucksvolles Beispiel stellt die Influenza-Pandemie von 1918 dar, in der mehr als 95 % der 50 Millionen oder mehr Todesfälle auf eine bakterielle Lungenentzündung zurückzuführen waren (94).

In frühen Veröffentlichungen im Jahr 2020 zum Thema bakterielle Superinfektionen und COVID-19 hingegen wurde von einer eher geringen Rate an bakteriellen Ko- und Superinfektionen von weniger als 10 % berichtet (95, 96), während die Rate an systemischem Antibiotika-Einsatz über 60 % betrug (95).

Antibiotic Stewardship (ABS) hat in Anbetracht der weltweit wachsenden Resistenzproblematik bei bakteriellen Krankheitserregern das Ziel die Entwicklung von Antibiotikaresistenzen nachhaltig zu reduzieren, indem ein Bewusstsein für einen rationalen Antibiotikaeinsatz und optimierte Antibiotikatherapiestrategien geschaffen wird.

Ein möglicher Einsatz weiterer Entzündungsmarker wie Procalcitonin (PCT) könnte bei der Unterscheidung zwischen bakteriellen und nicht-bakteriellen akuten Atemwegsinfektionen auch bei COVID-19 hilfreich sein. Bakterielle Infektionen erhöhen seine Produktion und Freisetzung aus extrathyreoidalen Quellen in den Blutkreislauf, und

ein niedriger PCT-Wert weist auf eine geringere Wahrscheinlichkeit einer bakteriellen Infektion hin (97-100).

Unsere Studie hatte das Ziel, den Zusammenhang zwischen dem Einsatz von Antibiotika und den Endpunkten Gesamtmortalität und klinische Verschlechterung bei Patienten in einer komplizierten Phase von COVID-19 und niedrigen PCT-Werten zu untersuchen.

Erneut wurde in der Studie die bereits zuvor beschriebene LEOSS-Kohorte genutzt. 6457 SARS-CoV-2-infizierte Fälle, die vom 18. März 2020 bis zum 16. Februar 2021 in der LEOSS-Kohorte dokumentiert wurden, wurden analysiert. Als primärer Endpunkt wurde die Korrelation zwischen der Verabreichung einer Antibiotikabehandlung und der Gesamtmortalität bzw. dem Fortschreiten in die nächsthöhere Krankheitsphase für erwachsene Patienten in der komplizierten Krankheitsphase und einem Procalcitonin-Wert von $\leq 0{,}5$ ng/ml berechnet. Bei der Analyse wurden die Einflussfaktoren Geschlecht, Alter und Komorbiditäten berücksichtigt.

Insgesamt 3627 Fälle erfüllten alle Einschlusskriterien für die Analysen. Für den primären Endpunkt korrelierte die Antibiotikabehandlung nicht mit einer geringeren Gesamtmortalität oder einem Fortschreiten in die nächsthöhere (kritische) Phase. Bei den sekundären Endpunkten hatten Patienten in der unkomplizierten Phase, unabhängig vom PCT-Wert, keine niedrigere Gesamtmortalität und keine geringere Progression in die nächsthöhere (komplizierte) Phase, wenn sie mit Antibiotika behandelt wurden. Des Weiteren hatten Patienten in der komplizierten Phase mit einem PCT-Wert von $> 0{,}5$ ng/ml und erfolgter Gabe von Antibiotika eine signifikant erhöhte Gesamtmortalität.

Zusammenfassend war der Einsatz von Antibiotika bei SARS-CoV-2-infizierten Patienten in der untersuchten Stichprobe der LEOSS-Kohorte nicht mit positiven Auswirkungen auf die Gesamtmortalität oder das Fortschreiten von COVID-19 verbunden. Ein empirischer Einsatz von Antibiotika bei Patienten mit COVID-19 ohne klare Hinweise auf eine bakterielle Koinfektion bzw. Superinfektion sollte daher nicht erfolgen.

4. Zusammenfassung

Mit Beginn der SARS-CoV-2 Pandemie stellte das neue Krankheitsbild COVID-19 die klinisch tätigen Ärzte weltweit vor große Herausforderungen. Gerade die hohe Komplexität von COVID-19 in Verbindung mit den, in Wellen auftretenden, hohen Patientenzahlen brachte die Gesundheitssysteme vieler Länder an und über ihre Grenzen. Gleichzeitig ergaben sich eine Vielzahl dringlicher wissenschaftlicher Fragestellungen, die in den Jahren 2020-2022 in hohem Maß weltweit die wissenschaftliche Arbeit dominierten.

Die hier beschriebenen Arbeiten konnten einerseits einen Beitrag liefern, um die Risikofaktoren für einen schweren Verlauf für COVID-19 besser zu definieren und damit einen gezielteren Einsatz der gerade in den pandemischen Wellen nur in beschränktem Maß verfügbaren Überwachungs-, Therapie- und Präventionsmaßnahmen zu ermöglichen. Zum anderen ist eine Kenntnis der Risikofaktoren unerlässlich, um bei Menschen mit einem erhöhten Risiko für einen schweren Verlauf bereits während der ersten Tage der SARS-CoV-2 Infektion eine antivirale Therapie einzuleiten und damit einen schweren Verlauf von COVID-19 deutlich weniger wahrscheinlich zu machen.

Gerade die Erkenntnis aus den hier vorgelegten Arbeiten und ähnlichen Veröffentlichungen anderer Arbeitsgruppen, dass die Risikofaktoren Adipositas, Diabetes und Bluthochdruck das Risiko einer COVID-19-bedingten Sterblichkeit auch bei jüngeren Patienten deutlich erhöhen, veränderte sowohl die Indikationsstellung für antivirale Therapien in der Frühphase als auch die Strategien bei der Priorisierung von Risikogruppen im Rahmen der Impfkampagnen gegen SARS-CoV-2. Obwohl der Zusammenhang zwischen chronischer Inflammation, Adipositas und metabolischem Syndrom gut bekannt ist, war der in dieser Arbeit und von anderen Gruppen beschriebene Einfluss dieser Risikofaktoren auf die Entwicklung der Folgeerkrankungen Long COVID/Post-COVID-Syndrom, bei deren Pathogenese Entzündungsprozesse nach unserem bisherigen Verständnis ebenfalls eine wichtige Rolle spielen, zumindest in seinem Ausmaß überraschend.

Die hier dargestellten Arbeiten zur Immunevasion von SARS-CoV-2 gegenüber monoklonalen Antikörpern im Kontext der Behandlung von Patienten mit Immundefizienz hatten einen wesentlichen Einfluss auf die Therapieregime, die wir während der SARS-CoV-Pandemie eingesetzt haben. Die sowohl bei Bamlanivimab zu Beginn des Jahres 2021, als auch bei Sotrovimab während der frühen Omikron-Welle Anfang 2022 erhobenen Daten

zu einer raschen Resistenzentwicklung gegenüber diesen wichtigen monoklonalen Antikörpern in einem hohen Anteil der behandelten Patienten mit Immundefizienz sind unter anderem in die Therapieempfehlungen der COVRIIN-Fachgruppe am Robert-Koch-Institut eingeflossen und wurden inzwischen von mehreren anderen Arbeitsgruppen bestätigt. Die Arbeit, in der eine Verminderung des Risikos der Selektion resistenter viraler Varianten bei Patienten mit Immundefizienz und verlängerter Virusausscheidung bei einer Kombinationstherapie mit dem monoklonalen Antikörper Sotrovimab und dem Polymeraseinhibitor Remdesivir gezeigt werden konnte, liefert dabei als eine der ersten klinischen Arbeiten Hinweise auf mögliche Vorteile einer Kombinationstherapie von SARS-CoV-2 bei Patienten mit Immundefizienz.

Abschließend konnte der Einsatz der, in der Frühphase der Pandemie intensiv und auch kontrovers diskutierten, Therapie mit Rekonvaleszentenplasma näher beleuchtet werden. Die Auswertung einer Kohorte am Universitätsklinikum Düsseldorf sowie der LEOSS-Kohorte bestätigte, passend zu unserem heutigen pathophysiologischen Verständnis der in Phasen verlaufenden Erkrankung COVID-19, dass Rekonvaleszentenplasma bei bereits schwer an COVID-19 erkrankten Patienten in der Spätphase des Krankheitsbildes mit im Vordergrund stehender Dysregulation des Immunsystems und Hyperinflammation keinen klinischen Nutzen hat. Andererseits ist die Beobachtung, dass eine frühe Therapie mit Rekonvaleszentenplasma mit einer niedrigen Mortalität von COVID-19 assoziiert war, sehr gut mit den Daten aus größeren randomisierten Studien vereinbar, die diesen Zusammenhang belegen konnten.

Zuletzt konnte in der LEOSS-Kohorte die Fragestellung beantwortet werden, ob im Rahmen von COVID-19, ähnlich wie bei der Influenza, bakterielle Superinfektionen eine hohe Bedeutung für die Mortalität bzw. den klinischen Verlauf haben. Da die Antibiotikabehandlung nicht mit einer geringeren Gesamtmortalität oder einer günstigen Beeinflussung des klinischen Verlaufs korrelierte, ist aufgrund dieser Daten der Einsatz von Antibiotika im Rahmen von COVID-19 nur bei klinischen, laborchemischen oder mikrobiologischen Hinweisen auf eine bakterielle Super- oder Koinfektion indiziert.

5. Literaturverzeichnis

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6. Lebenslauf

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7. Eigene Veröffentlichungen (Originalarbeiten)

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8. Anhang: Der Habilitationsschrift zugrundeliegende Publikationen

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ORIGINAL RESEARCH
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Obesity and Impaired Metabolic Health Increase Risk of COVID-19-Related Mortality in Young and Middle-Aged Adults to the Level Observed in Older People: The LEOSS Registry

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Advanced age, followed by male sex, by far poses the greatest risk for severe COVID-19. An unresolved question is the extent to which modifiable comorbidities increase the risk of COVID-19-related mortality among younger patients, in whom COVID-19-related hospitalization strongly increased in 2021. A total of 3,163 patients with SARS-COV-2

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diagnosis in the Lean European Open Survey on SARS-CoV-2-Infected Patients (LEOSS) cohort were studied. LEOSS is a European non-interventional multi-center cohort study established in March 2020 to investigate the epidemiology and clinical course of SARS-CoV-2 infection. Data from hospitalized patients and those who received ambulatory care, with a positive SARS-CoV-2 test, were included in the study. An additive effect of obesity, diabetes and hypertension on the risk of mortality was observed, which was particularly strong in young and middle-aged patients. Compared to young and middle-aged (18–55 years) patients without obesity, diabetes and hypertension (non-obese and metabolically healthy; $n = 593$), young and middle-aged adult patients with all three risk parameters (obese and metabolically unhealthy; $n = 31$) had a similar adjusted increased risk of mortality [OR 7.42 (95% CI 1.55–27.3)] as older (56–75 years) non-obese and metabolically healthy patients [$n = 339$; OR 8.21 (95% CI 4.10–18.3)]. Furthermore, increased CRP levels explained part of the elevated risk of COVID-19-related mortality with age, specifically in the absence of obesity and impaired metabolic health. In conclusion, the modifiable risk factors obesity, diabetes and hypertension increase the risk of COVID-19-related mortality in young and middle-aged patients to the level of risk observed in advanced age.

Keywords: obesity, diabetes, hypertension, impaired metabolic health, mortality, COVID-19

INTRODUCTION

As of 14 February 2022, more than 404 million people worldwide have been infected with SARS-CoV-2, resulting in more than 5.7 million deaths (1). Early in the SARS-CoV-2 pandemic, older age was identified as the strongest risk factor for COVID-19-related mortality. Furthermore, male sex and several comorbidities were found to be associated with an increased risk of mortality in patients with COVID-19 (2–4). Obesity and hyperglycemia in the non-diabetic range were additionally identified as potential risk factors for COVID-19 morbidity and mortality (5–9). Of note, these relationships were independent of age, sex and other comorbidities (10–14). Consequently, obesity and impaired metabolic health are now viewed as important modifiable risk factors for disease severity (15–17).

However, recently, in a large, international, multicenter study from 18 sites in 11 countries, of 7,244 patients hospitalized with COVID-19, obesity and diabetes were found to associate with increased adjusted odds of supplemental oxygen/non-invasive ventilatory support, yet, not with mortality (18). Furthermore, in a very large community-based cohort study from the United Kingdom that evaluated data from 6,910,695 patients with a positive SARS-CoV-2 test result, obesity strongly associated with mortality in the younger and middle-aged adults, but not in the older patients (19). Unfortunately, in that study no adjustment for comorbidities could be done. Thus, it is important to clarify whether obesity and other metabolic comorbidities may increase the risk of COVID-19-related mortality, independently of other diseases, specifically in younger and middle-aged patients.

These patients with COVID-19 are generally considered to have substantially lower risk of COVID-19-related mortality, than those older than 65 years. However, risk in younger age groups has become increasingly relevant, with initially selective vaccination of older individuals and rapidly rising incidence of infection and hospitalization among children, adolescents, and young adults (20). Data from the US Centers for Disease Control and Prevention (CDC) suggests that a 35-year-old with diabetes mellitus, hypertension, cardiovascular disease, obesity, or other chronic conditions had a similar risk of COVID-19-related death as a 65-year-old with none of these conditions (21). Furthermore, in an analysis of data from an US Premier Healthcare Database of hospital-based patients with COVID-19, younger patients (age 18–34 years) with morbid obesity, hypertension, and diabetes faced similar risk of death or need for mechanical ventilation, as that observed in middle-aged (age 35–64 years) adults (22). However, these did not consider potential confounding, and in the CDC report no information about comorbidities was available in 22% of the patients (21). Adjustment for sex and other comorbidities, such as cardiovascular, renal and liver disease, is essential, as these comorbidities are strongly related to impaired metabolic health.

To clarify the potential impact of obesity and impaired metabolic health on COVID-19 related mortality in younger adults, we have studied the determinants of COVID-19-related mortality in 3,163 patients with COVID-19 of the Lean European Open Survey on SARS-CoV-2-Infected Patients (LEOSS) cohort study.

RESEARCH DESIGN AND METHODS

Study Design and Patient Cohort

A total of 6,457 consecutive patients, who were included in the LEOSS registry between March 2020 and February 2021, were evaluated. LEOSS is a European non-interventional multi-center

cohort study established in March 2020 to address the lack of information on the epidemiology and clinical course of SARS-CoV-2 infection (23, 24). The registry collects data on hospitalized patients of all ages and patients who receive ambulatory medical consultation. As of July 2020, more than 125 sites from 7 different countries have been registered to LEOSS. Daily statistics are provided on the LEOSS website (https://leoss.net). To facilitate the rapid data acquisition needed during a pandemic, LEOSS involves autonomous, self-managed study sites that collect data in an anonymous form. To achieve this, no directly identifying data are stored in the registry and demographic data as well as timestamps are only collected in a rough form. Furthermore, data were documented categorically. Patient privacy was additional protected using the anonymization procedures described by Jakob et al. (24). Data collection is performed once per case, retrospectively after treatment has finished or the patient has died. Although this method precludes longitudinal data collection and follow-up of discharged patients, it has the advantage that no informed consent is necessary. Furthermore, this method provides for the inclusion of data on children and unconscious or deceased patients and avoids problems that could arise from language barriers. All patients had a diagnosis confirmed by positive results of PCR testing. Approval for LEOSS was obtained by the applicable local ethics committees of all participating centers and registered at the German Clinical Trails Register (DRKS, No. S00021145).

Clinical Data and Outcomes

Data were recorded in an electronic case report form operated using the online cohort platform ClinicalSurveys.net, which was developed by the University Hospital of Cologne (UHC), Germany. ClinicalSurveys.net was hosted by QuestBack, Oslo, Norway on servers of UHC, Cologne, as part of a software-as-a-service agreement. Baseline data closest to the first positive SARS-CoV-2 test were analyzed. Demographic, clinical, laboratory and outcome data were extracted from the in-hospital medical records. Operational definitions of the co-morbidities studied are based on the medical diagnosis guidelines that were applied by the treating physicians in the hospital. Diagnosis were either pre-known or newly made by the treating physicians based on the clinical in-hospital evaluation and/or laboratory results. Analyzed laboratory data were collected within 48 h of a positive SARS-CoV-2 PCR result, irrespective of the patient's status. Among the 6,457 patients evaluated only adult (age $\geq$18 years) patients who had complete information about sex, age, BMI and the comorbidities diabetes, hypertension, coronary artery disease, chronic kidney disease and chronic liver disease (N = 3,517) were considered eligible for the analyses. Among them, a total of 354 patients with missing information on survival were excluded, yielding a sample of 3,163 for the main analyses (**Supplementary Figure 1**).

Comorbidities were dichotomized (e.g., diabetes present/absent, coronary artery disease present/absent). Comorbidities were set to unknown/missing when all specific comorbidities of one group were unknown or missing. Values documented as unknown were defined as missing. Besides sex, age, BMI and the above-mentioned comorbidities, the following clinical parameters related to metabolic risk, which were not available in all patients, were evaluated: hemoglobin A1c (HbA1c), serum creatinine, serum C-reactive protein (CRP), serum interleukin-6 (IL-6), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum gamma-glutamyl transferase (GGT), as well as urine ketone bodies. Clinical parameters were set to unknown/missing if not available. The primary outcome was COVID-19-related mortality. In an exploratory approach disease severity, which is not a hard endpoint, was also studied (uncomplicated phase: patients were either asymptomatic, and had symptoms of upper respiratory tract infection, fever or nausea, emesis, or diarrhea; complicated phase: patients had at least one of the characteristics new need for oxygen supplementation or clinically relevant increase of prior oxygen home therapy, PaO2 at room air $<$ 70 mmHg, SO2 at room air $<$ 90%, increase of AST or ALT $>$ 5 $\times$ upper limit of normal, new cardiac arrythmia, new pericardial effusion $>$ 1 cm or new heart failure with pulmonary edema, congestive hepatopathy, or peripheral edema; critical phase patients were dependent on catecholamines, experienced life-threatening cardiac arrhythmia, had mechanical ventilation (invasive or non-invasive), or need for unplanned mechanical ventilation prolongation ($>$ 24 h) of planned mechanical ventilation, liver failure with an INR $>$ 3.5 (quick $<$ 50%), a qSOFA score of $>=$ 2, or acute renal failure with need of dialysis).

Statistical Analyses

We calculated and report patient characteristics as absolute numbers and percentages. For comparison of percentages between groups the χ^2-test was used. The odds ratios of baseline characteristics, comorbidities and laboratory parameters, with mortality were assessed in univariate and in multivariable logistic regression models. Univariate and multivariable relationships of baseline characteristics with mortality were also assessed after patients were stratified in young and middle aged (18–55 years; n = 1,068), older age (56–75 years; n = 1,220) and old age (>75 years; n = 875) groups. Then patients in each age group were further categorized by the presence or absence of obesity, of obesity+diabetes and of obesity+diabetes+hypertension. For the main analyses, patients in the three age groups were subdivided into those (i) without obesity (BMI<30 kg·m^{-2}) and without impaired metabolic health (no diabetes and no hypertension, n = 1,098) and in those (ii) having all three risk factors (BMI $\geq$30 kg·m^{-2}, diabetes and hypertension, n = 259). Kaplan-Meier analyses were used to compare the survival of the patients among these six subgroups. A $p < 0.05$ was considered to indicate statistical significance. Data management, statistical analysis, and computation of figures were conducted using R (R Development Core Team, Vienna, Austria, Version 3.5.2., 2019). Additional information about the LEOSS questionnaire can be found under https://leoss.net/.

RESULTS

Among the 3,163 patients included in the analyses, data were collected primarily from Germany (N = 95%), as well as from Turkey, Belgium, Switzerland, Spain, Austria, Italy, Bosnia and

TABLE 1 | Multivariable relationships of selected anthropometrics, comorbidities and laboratory parameters with COVID-19-related mortality.

Characteristics	Recovered/died	OR	Lower 95%CI	Upper 95%CI	*p*
Age 18–25 (years)	71/0	0.00	0.000	0.00	0.97
Age 26–35 (years) (ref)	199/3				
Age 36–45 (years)	290/4	0.82	0.18	4.22	0.80
Age 46–55 (years)	475/26	2.89	0.10	12.3	0.09
Age 56–65 (years)	578/83	7.14	2.60	29.5	0.001
Age 66–75 (years)	446/113	11.9	4.35	49.2	<0.0001
Age 76–85 (years)	478/196	17.4	6.37	71.7	<0.0001
Age >85 (years)	124/104	44.8	15.9	187	<0.0001
Sex female (ref)	1,059/171				
Sex male	1,602/331	1.62	1.30	2.04	<0.0001
BMI 18.5–24.9 (kg·m^{-2}) (ref)	873/167				
BMI 25–29.9 (kg·m^{-2})	977/178	0.99	0.78	1.29	0.99
BMI 30–34.9 (kg·m^{-2})	534/94	1.04	0.76	1.40	0.81
BMI ≥35 (kg·m^{-2})	277/63	1.77	1.22	2.56	0.003
No diabetes (ref)	2,119/333				
Diabetes	542/169	1.44	1.09	1.89	0.009
HbA1c <6.4% (ref)	48/6				
HbA1c 6.4–8 %	118/27	2.04	0.82	5.88	0.15
HbA1c 8.1–10%	61/14	2.65	0.95	8.16	0.07
HbA1c >10%	30/12	6.37	2.13	20.8	0.001
HbA1c not available	2,404/443	3.96	1.73	10.8	0.003
No hypertension (ref)	1,416/138				
Hypertension	1,245/364	1.27	0.99	1.61	0.056
No coronary artery disease (ref)	2,340/376				
Coronary artery disease	321/126	1.14	0.88	1.48	0.31
No chronic kidney disease (ref)	2,322/359				
Chronic kidney disease	339/143	1.42	1.10	1.82	0.007
No liver cirrhosis (ref)	2,643/493				
Liver cirrhosis	18/9	2.41	0.97	5.70	0.048

OR, odds ratio; CI, confidence interval.

Herzegovina, United Kingdom and Latvia. A total of 2,989 from 3,144 patients (19 patients with missing information) had an inpatient stay. Disease course was classified as uncomplicated (N = 1,284) complicated (N = 1,130) and critical (N = 749) (24). From the 3,163 patients studied, 2,661 patients recovered from the disease while 502 patients died (**Supplementary Table 1**).

Univariable and Multivariable Relationships of Patient Characteristics With Mortality

In univariable analyses, among the parameters age, sex, BMI, comorbidities and selected laboratory variables, determined at the day of SARS-COV-2 diagnosis, higher age, male sex, diabetes, hypertension, HbA1c >10%, coronary artery disease, chronic liver disease and liver cirrhosis were associated with an increased risk of mortality (**Supplementary Table 2**). In a multivariable regression model including all studied parameters, higher age, male sex, BMI ≥35 kg·m^{-2}, diabetes, HbA1c >8.1%, CRP ≥30 mg/L and GGT >10 upper limit of normal were independently associated with an increased risk of mortality (**Supplementary Table 3**).

To avoid over-adjustment in the statistical models by including variables that are highly related to each other, e.g., the diagnosis of liver cirrhosis and elevated transaminases or chronic kidney disease and elevated serum creatinine, we further focused in the multivariate regression models on the parameters reported in the **Table 1**. In that parsimonious multivariable regression model higher age, male sex, BMI ≥ 35 kg·m^{-2}, HbA1c >10%, chronic kidney disease and liver cirrhosis were independently associated with an increased risk of mortality. The association with hypertension was borderline, with an adjusted p-value of 0.056 (**Table 1**, **Supplementary Figure 2**).

Risk of Mortality in Young/Middle-Aged, Older and Old Patients

To investigate the relationships of obesity and impaired metabolic health with the risk of mortality in different age groups, patients were divided into three age groups (**Supplementary Table 4**), with 1,068 young and middle-aged, 1,220 older age and 875 old age groups. Based on the similar sample sizes these three groups were equally strong powered for

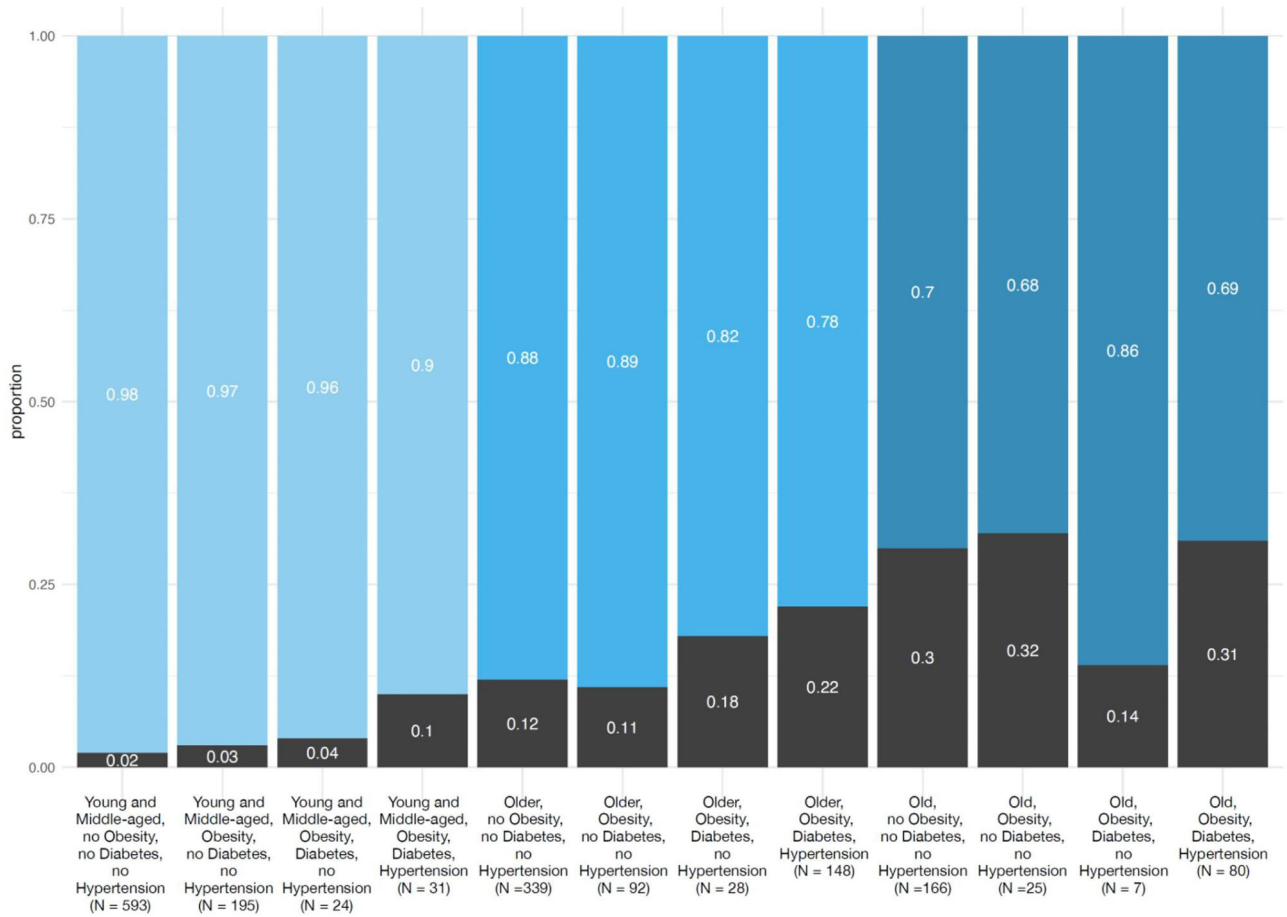

FIGURE 1 | Proportion of COVID-19 patients who recovered and died divided in three age groups based on the presence or absence of obesity, diabetes and hypertension. All COVID-19 patients who recovered and died ($n = 3,163$) were first divided in three age groups (young and middle aged, 18–55 years, $n = 1,068$; older age, 56–75 years; $n = 1,220$ and old age, >75 years; $n = 875$) and subsequently divided in four groups based on the presence or absence of obesity (BMI ≥ 30 kg·m^{-2}), and impaired metabolic health (diabetes and hypertension).

the investigation of the patient's characteristics with mortality in the statistical analyses. In multivariable regression analyses male sex was associated with a higher risk of mortality in the young/middle-aged and in the old age groups, but not in the older age group. BMI $\geq$35 kg·m^{-2} was associated with increased mortality in the young/middle-aged and in the older age groups, but not in the old age group. Diabetes was associated with increased mortality only in the old age group (**Supplementary Table 5**).

Risk of Mortality in Subjects Stratified by Age and Obesity/Metabolic Health

To compare the contributions of advanced age vs. obesity and impaired metabolic health (diabetes and hypertension) to the mortality risk, we divided the patients into 12 subgroups based upon age and presence or absence of obesity, diabetes and hypertension. First, to investigate an additive effect of these parameters on the mortality risk, we divided the subjects in the three age groups based on the presence or absence of obesity, obesity + diabetes and obesity + diabetes + hypertension. Second, to investigate the impact of obesity + impaired metabolic health (diabetes and hypertension) on the risk of mortality more in detail, we compared the following 6 groups: (1) young and middle-aged without obesity, diabetes and hypertension ($N = 593$), (2) young and middle-aged with obesity, diabetes and hypertension ($N = 31$), (3) older age without obesity, diabetes and hypertension ($N = 339$), (4) older age with obesity, diabetes and hypertension ($N = 148$), (5) old age without obesity, diabetes and hypertension ($N = 166$) and (6) old age with obesity, diabetes and hypertension ($N = 80$).

When the age groups were stratified by the presence or absence of obesity and impaired metabolic health, both, older age and the presence of obesity and impaired metabolic health associated with increased risk of mortality (**Figure 1**). In the multivariable statistical model (**Table 2**, Model 1) moderately higher adjusted risks of mortality were observed in the young and middle-aged patients with obesity [$N = 195$; OR 1.75 (95% CI 0.53–5.13)] and obesity + diabetes [$N = 24$; OR 2.96

TABLE 2 | Multivariable relationships of three age groups based on the presence (unhealthy) or absence (healthy) of obesity, diabetes and hypertension and selected anthropometrics, comorbidities and laboratory parameters with COVID-19-related mortality.

Characteristics	Model 1				Model 2			
	OR	Lower 95%CI	Upper 95%CI	*p*	OR	Lower 95%CI	Upper 95%CI	*p*
Young/middle-aged–no obesity, no diabetes, no hypertension (ref.) (*N* = 593)								
Young/middle-age–obesity, no diabetes, no hypertension (*N* = 195)	1.75	0.53	5.13	0.32	1.55	0.47	4.60	0.45
Young/middle-aged–obesity, diabetes, no hypertension (*N* = 24)	2.96	0.16	17.3	0.32	2.81	0.14	17.1	0.35
Young/middle-aged–obesity, diabetes, hypertension (*N* = 31)	6.95	1.45	25.6	0.006	5.99	1.23	23.0	0.014
Older–no obesity, no diabetes, no hypertension (*N* = 339)	8.24	4.12	18.4	<0.0001	6.88	3.40	155	<0.0001
Older–obesity, no diabetes, no hypertension (*N* = 92)	7.70	3.01	20.0	<0.0001	5.88	2.25	15.5	0.0003
Older–obesity, diabetes, no hypertension (*N* = 28)	13.4	3.61	44.9	<0.0001	13.6	3.53	48.2	0.0001
Older–obesity, diabetes, hypertension (*N* = 148)	18.0	8.16	43.0	<0.0001	14.7	6.55	35.9	<0.0001
Old–no obesity, no diabetes, no hypertension (*N* = 166)	24.4	12.1	54.9	<0.0001	21.6	10.5	49.5	<0.0001
Old–obesity, no diabetes, no hypertension (*N* = 25)	29.6	9.88	88.8	<0.0000	24.6	7.94	75.6	<0.0001
Old–obesity, diabetes, no hypertension (*N* = 7)	7.47	0.37	52.4	0.08	6.62	0.32	48.5	0.10
Old–obesity, diabetes, hypertension (*N* = 80)	28.4	12.1	71.5	<0.0001	27.1	11.3	69.6	<0.0001
Sex male	1.38	0.98	1.95	0.07	1.28	0.90	1.83	0.18
HbA1c 6.4–8%	1.40	0.38	6.78	0.64	1.45	0.38	7.23	0.61
HbA1c 8.1–10%	1.99	0.50	10.1	0.36	2.78	0.67	14.6	0.19
HbA1c >10%	3.47	0.67	20.7	0.14	2.98	0.55	18.8	0.22
HbA1c unknown	2.34	0.72	10.6	0.20	2.48	0.74	11.5	0.18
Coronary artery disease	1.13	0.70	1.78	0.61	1.08	0.66	1.74	0.74
Chronic kidney disease	1.75	1.14	2.66	0.009	1.76	1.13	2.73	0.012
Liver cirrhosis	1.55	0.32	5.63	0.53	2.76	0.54	10.7	0.17
CRP 3–29 mg/L	-	-	-	-	1.77	0.58	7.71	0.37
CRP 30–69 mg/L	-	-	-	-	4.95	1.66	21.4	0.011
CRP 70–119 mg/L	-	-	-	-	5.32	1.74	23.3	0.009
CRP 120–179 mg/L	-	-	-	-	6.54	2.05	29.2	0.004
CRP 180–249 mg/L	-	-	-	-	17.4	5.01	81.8	<0.0001
CRP >249 mg/L	-	-	-	-	23.4	6.43	113	<0.0001
CRP unknown	-	-	-	-	6.56	2.31	27.6	0.002

OR, odds ratio; CI, confidence interval; Model 1, adjusted for sex, HbA1c, coronary artery disease, chronic kidney disease and liver cirrhosis; Model 2, adjusted for sex, HbA1c, coronary artery disease, chronic kidney disease, liver cirrhosis and CRP.

(95% CI 0.16–17.3)], which were statistically not significant, when compared to the young and middle-aged patients without obesity, diabetes or hypertension. However, when compared to the latter group, the adjusted risk of mortality was strongly increased in the young and middle-aged patients with obesity+impaired metabolic health [diabetes + hypertension; *N* = 31; OR 6.95 (95% CI 1.45–25.6)]. This group had a nearly 7-fold higher risk of mortality, compared to the young and middle-aged patients without obesity, diabetes or hypertension (**Table 2**, Model 1 and **Figure 2**).

Older patients without obesity, diabetes or hypertension had a higher adjusted risk of mortality [*N* = 339; OR 8.24 (95% CI 4.12–18.4)], compared to young and middle-aged patients without obesity, diabetes or hypertension. This risk increased in the presence of obesity, diabetes and hypertension and older patients having all three risk factors (*N* = 148) had an adjusted OR for mortality of 18.0 (95% CI 8.16–43.0), compared to young and middle-aged patients without obesity, diabetes or hypertension. Interestingly, this risk was merely 2.2-fold higher than the risk of older patients without obesity, diabetes and hypertension (**Table 2**, Model 1 and **Figure 2**).

Old patients without obesity, diabetes and hypertension had a very high adjusted risk of mortality [*N* = 166; OR 24.4 (95% CI 12.1–54.9)], compared to young and middle-aged patients without obesity, diabetes or hypertension. However, in the old patients, obesity, diabetes or hypertension only weakly increased this risk [1.2-fold higher; *N* = 80; OR 28.4 (95% CI 12.1–71.5)] (**Table 2**, Model 1 and **Figure 2**).

Similar relationships were observed when patients were stratified in those with an uncomplicated and a severe

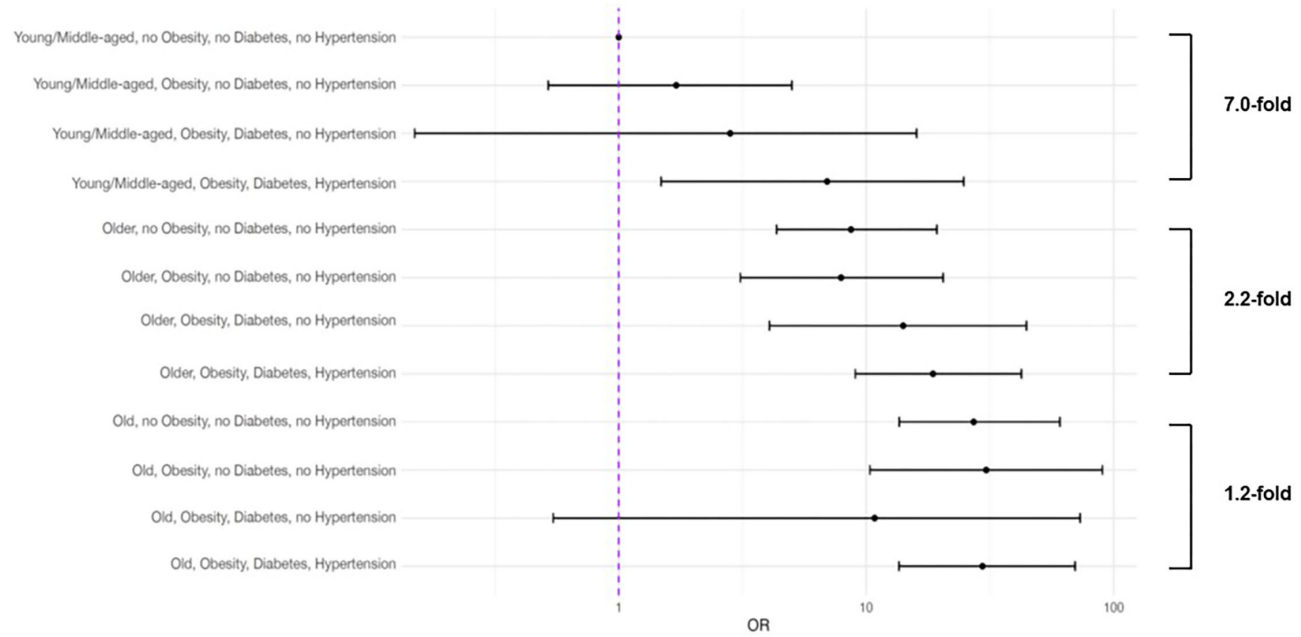

FIGURE 2 | Multivariable relationships of selected anthropometrics, comorbidities and laboratory parameters with COVID-19-related mortality in three age groups based on the presence (unhealthy) or absence (healthy) of obesity, diabetes and hypertension. All COVID-19 patients who recovered and died ($n = 3,163$) were first divided in three age groups (young and middle aged, 18–55 years, $n = 1,068$; older age, 56–75 years; $n = 1,220$ and old age, >75 years; $n = 875$) and subsequently divided in two groups ($n = 1,357$) based on the presence (unhealthy) or absence (healthy) of obesity (BMI $\geq$ 30 kg·m^{-2}), and impaired metabolic health (diabetes and hypertension). All parameters shown were included in the multivariable regression analysis.

(complicated phase and critical phase) course of the disease. For example, when compared to the young and middle-aged patients without obesity, diabetes or hypertension, the adjusted risk of severe COVID-19 was increased in the young and middle-aged patients with obesity + impaired metabolic health [diabetes + hypertension; $N = 31$; OR 2.60 (95% CI 1.87–3.64)]. Furthermore, this risk was comparable to the risk observed in older non-obese and metabolically healthy patients [$n = 339$; OR 2.66 (95% CI 2.01–3.52)] (**Supplementary Table 6**).

Among the patients who died, most deaths occurred within the first 2 weeks of follow-up. In Kaplan-Meier survival analyses young and middle-aged patients with obesity and impaired metabolic (diabetes + hypertension) health had a similar time-to-death to those in the older age group without obesity and impaired metabolic health (**Figure 3**). Compared to young and middle-aged patients without obesity and impaired metabolic health (group 1), the adjusted OR of mortality was 6.95 (95% CI 1.45–25.6) in the young and middle-aged group with obesity and impaired metabolic health (group 2), which was not statistically different from the risk in the older age group without obesity and impaired metabolic health [OR 8.24 (95% CI 4.12–18.4)] (**Table 2**, Model 1 and **Figure 2**).

We then explored parameters that may explain the elevated risk of COVID-19-related mortality with age, specifically in the absence of obesity and impaired metabolic health. We additionally adjusted our multivariable regression model for CRP levels (**Table 2**, Model 2). This resulted in an attenuation of the elevated risk of mortality observed in older and old patients without obesity and impaired metabolic health, when compared to young and middle-aged patients without obesity and impaired metabolic health, by 17 and 11%, respectively.

To address, whether the increased risk of mortality that associated with obesity and impaired metabolic health and that was very high, particularly in the group of young and middle-aged patients, may be predominantly driven by the risk in middle-aged patients, we also divided the patients in a younger (age 18–35 years) group and a middle-aged (age 36–55 years) group. Although the sample size was very low, only allowing an exploratory evaluation, we found that young patients with obesity and impaired metabolic health had a 4.2-fold higher risk of mortality, compared to the young patients without obesity and impaired metabolic health. A similarly increased risk (3.5-fold) was observed in the middle-aged patients with obesity and impaired metabolic health, compared to the middle-aged patients without obesity and impaired metabolic health (**Supplementary Table 7**).

DISCUSSION

Both, high BMI and adverse cardiometabolic status, are now established risk factors for severe COVID-19 (25). However, the risk attributed to these factors is considered to be lower than that of advanced age and perhaps also male sex. Nevertheless, the relative importance of these risk factors has not been well-studied. This knowledge gap may have direct public health

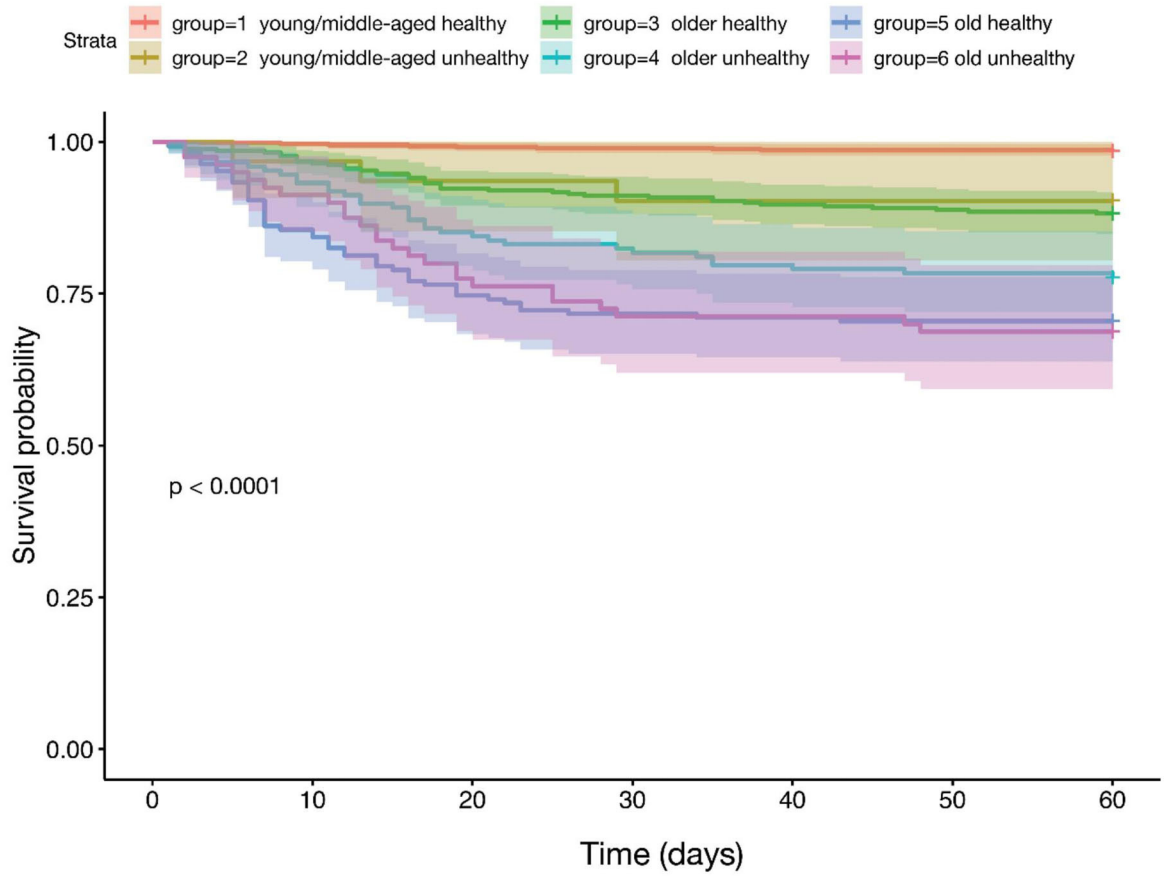

FIGURE 3 | Kaplan-Meier survival comparing three age groups based on the presence (unhealthy) or absence (healthy) of obesity, diabetes and hypertension. All COVID-19 patients who recovered and died ($n = 3{,}163$) were first divided in three age groups (young and middle aged, 18–55 years, $n = 1{,}068$; older age, 56–75 years; $n = 1{,}220$ and old age, >75 years; $n = 875$) and subsequently divided in two groups ($n = 1{,}357$) based on the presence (unhealthy) or absence (healthy) of obesity (BMI $\geq$ 30 kg·m^{-2}), and impaired metabolic health (diabetes and hypertension).

implications, as metabolic risk factors–unlike age and sex–are modifiable (15–17). In this multi-national study, mostly including hospitalized patients with COVID-19, we found similar relationships of metabolic risk factors and adiposity, with COVID-19-related mortality, as were reported by previous studies (2–14). This allowed us to address an important question: to what extent does obesity, diabetes and hypertension, which were recently found to account for almost 60% of the COVID-19 hospitalizations in the United States (26), increase the risk of COVID-19-related mortality in younger patients, when compared to older patients. We found that an additive effect of obesity, diabetes and hypertension on the risk of COVID-19-related mortality exists. Compared to the respective older and old groups without these risk factors, the adjusted risk of mortality increased particularly strong in the young and middle-aged groups with these risk factors. In this respect, compared to young and middle-aged patients without obesity, patients merely having obesity only had a moderately increased adjusted mortality risk. This risk increased considerably in young and middle-aged patients with obesity and diabetes. Such an increase in risk was not observed in the older and old patients. Importantly, the presence of all three risk factors, obesity diabetes and hypertension, independently of other comorbidities and of sex, increased the risk of COVID-19-related mortality in younger and middle-aged patients to the risk level that we observed in older patients without these diseases. This finding is potentially of major public health relevance, as younger age is considered to protect from severe COVID-19.

Studies including COVID-19 patients from the United Kingdom reported that diabetes most strongly increased the risk of COVID-19-related mortality in younger patients (27, 28). Furthermore, data from the US CDC and the US Premier Healthcare Database of hospital-based patients with COVID-19 previously suggested that younger patients with obesity, diabetes or other comorbidities, have an increased risk of COVID-19-related death, that amounted to the risk often observed in older patients (21, 22). However, in those studies no adjustment for sex and comorbidities was done. In our study, diabetes was associated with an increased risk of COVID-19-related mortality in younger and in middle-aged patients, but this relationship was attenuated with adjustment for sex, BMI and other comorbidities. Thus, our findings indicate

that obesity, diabetes and hypertension comprise a phenotype strongly associated with increased risk of COVID-19-related mortality in young and middle-aged patients, independently of other important determinants of severe COVID-19.

These findings may have several clinical implications. First, they support the recommendations of international medical societies, that obesity, diabetes and hypertension are important risk factors that should be critically considered by health care providers, when COVID-19 is being diagnosed in a patient. Intense clinical surveillance of these patients, particularly during the early stages of the disease, should be ensured. This approach is also supported by our findings of an increased mortality of obese and metabolically unhealthy COVID-19 patients during the first 2 weeks after diagnosis, independently of age.

Second, in view of the changing demographics of hospitalizations–with a substantial increase among patients <55 years relative to older people (21)–health care providers should not assume that younger individuals generally are at lower risk for severity of COVID-19. Consequently, younger people with these common risk factors should also be prioritized in vaccination strategies.

Third, there is increasing concern that SARS-CoV-2 will not only become an endemic virus and that an emergent coronavirus may cause severe disease in children (29–31), but that new variants of SARS-CoV-2 may evade the body's immune response, both in vaccinated and in not yet vaccinated people (29–35). Particularly the second year of the COVID-19 pandemic has been dominated by variants of concern (36, 37). Among them, mutations of the SARS-CoV-2 spike protein, the primary antigen, may be problematic, as most recently suggested for the Omicron (B.1.1.529) SARS-CoV-2 variant of concern[1]. In this respect obesity and diabetes may become even more important risk factors than currently considered. Obesity and impaired metabolic health may adversely influence the efficacy of SARS-CoV-2 vaccines (38, 39). In this respect, most recently some preliminary data indicate that obesity, diabetes and CVD may predispose for vaccine breakthrough COVID-19 infections (40–42). Premature immunesenescence, accelerated aging of the immune system, particularly of the CD4+ and CD8+ T cell compartments, has been found in people with obesity or type 2 diabetes (43–45). Intriguingly, as a mechanism explaining this observation, intact insulin signaling was observed to play an important role in modulating the body's immune response. Insulin receptor signaling has an impact on T cell glucose metabolism and amino acid handling. In rodents, insulin receptor-deficient T cells were found to have reduced inflammatory potential and poor protective immunity against H1N1 influenza infection (46). Considering that obesity, especially central adiposity, and impaired metabolic health, strongly associate with insulin resistance (47–49), and a healthy diet and exercise (50), as well as new dietary concepts to improve the gut microbiome (51) are very helpful to improve metabolic health, reduction of fat mass and a healthy diet may be critical for the coming months of the SARS-CoV-2 pandemic.

[1]https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern

Fourth, most recently it was shown that, beyond the acute illness, substantial burden of health loss, including disorders of lipid metabolism, diabetes and obesity, is observed in COVID-19 survivors (52, 53). Although, this has not been investigated, yet, the presence of obesity and impaired metabolic health prior to the SARS-CoV-2 infection may particularly increase the burden of health loss in COVID-19 survivors. This may be problematic especially for younger patients, who may, thereby, experience a larger amount of years of life lost, than older patients.

A strength of our study is that the multi-center LEOSS registry prospectively collects epidemiological and clinical data based on a pre-specified protocol. Furthermore, the hospitals have the capacity to also monitor patients with asymptomatic or mild SARS-CoV-2 infections. However, there are several limitations. This study analyzed factors associated with disease course at initial presentation, not treatment, and cannot assess causality. We cannot rule out the presence of confounding from socioeconomic status, health insurance issues and access to health services and country specific testing capacities, among other factors. Some of these factors could be correlated with delayed diagnosis and therefore a more complicated clinical stage at initial presentation. Furthermore, the highest documentation rates were performed by University hospitals in larger cities; consequently, rural areas might be underrepresented. Finally, the sample size in the younger age groups was relatively small, most probably resulting from the fact that younger people generally are less often hospitalized with COVID-19 compared to middle-aged and older people. The small sample size in some of the groups may result in that a statistical error may occur from skewed group comparisons.

In conclusion, we found that obesity, diabetes and hypertension have an additive effect on COVID-19-related mortality and that this effect is particularly strong in young and middle-aged patients. Furthermore, we found that obesity, diabetes and hypertension increased the risk of COVID-19-related mortality in young and middle-aged patients to the risk level that we observed in older but metabolically healthy patients. Importantly, this increased risk was independent of other comorbidities and of sex. Awareness of health care providers about this strong impact of obesity and impaired metabolic health on the risk of COVID-19-related mortality may be critical to intensify surveillance of younger patients infected with SARS-CoV-2 and to motivate subjects at risk to lose weight and improve their metabolic health.

DATA AVAILABILITY STATEMENT

Patient data from the LEOSS registry are subject to the LEOSS governance, data use, and access policy (policy text available on https://leoss.net). Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Ethics Committees of all participating centers

and registered at the German Clinical Trails Register (DRKS, No. S00021145). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

NS and KS analyzed the data and wrote the manuscript. NS, KS, MHe, and AB designed the study. CJ, AW, YK, SB, MMR, FH, MHa, CP, MHo, JD, KW, CR, JV, MSt, and BJ collected the data and contributed to the discussion. MHe, AF, RW, HP, MSo, MRo, AS, BG, MA, DL, MSc, and AB critically reviewed the manuscript and contributed to the discussion. NS is the guarantor of this study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.875430/full#supplementary-material

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ORIGINAL PAPER

Obesity and lipid metabolism disorders determine the risk for development of long COVID syndrome: a cross-sectional study from 50,402 COVID-19 patients

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Abstract

Purpose Metabolic disorders have been identified as major risk factors for severe acute courses of COVID-19. With decreasing numbers of infections in many countries, the long COVID syndrome (LCS) represents the next major challenge in pandemic management, warranting the precise definition of risk factors for LCS development.

Methods We identified 50,402 COVID-19 patients in the Disease Analyzer database (IQVIA) featuring data from 1056 general practices in Germany. Multivariate logistic regression analysis was used to identify risk factors for the development of LCS.

Results Of the 50,402 COVID-19 patients included into this analysis, 1,708 (3.4%) were diagnosed with LCS. In a multivariate regression analysis, we identified lipid metabolism disorders (OR 1.46, 95% CI 1.28–1.65, $p < 0.001$) and obesity (OR 1.25, 95% CI 1.08–1.44, $p = 0.003$) as strong risk factors for the development of LCS. Besides these metabolic factors, patients' age between 46 and 60 years (compared to age ≤ 30, (OR 1.81 95% CI 1.54–2.13, $p < 0.001$), female sex (OR 1.33, 95% CI 1.20–1.47, $p < 0.001$) as well as pre-existing asthma (OR 1.67, 95% CI 1.39–2.00, $p < 0.001$) and depression (OR 1.27, 95% CI 1.09–1.47, $p = < 0.002$) in women, and cancer (OR 1.4, 95% CI 1.09–1.95, $p = < 0.012$) in men were associated with an increased likelihood of developing LCS.

Conclusion Lipid metabolism disorders and obesity represent age-independent risk factors for the development of LCS, suggesting that metabolic alterations determine the risk for unfavorable disease courses along all phases of COVID-19.

Keywords LCS · Long COVID · Post-COVID syndrome · SARS-CoV-2 · BMI · Diabetes

Introduction

The SARS-CoV-2 pandemic represents an unprecedented global challenge. By November 2021, over 247 million confirmed cases of SARS-CoV-2 have been reported and more than 5 million patients have died in association with the coronavirus disease 2019 (COVID-19) [1]. Even with infection rates and numbers of patients hospitalized for COVID-19 decreasing in some countries, the long-term consequences of SARS-CoV-2 infections, often referred to as long COVID syndrome (LCS), represent a growing medical and socioeconomic problem, worldwide [2].

The LCS can affect a wide range of organ systems such as the respiratory system or the nervous system [3]. Commonly observed symptoms include shortness of breath, fatigue, anosmia, muscle weakness or cognitive impairment [3]. However, a broad variety of at least partly unspecific symptoms have been described in the context of LCS

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and LCS has only been poorly defined to date and systematic data on incidence rates are largely missing [4, 5]. Current data from the United Kingdome and the United States of America indicate that the incidence of the LCS range between 7 and 13.3%, depending on the definition of LCS as well as the length of the follow-up period after initial diagnosis of COVID-19 [4, 5]. The WHO has recently published a clinical case definition of post-COVID-19 syndrome, which also includes a review of several other definitions of LCS/post-COVID syndrome [6]

Risk factors for LCS are widely unclear. In particular, it is only poorly understood if the risks for incidence and severity of LCS correlate with disease severity of acute SARS-CoV-2 infection, warranting a clear definition of risk factors for the development of LCS [2]. In addition, most existing data on LCS are primarily focusing on patients hospitalized for COVID-19, while less severe courses that are treated by general practitioners only are less frequently considered. In the present study, we, therefore, used the Disease Analyzer database (IQVIA), which features diagnoses and basic medical as well as demographic data of outpatients treated in general practices in Germany, to study the prevalence of LCS in Germany and to identify clinical factors associated with its development.

Materials and methods

Study design and database

This retrospective observational study was based on cross-sectional medical record data from the Disease Analyzer database (IQVIA), which compiles diagnoses as well as general medical and demographic data that are anonymously obtained from computer systems of general practitioners and specialists in Germany [7]. The sampling method for the Disease Analyzer database is based on summary statistics from all medical doctors in Germany that are published yearly by the German Medical Association and is defined according to the specialist group, the German federal state, the community size category, and the physicians' age. The database covers ~ 3% of all outpatient practices in Germany. The sampling methods used to select physicians' practices have been shown to be appropriate for obtaining a population-representative database of primary and specialized care in Germany [7]. Diagnoses [(according to the International Classification of Diseases, 10th revision (ICD-10)], prescriptions (according to the Anatomical Therapeutic Chemical (ATC) Classification system), and the quality of reported data are constantly monitored by IQVIA.

Study population and outcomes

The analysis included 50,402 patients with a confirmed diagnosis of COVID-19 (ICD-10: U07.1) between March 1, 2020 and March 31, 2021 (index date) from one of 1056 GP practices that routinely send data to the Disease Analyzer database. The study's primary outcome was the proportion of patients with a documentation of long COVID syndrome (LCS) or a diagnosis suggestive for LCS. Since there was no specific ICD-10 code for LCS during this period of time, LCS was identified based on the original diagnosis text of the physicians ("long COVID syndrome", "post COVID syndrome, "post COVID complications"). The following ICD-10 diagnoses were additionally used as surrogates for LCS: chronic fatigue (ICD-10: G93.3), abnormalities of breathing (ICD-10: R06), disturbances of smell and taste (ICD-10: R43), malaise and fatigue (ICD-10: R53, disturbances in attention (ICD-10: R41.8). Patients with a diagnosis of one or more of these diagnoses documented within the time period between 90 and 183 days after the diagnosis of COVID-19 were enrolled. Patients with a diagnosis of one or more of these diagnoses within 12 months prior to diagnosis of COVID-19 were excluded.

Statistical analyses

The proportion of patients with LCS was analyzed for the total study population as well as for men, women and four age groups (≤ 30, 31–45, 46–60 and > 60 years). The association between predefined variables and the incidence of LCS was investigated in a multivariable logistic regression model. This model included age, sex, and the following diagnoses documented within 12 months prior to the index date: arterial hypertension (ICD-10: I10), lipid metabolism disorders (ICD-10: E78), obesity (ICD-10: E66), cancer (ICD-10: C00–C99), type 1 diabetes mellitus (ICD-10: E10), type 2 diabetes mellitus (ICD-10: E11, E14), depression (ICD-10: F32, F33), asthma (ICD-10: J45), and chronic obstructive bronchitis or lung disease (ICD-10: J42–J44). In a subgroup of patients with available body mass index (BMI) values documented within 6 months prior to the index date ($n = 7732$), the association between BMI and LCS was analyzed in a second multivariable logistic regression model. Results from the logistic regression analyses are shown as odds ratios (ORs) and 95% confidence intervals (CI). A p value lower than 0.05 was considered statistically significant. All analyses were performed using SAS 9.4. (Cary, NC: SAS Institute Inc).

Results

Characteristics of study cohort

To identify risk factors for the development of long COVID syndrome (LCS), we performed a retrospective observational study based on cross-sectional medical record data from the Disease Analyzer database (IQVIA), which compiles diagnoses as well as general medical and demographic data obtained anonymously from computer systems of general practitioners in Germany [7]. Of the 50,402 patients with a confirmed SARS-CoV-2 infection (ICD-10: U07.1), 1708 (3.4%) were diagnosed with LCS or one of the related diagnoses (ICD-10: G93.3, R06, R43, R53; Table 1). The average time between the diagnosis of COVID-19 and the diagnosis of LCS was 82 days (SD 28 days). Each patient had a least one diagnosis of LCS or the related diagnoses > 90 days after the initial diagnosis of COVID-19. The mean age of all COVID-19 patients was 48.8 years (SD: 19.3 years. 27,512 (54.5%) of patients were female. Arterial hypertension ($n = 12{,}898$, 25.6%) was the most prevalent comorbidity, followed by lipid metabolism disorders ($n = 8580$, 17.0%), depression ($n = 8529$, 16.9%), diabetes mellitus type 2 ($n = 5060$, 10.0%), obesity ($n = 4995$, 9.90%), and chronic bronchitis or chronic obstructive pulmonary disease ($n = 4399$, 8.7%).

Clinical factors associated with the development of long COVID syndrome

To identify independent risk factors for LCS, we performed multivariate logistic regression analyses (Table 2). These analyses revealed that lipid metabolism disorders (OR 1.46, 95% CI 1.28–1.65, $p < 0.001$) and obesity (OR 1.25 95% CI 1.08–1.44, $p = 0.003$) displayed a strong association with the development of LCS. Notably, the age group between 46 and 60 years (OR 1.81, 95% CI 1.54–2.13, $p < 0.001$) was associated with a 1.8-fold higher risk of LCS compared to patients ≤ 30 years. Moreover, the risk for LCS rose gradually with increasing BMI and was highest among patients with a BMI ≥ 35 kg/m^2; however, this association was not significant due to the small sample sizes of documented

Table 1 Baseline characteristics of the study sample

Variable	Category	Number of patients	Proportion (%)
n		50,402	
Age in years	Mean (standard deviation)	48.8 (19.3)	
	≤ 30	10,443	20.7
	31–45	12,963	25.7
	46–60	14,424	28.6
	> 60	12,572	25.0
Sex	Female	27,512	54.5
	Male	22,890	45.5
Comorbidities documented within 12 months prior to the index date	Hypertension	12,898	25.6
	Lipid metabolism disorder	8,580	17.0
	Diabetes mellitus type 1	364	0.7
	Diabetes mellitus type 2	5060	10.0
	Obesity	4995	9.9
	Ischemic heart diseases	3803	7.6
	Asthma	4073	8.1
	Chronic bronchitis/COPD	4399	8.7
	Cancer	2605	5.2
	Depression	8529	16.9
Month of the first COVID-19 diagnosis	March–September 2020	7340	14.6
	October 2020	4351	8.6
	November 2020	9437	18.7
	December 2020	11,347	22.5
	January 2021	8252	16.4
	February 2021	4032	8.0
	March 2021	5643	11.2

Data represent percentages unless otherwise stated

Table 2 Association between predefined variables and the incidence of long COVID syndrome in patients diagnosed with COVID-19 (multivariate logistic regression model)

Variable	Number of patients in the subgroup (*n*)	Proportion of patients with post-COVID-19 syndrome (*n*, %)	Total		Women		Men	
			Odds ratio (95% confidence interval)[1]	*p* value	Odds ratio (95% confidence interval)[1]	*p* value	Odds ratio (95% confidence interval)[1]	*p* value
Total	50,402	1708 (3.4)						
Age ≤ 30 years	10,443	213 (2.0)	Reference					
Age 31–45 years	12,963	379 (2.9)	1.33 (1.12–1.58)	0.001	1.29 (1.04–1.60)	0.023	1.39 (1.05–1.84)	0.021
Age 46–60 years	14,424	664 (4.6)	1.81 (1.54–2.13)	< 0.001	1.67 (1.36–2.05)	< 0.001	2.04 (1.56–2.67)	< 0.001
Age > 60 years	12,572	452 (3.6)	1.19 (0.99–1.43)	0.071	1.09 (0.86–1.39)	0.460	1.37 (1.01–1.86)	0.045
Female	27,512	1056 (3.8)	1.33 (1.20–1.47)	< 0.001	–		–	
Male	22,890	652 (2.9)	Reference		–		–	
Hypertension	12,898	634 (4.9)	1.31 (1.15–1.48)	< 0.001	1.27 (1.08–1.49)	0.004	1.39 (1.13–1.70)	0.001
Lipid metabolism disorder	8580	472 (5.5)	1.46 (1.28–1.65)	< 0.001	1.43 (1.21–1.68)	< 0.001	1.49 (1.22–1.81)	< 0.001
Diabetes mellitus type 1	364	15 (4.1)	1.00 (0.59–1.69)	0.987	0.98 (0.45–2.11)	0.950	0.99 (0.48–2.05)	0.978
Diabetes mellitus type 2	5060	233 (4.6)	0.93 (0.79–1.10)	0.389	0.80 (0.64–1.02)	0.069	1.10 (0.87–141)	0.440
Obesity	4995	271 (5.4)	1.25 (1.08–1.44)	0.003	1.28 (1.06–1.53)	0.010	1.19 (0.94–1.51)	0.153
BMI < 25.0 kg/m^2	2521	136 (5.4)	Reference		Reference		Reference	
BMI 25.0–29.9 kg/m^2	2693	138 (5.1)	0.95 (0.74–1.21)	0.662	0.92 (0.67–1.26)	0.611	1.14 (0.75–1.73)	0.543
BMI 30.0–34.9 kg/m^2	1549	95 (6.1)	1.15 (0.88–1.50)	0.323	1.20 (0.85–1.69)	0.301	1.25 (0.79–2.00)	0.342
BMI ≥ 35.0 kg/m^2	969	63 (6.5)	1.22 (0.90–1.66)	0.207	1.20 (0.83–1.73)	0.337	1.34 (0.76–2.36)	0.311
Ischemic heart diseases	3803	187 (4.9)	1.08 (0.91–1.29)	0.391	1.21 (0.96–1.52)	0.115	0.90 (0.69–1.18)	0.458
Asthma	4073	231 (5.7)	1.49 (1.28–1.73)	< 0.001	1.67 (1.39–2.00)	< 0.001	1.22 (0.93–1.59)	0.155
Chronic bronchitis/COPD	4399	187 (4.3)	0.93 (0.79–1.10)	0.379	0.82 (0.64–1.04)	0.056	1.13 (0.88–1.46)	0.328
Cancer	2605	132 (5.1)	1.21 (1.00–1.46)	0.054	1.04 (0.81–1.35)	0.737	1.46 (1.09–1.95)	0.012
Depression	8529	416 (4.9)	1.21 (1.07–1.37)	0.002	1.27 (1.09–1.47)	0.002	1.14 (0.92–1.41)	0.223

[1]The logistic regression analysis was adjusted for age, sex, comorbidities including hypertension, lipid metabolism disorder, diabetes mellitus, obesity, ischemic heart diseases, asthma, chronic bronchitis/COPD, cancer, depression. BMI values were available for 7732 patients

BMI values. Besides these metabolic factors, we identified that female sex (OR 1.33, 95% CI 1.20–1.47, $p < 0.001$) was significantly associated with the likelihood of being diagnosed with LCS. In addition, pre-existing asthma (OR 1.49, 95% CI 1.28–1.73, $p < 0.001$), hypertension (OR 1.31, 95% CI 1.15–1.48, $p < 0.001$), and depression (OR 1.21, 95% CI 1.07–1.37, $p = 0.002$) turned out as risk factors for the development of LCS. In contrast, pre-existing diabetes mellitus type 1 or 2, ischemic heart disease, or cancer did not influence the development of LCS (Table 2). Finally, we observed differences regarding the development of LCS between female and male COVID-19 patients. As such, obesity had stronger effect in women than in men and a pre-existing cancer diagnosis had a significant effect on the development of LCS in men but not women. In contrast, asthma and depression were significantly associated with LCS in female but not male COVID-19 patients (Table 2).

Discussion

Our data suggest that lipid metabolism disorders and obesity but not diabetes represent strong age-independent risk factors for LCS. As the pathophysiology of LCS is presently unclear, this finding provides important information about a possible pathophysiological relationship of metabolic risks and the development and severity of LCS. This would support the hypothesis that obesity-related chronic

inflammation and immune-metabolic processes promote not only severe clinical courses of acute SARS-CoV-2 infection [8], but also the development of LCS. In this context, it cannot be excluded that in our statistical analysis, there might have been an indirect association between severe courses of COVID-19 and the occurrence of LCS. However, it should be noted that the data source of outpatients with SARS-CoV-2 infection makes it unlikely that severe clinical courses had accumulated in our cohort of LCS patients. Moreover, diabetes or age > 60 years, known risk factors for severe courses of acute COVID-19 [9, 10], were not associated with LCS in our cohort, arguing against a linear concordance between risk-profiles of acute COVID-19 and LCS.

Post-acute sequelae (PAS) in the context of viral respiratory infections do not represent a fundamentally new observation, since PAS were already described as a consequence of other non-persistent viral infections in the pre-COVID era [11]. Of note, recent data suggest that the clinical symptoms, which are now referred to as LCS, likewise occur after infection with seasonal influenza [12]. Interestingly, metabolic factors are also discussed as potential risk factors for short and long-term mortality and morbidity for other viral infections as well [13–15], highlighting the general role of metabolic diseases as determinants for patients' long-term outcome after viral infections. Besides metabolic risk factors, we identified other pre-existing medical conditions such as asthma, arterial hypertension and depression as important risk factors for the development of LCS. Our observation that female sex and patients' age between 46 and 60 years indicate an increased risk of LCS is consistent with other published data from non-hospitalized [16] or hospitalized cohorts [17] of COVID-19 patients. Sigfrid et al. showed that women under age 50 were up to five times less likely to report feeling recovered and twice as likely to report worse fatigue than men of the same age [17]. A recent study of a cohort of healthcare workers (HCW) made observations that point in a similar direction to our data on a larger and more representative population, showing an OR of 1.6 for HCWs who were overweight and an OR of 3.7 for HCWs who had lung disease [18].

In contrast to previous studies of LCS, which have focused predominantly on specific patient groups and tended to study cohorts cared for at specialized COVID-19 centers, our study features a large cohort of COVID-19 outpatients that are representative for the sociodemographic situation in Germany and other high-income countries. However, we acknowledge some limitations. First, during the study period, LCS represented a novel diagnosis that evolved over time and had not yet been assigned to a specific ICD code. Clear diagnostic criteria as nowadays provided by the WHO were lacking, which may have led to overestimation or underestimation of LCS cases. Second, besides diagnosis of LCS in the original diagnosis text, we included diagnoses suggestive for LCS (e.g., "abnormalities of breathing") that could also occur independently of COVID-19 and there is no valid information if these symptoms were associated with COVID-19 or not. In contrast, some diagnoses that are also consistent with LCS may not have been sufficiently accounted for. Moreover, data starting from March 2020 were used while LCS has really came to light at the end of 2020, which explains why this diagnosis was documented more often in the last months. Finally, we were unable to include a control group as the diagnosis LCS cannot occur in people who were not diagnosed with COVID-19 previously. Nevertheless, our database of currently more than 50,000 COVID-19 patients is a valuable source to identify risk factors for the development of LCS. The overlap with previously published results [16, 17] strengthens the validity of our results and supports the usability of our database in the context of LCS research.

In summary, since obesity and lipid disorders represent modifiable risk factors, our data suggest that lifestyle and metabolic interventions could be part of future strategies for pandemic preparedness. Moreover, our data clearly support the fact that patients with metabolic diseases should be considered as risk patients in all phases of COVID-19, and therefore, need a close clinical supervision even after overcoming the acute phase of COVID-19.

Author contribution KK, SHL and CR designed the study; KK performed statistical analyses and generated figures and tables; SHL, CR, BOJ and KK wrote the manuscript; TL and CT provided intellectual input and corrected the manuscript; SHL, KK, and CR revised the manuscript; all the authors agreed to the final version of the manuscript.

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Declarations

Conflict of interest The authors declare no competing interest.

Ethical approval The "Disease Analyzer" database, used for analysis, contains anonymized electronic patient records. Patient data were analyzed in aggregated form without individual data being available. An individual consent form was not obtained following national and European legislation.

Consent for publication All the authors approved the publication of this manuscript.

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Research paper

Emergence of the E484K mutation in SARS-COV-2-infected immunocompromised patients treated with bamlanivimab in Germany

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ABSTRACT

Background: Monoclonal antibodies (mAb) have been introduced as a promising new therapeutic approach against SARS-CoV-2. At present, there is little experience regarding their clinical effects in patient populations underrepresented in clinical trials, e.g. immunocompromised patients. Additionally, it is not well known to what extent SARS-CoV-2 treatment with monoclonal antibodies could trigger the selection of immune escape viral variants.
Methods: After identifying immunocompromised patients with viral rebound under treatment with bamlanivimab, we characterized the SARS-CoV-2-isolates by whole genome sequencing. Viral load measurements and sequence analysis were performed consecutively before and after bamlanivimab administration.
Findings: After initial decrease of viral load, viral clearance was not achieved in five of six immunocompromised patients treated with bamlanivimab. Instead, viral replication increased again over the course of the following one to two weeks. In these five patients, the E484K substitution – known to confer immune escape – was detected at the time of viral rebound but not before bamlanivimab treatment.
Interpretation: Treatment of SARS-CoV-2 with bamlanivimab in immunocompromised patients results in the rapid development of immune escape variants in a significant proportion of cases. Given that the E484K mutation can hamper natural immunity, the effectiveness of vaccination as well as antibody-based therapies, these findings may have important implications not only for individual treatment decisions but may also pose a risk to general prevention and treatment strategies.
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1. Introduction

Monoclonal antibodies (mAb), such as bamlanivimab (LY-CoV555), represent a promising new treatment option for early SARS-CoV-2 infection. They have the potential to prevent complications of severe COVID-19 disease for the individual patient and therefore might help to reduce the burden on health systems [1,2]. Compared to previous approaches of using convalescent plasma, monoclonal antibodies have several advantages, for example, their

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Research in context

Evidence before this study

We searched PubMed Central for articles published until April 27, 2021, using the key words "SARS-CoV-2″, "bamlanivimab", "E484K" and "immune escape". Moreover, we screened preprint servers such as medrxiv, biorxiv and SSRN for relevant articles. There are no clinical reports describing the emergence of the E484K mutant after treatment with bamlanivimab, with the exception of data from a pivotal large randomized trial (BLAZE-1), which reported low frequencies of newly detected bamlanivimab immune escape mutants in patients receiving different doses of bamlanivimab and significantly lower frequencies in patients in the placebo arm. Several publications describe mutations associated with bamlanivimab immune escape in vitro.

Added value of this study

In the context of using the SARS-CoV-2-specific mAb bamlanivimab in patients at increased risk for severe course of COVID-19, we observed viral rebound in five of six severely immunodeficient patients treated with bamlanivimab. Whole genome sequencing revealed the rapid emergence of the immune escape mutation at position 484 within days of bamlanivimab administration in all five patients with viral rebound. Severe clinical course of COVID-19 was not prevented in two of our patients despite early administration of bamlanivimab, one of whom had a fatal outcome. While it is known that escape mutations can evolve upon treatment with monoclonal antibodies, convalescent plasma, or under the selection pressure of natural immunity, our study provides, to our knowledge, for the first time data on a clinical constellation in which selection of the immune escape mutation E484K, which also occurs in epidemiologically important "variants of concern," occurs in a very high proportion of patients.

Implications of all the available evidence

SARS-CoV-2 immune escape mutants can evolve rapidly in the context of prolonged narrowly focused selection pressure, such as in the treatment of SARS-CoV-2 with single monoclonal antibodies in immunocompromised patients. Viral variants harbouring immune escape mutations not only threaten the individual response to treatment, but also potentially pose a threat to general prevention and treatment strategies due to their transmissibility. From the experience with bamlanivimab presented here, it can be inferred that treatments of immunocompromised SARS-CoV-2 patients with single monoclonal antibodies should be applied with utmost caution.

high specificity of binding, homogeneity and the lack of potential transmission of infectious agents. However, in other disease contexts, such as HIV therapy, potential drawbacks of monoclonal antibodies have also become clear, such as immune escape by selection of viral mutations [3]. In SARS-CoV-2 infection, several viral variants of concern (VOCs) are associated with immune evasion from neutralizing antibodies after resolved infection or vaccination and therefore potentially compromise the efficacy of prevention and treatment strategies [4,5]. Based on pathophysiological considerations, immunocompromised COVID-19-patients may be at increased risk of not achieving rapid viral clearance, thereby favoring the selection of viral mutations under therapy pressure. For influenza, it has been shown that antiviral resistance to neuraminidase inhibitors readily develops in immunocompromised individuals with persistent viral shedding [6]. It has been already reported that viral persistence and evolution of SARS-CoV-2 with selection of immune escape mutants can occur in immunocompromised hosts [4,7]. Since this patient group is underrepresented in clinical trials with monoclonal antibodies, we characterized viral evolution in five immunocompromised patients with delayed viral clearance after treatment with the monoclonal antibody bamlanivimab.

2. Methods

2.1. Design and setting

All patients were treated with bamlanivimab for SARS-CoV-2 infection at an academic tertiary referral center in Germany because of their increased individual risk for progression to severe COVID-19. Viral load was measured consecutively before and after bamlanivimab administration. After identifying immunocompromised patients with viral rebound under treatment with bamlanivimab, we characterized the available SARS-CoV-2-isolates with cycle threshold < 30 by whole genome sequencing.

2.2. Isolation of viral genomic material and SARS-CoV-2 quantification

Respiratory samples from nasopharyngeal swabs were used for total nucleic acid extraction using the EZ1 Virus Mini Kit v2.0 on an EZ1 Advanced XL (Qiagen, Germany) according to the manufacturer's instructions. SARS-CoV-2 was detected as previously described [8] by the cobas® SARS-CoV-2 test on the cobas®6800 system (Roche), or by the SARS-CoV-2 test on the NeuMoDx™ platform (Qiagen) with a plasmid-standard for quantification [9].

2.3. SARS-CoV-2 whole genome sequencing

Viral RNA was reversely transcribed to single-strand cDNA using random hexamers and SuperScript reverse transcriptase (ThermoFisher) [10]. Viral cDNA was PCR-amplified using the Artic network SARS-CoV-2 protocol with V3 primers [11,12], employing an extended annealing/extension time of 10 min. Prior to library preparation, for each sample, Artic PCR pools 1 and 2 were combined (500 ng DNA per pool). Sequencing was carried out on the Oxford Nanopore MinION device, utilizing MIN106 flow cells and the SQL-LSK109 ligation sequencing kit. Barcoding was carried out with the native Barcoding Expansion 96 Kit (EXP-NBD196).

Data analysis and generation of consensus sequences were carried out as previously described [13]. Briefly, after base-calling with Guppy v3.4.5+fb1fbfb, the Artic pipeline1 with default settings was applied to each sequencing run, analyzing each sample independently with Nanopolish and Medaka [14]. Generated VCF files and consensus FASTA sequences were manually curated by a) carrying out a comparison between the Nanopolish- and Medaka-based VCF files and b) visual inspection with IGV [15], checking for i) false-positive calls; ii) polymorphic positions with more than one plausible allele; iii) false-negative calls.

2.4. Role of the funding source

The funders had no role in study design, data collection, data analysis, interpretation or writing of the report.

3. Results

After bamlanivimab became available for clinical use in Germany as the first monoclonal antibody directed against SARS-CoV-2, our clinic treated six patients in whom we feared a severe course in the setting of SARS-CoV-2 with known severe humoral and/or cellular immunodeficiency. The main clinical characteristics of the six patients are presented in Table 1.

Table 1
Patient characteristics.

	Age	Sex	Medical Condition	Immunosuppressive medication	Days post first positive SARS-CoV-2 qPCR at the time of bamlanivimab administration	Immune escape	Viral strain	Outcome
Patient 1	early seventies	male	ANCA-associated vasculitis with end-stage renal disease	rituximab, prednisolon	D2	yes, E484K and E484Q	B.1	Died
Patient 2	early forties	female	AIDS		D3	yes, E484K	B.1.1	Discharge from Hospital
Patient 3	early sixties	male	relapsed follicular lymphoma	obinutuzumab, thiotepa, cytarabine, etoposide	D76	yes, E484K	B.1.177	Discharge from Hospital
Patient 4	late sixties	male	Heart transplant recipient (about 30 years ago)	cyclosporine, azathioprine, prednisolone	D2	yes, E484K	B.1.177	Discharge from Hospital
Patient 5	late sixties	male	Chronic lymphatic leukemia	–	D45	yes, E484K	B.1.258	Discharge from Hospital
Patient 6	mid sixties	female	Kidney transplant recipient (about 2 months ago)	tacrolimus, mycophenolate mofetil, prednisolone	D17	no	B.1.160	Discharge from Hospital

Patient 1 (Fig. 1A) was a caucasian male in his early seventies, suffering from ANCA-associated vasculitis with end-stage renal disease. He was on therapy with the CD20-directed antibody rituximab (last infusion approximately three months before admission) and additionally was on prednisolone 20 mg qd. Upon hospital admission (day 1), he presented with mild symptoms of upper respiratory tract infection and fever to the emergency department, leading to the rapid diagnosis of SARS-CoV-2 infection with an initial viral load of $8•47 \times 10^7$ copies/mL. Being a patient with significantly increased risk for a severe course of COVID-19 due to his past medical history, he was treated with bamlanivimab (700 mg) intravenously at day 2. On day 4, his respiratory situation deteriorated with the need for oxygen supplementation, thus prednisolone was switched to dexamethasone 6 mg qd. Viral load dropped to $4{\cdot}62 \times 10^5$ copies/mL on day 8. However, his clinical condition further deteriorated and by day 12, the viral load had increased again by four log levels ($2{\cdot}27 \times 10^9$ copies/mL). This prompted us to perform whole genome sequence analysis, which revealed the presence of the E484K immune escape mutation in strain B.1. Of note, this substitution was not present before the treatment with bamlanivimab (Fig. 1A). Interestingly, at day 15 we observed continuous evolution to E484Q, reverting back to E484K on day 16 after administration of three units of convalescent plasma (CP). Unfortunately, the patient could not be stabilized and died on day 20 due to multi-organ failure.

Patient 2 (Fig. 1B) was a caucasian female in her early forties presenting with advanced HIV-1 infection (CD4+ cells 0/μL) and cerebral toxoplasmosis. Antiretroviral treatment was initiated with tenofovir alafenamide, emtricitabine and bictegravir for HIV-1 as well as a combination regimen of pyrimethamine and clindamycin for cerebral toxoplasmosis. Admission screening (d1) revealed a SARS-CoV-2 infection with $2{\cdot}22 \times 10^7$ copies/ml (strain B.1.1, harbouring E484E). The patient reported only mild symptoms (impaired taste). In the high risk setting of severely impaired T-cell response, bamlanivimab 700 mg was administered intravenously on day 3. SARS-CoV-2-RNA levels remained high for about one week and then further increased up to $2{\cdot}9 \times 10^9$ copies/mL with simultaneous detection of the E484K substitution. Due to persistently high viral replication levels, the patient was treated with the antiviral drug remdesivir and two units of CP, resulting in a decrease of SARS-CoV-2 RNA by three log levels over the following 10 days. At day 32 the patient could be discharged from hospital with two consecutive negative SARS-CoV-2 qPCR tests.

Patient 3 (Fig. 1C) was a caucasian male in his early sixties with relapsed follicular lymphoma, who had persistent positive SARS-CoV-2 qPCR approximately 2 months after the first positive SARS-CoV-2 RT-qPCR in November 2020 (day 1). In order to ensure viral clearance before a scheduled and urgently indicated high-dose chemotherapy and autologous hematopoietic stem cell transplantation (HSCT), 2 units of CP were administered on day 57 and one unit on day 59. After nasopharyngeal swabs repeatedly tested negative for SARS-CoV-2, high-dose chemotherapy with obinutuzumab, thiotepa, cytarabine and etoposide was started on day 69. On day 74, SARS-CoV-2 RT-qPCR was positive again on a nasopharyngeal swab with $2{\cdot}0 \times 10^5$ copies/mL (strain B.1.177, harbouring E484E). Although the initial SARS-CoV-2-positive sample was not available for viral sequencing and therefore reinfection cannot be formally excluded, a persistent but undetected infection with loss of immune control after high-dose chemotherapy seems more likely. The patient reported mild symptoms (fatigue), and bamlanivimab 700 mg was administered intravenously on day 76. The patient received autologous HSCT on day 78. Viral load further increased and peaked at day 79 with $1{\cdot}47 \times 10^8$ copies/mL. Whole genome sequencing revealed the E484K mutant on day 87. Following stem cell engraftment and subsequent improvement of cellular immunity, viral titers decreased, and viral clearance was achieved at day 103. The patient could be discharged from hospital in good clinical condition.

Patient 4 (Fig. 1D) was a caucasian male in his late sixties with successful heart transplant for severe myocarditis almost 30 years before. He received 60 mg cyclosporine qd, 12·5 mg azathioprine qod, and 5 mg prednisolone qd as long-term immunosuppressive therapy. He had presented to the emergency room of a peripheral hospital due to unexplained abdominal pain and was transferred to our center after a positive rapid test due to his complex medical history. Two days before the positive SARS-CoV-2 test, the patient reported mild fatigue but no further symptoms. Bamlanivimab 700 mg was administered on day 2. Viral load was initially low with $5{\cdot}27 \times 10^4$ copies/mL (strain B.1.177, harbouring E484E), increased on the day of bamlanivimab administration, then dropped and became negative over the next few days. In parallel, clinical and radiological signs of pneumonia developed during the same period, resulting in short-term therapy with high-flow oxygen and dexamethasone for 10 days. Due to the lack of a thorough clinical improvement, a new nasopharyngeal swab was taken on day 19, which was again positive, with a high viral load of $6{\cdot}89 \times 10^7$ copies/mL. At this point, whole genome sequencing revealed the E484K mutant. Without further specific antiviral therapy the patient improved slowly and could be discharged from the hospital at day 40 with two negative consecutive SARS-CoV-2 qPCR tests.

Patient 5 (Fig. 1E) was a caucasian male in his late sixties with B-cell chronic leukemia (CLL) stage Binet C who had persistent positive

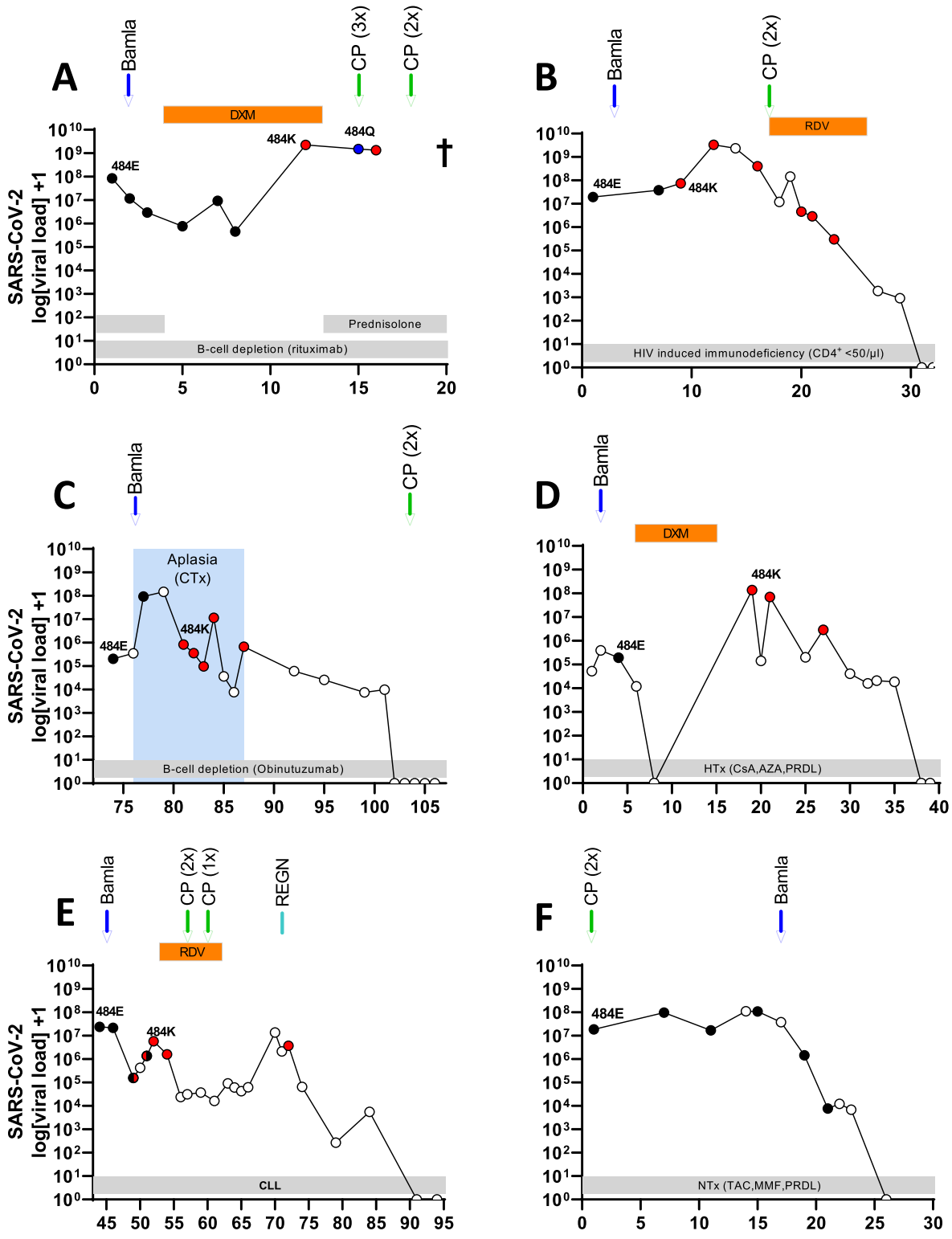

Fig. 1. Selection of E484K in SARS-CoV-2 infected patients with severe immunosuppression.

(A) Patient 1 with ANCA-associated vasculitis and pronounced immunosuppression; (B) Patient 2 with severe HIV-associated immunosuppression; (C) Patient 3 with follicular lymphoma and severe immunosuppression due to high-dose chemotherapy; (D) Patient 4 with severe immunosuppression due to heart transplantation; (E) Patient 5 with severe immunosuppression due to chronic lymphocytic leukemia; (F) Patient 6 with severe immunosuppression due to kidney transplantation. Bamla, bamlanivimab; CP, convalescent plasma; RDV, remdesivir; REGN, REGN—COV2, casirivimab/imdevimab; CTx, chemotherapy; HTx heart transplantation; PRDL, prednisolone; DXM, dexamethasone; MMF, mycophenolate mofetil; TAC, tacrolimus; CsA, cyclosporin A; AZA, azathioprine; CCL, chronic lymphocytic leukemia; E484K, substitution in the receptor-binding domain (RDB) associated with immune escape. Time points are color coded according their sequence. White, not determined; black, 484E; red, 484 K; blue, 484Q (for details see suppl. Table 1).

SARS-CoV-2 RT-qPCR ($3{\cdot}12 \times 10^7$copies/mL, strain B.1.258, harbouring E484E) 44 days after the first positive SARS-CoV-2 RT-qPCR in December 2020 before scheduled initiation of CLL treatment with ibrutinib. In view of the current risk constellation and the previous illnesses, bamlanivimab 700 mg was applied on day 45, which resulted in only a transient decrease in SARS-CoV-2 viral load. A subsequent increase of the viral load to $4{\cdot}82 \times 10^6$ copies/mL prompted us to perform whole-genome sequencing on day 52 (day 8 after bamlanivimab administration), which identified the E484K mutant. An off-label-use therapy with remdesivir was then initiated for a total of 10 days. In addition, cumulatively 3 units of CP were administered (Fig 1E). In the further course a high SARS-CoV-2 viral load persisted ($2{\cdot}26 \times 10^6$copies/mL, still harbouring E484K) so that we decided to administer imdevimab/casirivimab, which was well tolerated by the patient. Viral load subsequently decreased and became negative on day 91. The patient could be discharged from hospital in moderately reduced clinical condition.

Patient 6 (Fig. 1F) was a caucasian female in her mid-sixties with allogeneic cadaveric kidney transplantation performed about 2 months before hospital admission for SARS-CoV2-infection. Her immunosuppressive therapy consisted of 3 mg tacrolimus bid, mycophenolate mofetil 1 g bid (paused after admission) and prednisolone 20 mg qd. At admission, her SARS-CoV-2 viral load was $9{\cdot}36 \times 10^6$ (strain B.1.160, harbouring E484E). Considering the increased risk for a severe course of COVID-19, we decided to administer 2 units of CP on the day of admission. Because viral load did not decrease over the next 2 weeks, we additionally administered 700 mg of bamlanivimab. Viral load subsequently dropped and became negative on day 26. Without further complications, the patient could be discharged from hospital in good clinical condition.

4. Discussion

Of six severely immunosuppressed SARS-CoV-2-infected patients treated with bamlanivimab, we observed viral immune escape in five patients. The E484K mutation selected here in common SARS-CoV-2 variants is also present in different VOCs associated with immune evasion, including the variant B.1.351 initially described in South Africa (WHO label Beta) and the variants P1 (WHO label Gamma) and P2 (WHO label Zeta) detected in Brazil [16-18] which are currently rare in Germany. To investigate the local baseline prevalence of the E484K mutation, all SARS-CoV-2 whole genome sequences available from the Dusseldorf region at that time were screened. Remarkably, only 3 out of the 1270 available sequences presented the E484K mutation and were characterized as B.1.351 isolates. This observation and the fact that all our patients harboured variants with the E484E mutation at baseline support our hypothesis that the E484K mutation was indeed newly selected under the specific immune pressure of bamlanivimab in five of six patients with impaired humoral and cell-mediated immunity. It should also be mentioned that the recently described variant B.1.617.1 (first documented in India, WHO label Kappa) harbours the E484Q mutation [19], which was also selected in patient 1.

While it was reported in vitro that SARS-CoV-2 variants harbouring the mutations E484K or E484Q are resistant against neutralization by the monoclonal antibody bamlanivimab [16,18], our clinical observation that these mutations newly emerged under bamlanivimab therapy and potentially impaired clinical outcomes of patients could have important implications not only for the clinical management of individual patients but also concerning epidemiological measures for pandemic control. Especially when used in immunocompromised patients in the outpatient setting, there would be a risk of transmission of viruses with immune escape mutations, which may become highly relevant when such mutations are selected in VOCs associated with increased viral transmission such as the variant B.1.1.7. Indeed, there are already different reports that describe the E484K substitution in the context of B.1.1.7. Due to the complexity of the immunosuppressed patients treated with bamlanivimab, we treated them exclusively as inpatients in single rooms in a dedicated COVID-19 isolation ward with staff trained and highly experienced in PSA, and there was no evidence of transmission of these emerging viral strains harbouring E484K in this setting. After the potential threat was recognized upon receipt of sequencing results, all patients were followed for an extended period of time and only discharged after persistently low and eventually negative SARS-CoV-2 PCR results.

Cautious management and strict adherence to infection prevention and control practices appear to be highly advisable in the context of mAb treatment of SARS-CoV-2-positive immunosuppressed individuals.

Gottlieb and colleagues reported an emergence of escape mutants (E484K; E484Q; F490S and S494P) in 28/297 (9·4%) patients who received bamlanivimab monotherapy and even in 7/145 (4·8%) of patients receiving placebo in the phase 2/3 BLAZE-1 trial [2]. The fact that we observed the occurrence of E484K in a much higher percentage (5/6; 83·3%) when treating severely immunosuppressed patients suggests a significantly higher risk of viral escape in this setting. While the exact reason for the observed emergence of immune escape mutants predominantly in immunocompromised patients is unclear, it is likely that the persistent impairment of humoral and cellular immune control in these patients results in prolonged intervals of viral replication. In addition, with mAb targeting specific epitopes of SARS-CoV-2, escaping antibody neutralization by mutation is easier in the context of a single mAb compared to e.g. polyclonal immune sera and natural immunity. The combination of prolonged intervals of viral replication under narrowly focused selection pressure may explain the rapid viral immune escape observed in our patients.

The differential therapeutic response to bamlanivimab in terms of viral load with at least initial decrease in patients 1, 4, 5, and 6, but on the other hand, unchanged or increasing SARS-CoV-2 viral load after bamlanivimab administration in patients 2 and 3 could be explained by a combination of several factors. First, therapy occurred at different time points within the natural history of SARS-CoV-2 infection, with typically an initial rapid increase in viral load, subsequent stabilization at a high level, and subsequent clearance by the onset of the adaptive immune response. However, this would certainly not explain the course of patient 3, who had been SARS-CoV-2 positive for an extended period of time. Similarly, the development of the immune escape mutation E484K during the course of the disease cannot conclusively explain the lack of timely response to mAb therapy in patients 2 and 3.

We therefore hypothesize that in the context of the severe immunosuppression of these bamlanivimab-treated patients, their own immune function, which changed significantly in some of the patients during the course of the disease, contributed quite substantially to these viral load courses. Patient 2 initially had a very severe cellular immunodeficiency with CD4+ cells of 0/μl, which improved only gradually after initiation of antiretroviral therapy, whereas in patient 3 cellular immunity was transiently profoundly impaired by high-dose chemotherapy with consecutive aplasia. In our view, it should therefore be discussed to what extent a certain degree of cellular immune function may be essential for a successful therapeutic response after administration of monoclonal antibodies.

CP and casirivimab/imdevimab (REGN—COV2) appeared to remain at least partially effective from a clinical perspective when used in our patients with viral rebound even in the presence of E484K (Fig. 1). However, due to the different disease courses, the additional use of remdesivir in two cases and the small number of patients, further data are needed regarding the efficacy in this specific clinical setting.

It is also noteworthy that the only severely immunosuppressed patient without viral rebound (patient 6) had previously been given CP

and thus had not actually received mAb monotherapy. Combinations of two or more mAbs or polyclonal antisera may therefore increase the genetic barrier sufficiently to largely prevent escape of the immune system as known from other viral infections [20]. Furthermore, in the context of variants of concern that already harbour immune escape mutations, it should be kept in mind that functional monotherapy may also be present when a combination of two mAbs is used. However, this needs to be thoroughly evaluated, especially when treating severely immunocompromised patients infected with SARS-CoV-2. The U.S. Food and Drug Administration has recently withdrawn the Emergency Use Authorization for bamlanivimab in consideration of the increasing prevalence of immune escape mutants in the USA. The European Medicines Agency (EMA) issued a recommendation on treatment with bamlanivimab and etesevimab in early March 2021. The EMA concludes that this mAb combination can be used to treat confirmed COVID-19 in patients who do not require supplemental oxygen and who are at high risk for progression of COVID-19 to a severe disease course. The agency also examined the use of bamlanivimab alone, which was available as monotherapy in Germany from the end of January 2021, and concluded that it could be also considered as a treatment option despite uncertainties about the benefits of monotherapy.

Until further data will be available, our results suggest that caution is warranted in the use of monoclonal antibodies in immunocompromised patients infected with SARS-CoV-2.

Contributors

BJ, NL, TF, AW, JT, TL were responsible for conceptualization and supervised the study. BJ, TF, VK, TL, ML, NF, DS, TB, DK, RH, NL, AW, JT contributed to investigation and data curation. BJ, NL, AW, OA, AD, JT conducted the formal analysis. BJ, TF, NL, AW, JT were responsible for methodology, data validation and visualization. BJ, NL, TF, VK, AW, JT, TL contributed to the original draft. All authors critically revised the manuscript and approved the final version of the manuscript.

Data availability statement

Raw data were generated at University Hospital Duesseldorf. Derived or additional data supporting the findings of this study are available from the corresponding author [BJ] upon reasonable request.

Declaration of interests

BJ received honoraria for presentations from Gilead (Remdesivir) as well as Falk, Janssen-Cilag, MSD, BMS, Abbvie, ViiV, Gilead, Boehringer, Fresenius Medical Care (outside the submitted work) and served on advisory boards for ViiV, BMS, Gilead, Theratechnologies (outside the submitted work). VK received lecture fees from Abbvie, Falk, Albireo, Gilead (outside the submitted work). TF was PI for a Gilead clinical trial (Remdesivir) and served on Gilead advisory boards. All other authors declare no competing interests regarding this work.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.lanepe.2021.100164.

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Clinical Infectious Diseases

MAJOR ARTICLE

Rapid Selection of Sotrovimab Escape Variants in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Omicron-Infected Immunocompromised Patients

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Background. Monoclonal antibodies (mAbs) that target severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are predominantly less effective against Omicron variants. Immunocompromised patients often experience prolonged viral shedding, resulting in an increased risk of viral escape.

Methods. In an observational, prospective cohort, 57 patients infected with Omicron variants who received sotrovimab alone or in combination with remdesivir were followed. The study end points were a decrease in SARS-CoV-2 RNA $<10^6$ copies/mL in nasopharyngeal swabs at day 21 and the emergence of escape mutations at days 7, 14, and 21 after sotrovimab administration. All SARS-CoV-2 samples were analyzed using whole-genome sequencing. Individual variants within the quasispecies were subsequently quantified and further characterized using a pseudovirus neutralization assay.

Results. The majority of patients (43 of 57, 75.4%) were immunodeficient, predominantly due to immunosuppression after organ transplantation or hematologic malignancies. Infections by Omicron/BA.1 comprised 82.5%, while 17.5% were infected by Omicron/BA.2. Twenty-one days after sotrovimab administration, 12 of 43 (27.9%) immunodeficient patients had prolonged viral shedding compared with 1 of 14 (7.1%) immunocompetent patients ($P = .011$). Viral spike protein mutations, some specific for Omicron (e.g., P337S and/or E340D/V), emerged in 14 of 43 (32.6%) immunodeficient patients, substantially reducing sensitivity to sotrovimab in a pseudovirus neutralization assay. Combination therapy with remdesivir significantly reduced emergence of escape variants.

Conclusions. Immunocompromised patients face a considerable risk of prolonged viral shedding and emergence of escape mutations after early therapy with sotrovimab. These findings underscore the importance of careful monitoring and the need for dedicated clinical trials in this patient population.

Keywords. immunodeficiency; sotrovimab; SARS-CoV-2; Omicron; escape.

During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, numerous studies showed that treatment options that directly target SARS-CoV-2 are most successful in the early phase of coronavirus disease 2019 (COVID-19), whereas in the late phases of COVID-19 with pneumonia and hyperinflammation, immunomodulation is the main therapeutic principle. Several monoclonal antibodies (mAbs) that target SARS-COV-2, such as bamlanivimab/etesevimab or casirivimab/imdevimab, became available starting in late 2020 and were successfully used in the early phase of COVID-19 to prevent disease progression in high-risk patients [1]. With the emergence of the currently dominating variant of concern Omicron in November 2021, a significant rise in infection rates was observed. This went along with a loss of in vitro activity of the mAb combination casirivimab/imdevimab, commonly used until then, because the target regions in the spike protein were altered through several mutations [2]. In January 2022, sotrovimab became available in Germany. It was one of the few mAbs found to be effective against the Omicron variant in vitro and thus represented a promising treatment option for early SARS-CoV-2 infection [3–5].

Sotrovimab was approved for use in children aged >12 years and in adults at high to moderate risk for developing severe

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infection [6]. To date, only 2 randomized, controlled trials have evaluated the efficacy of sotrovimab in preventing hospitalization and disease progression, but only the COVID-19 Monoclonal Antibody Efficacy Trial–Intent to Care Early (COMET-ICE) trial showed a benefit [3, 4, 7]. However, these trials did not include severely immunodeficient patients such as solid organ transplant (SOT) recipients. Case series as well as 2 cohort studies evaluated the efficacy and safety of sotrovimab in SOT patients in the context of Omicron and reported a reduction in disease severity [8, 9]. However, it was suggested that therapy of SARS-CoV-2 infections with a single mAb might promote the emergence of escape mutations in the spike protein, especially in immunocompromised patients [10]. Recently, mutations have been reported after sotrovimab therapy in patients infected with the Omicron variant, but the risk factors for the occurrence and the longitudinal development of resistance are still largely unclear [11, 12]. Therefore, we analyzed the outcome and risk factors for viral persistence after treatment with sotrovimab in our cohort of patients treated since January 2022, focusing specifically on the emergence of escape mutations.

METHODS

Study Design

We performed a prospective, observational cohort study in patients diagnosed with SARS-CoV-2 infection who received sotrovimab therapy between 20 January 2022 and 25 February 2022. Patients were either hospitalized or presented to the outpatient clinic at the University Hospital Düsseldorf. Inclusion criteria were polymerase chain reaction (PCR)–confirmed SARS-CoV-2 infection, age >12 years, weight >40 kg, and risk factors for developing a severe course of COVID-19. All patients provided informed consent. Patients were pseudonymized with an ID number. A single dose of 500 mg of sotrovimab was administered intravenously over a 1-hour period as part of routine clinical practice.

Baseline was defined as the day of sotrovimab administration. Nasopharyngeal swabs and clinical parameters were collected at baseline and during the follow-up period: every 7 days (±2 days) until viral clearance was achieved. The main study end points were percentage of patients with a decrease in SARS-CoV-2 RNA $<10^6$ copies/mL in nasopharyngeal swabs 21 days after sotrovimab administration and characterization of the viral variants including screening for escape mutations during the observation period of 28 days. Patients who did not attend their follow-up appointments and patients for whom viral genome sequencing was unsuccessful at any time during the study were excluded from the statistical analyses.

Definition of Prolonged Viral Shedding

Prolonged viral shedding was defined as a persistent SARS-CoV-2 RNA concentration above 10^6 copies/mL 21 days after sotrovimab administration. The threshold of 10^6 SARS-CoV-2 RNA copies/mL or a cycle threshold value >25 is considered a measure of infectivity based on in vitro cell culture data that show a correlation between viral load and viral cultivability and the associated probability of transmission [13]. This cutoff value as a correlate of contagiousness was also chosen following the German recommendations of the Robert Koch Institute for the isolation of SARS-CoV-2–infected hospitalized patients.

Laboratory SARS-CoV-2 Analyses

All detailed information on SARS-CoV-2 detection and quantification, SARS-CoV-2 whole-genome sequencing and resistance analysis, pseudovirus cloning, production, and neutralization assays is provided in the Supplementary Appendix.

Statistical Analyses

Detailed information on the statistical programs used and the statistical tests performed is provided in the Supplementary Appendix.

All investigations were performed in accordance with the Declaration of Helsinki. The study was approved via ethics vote of the local ethics committee of the medical faculty of Heinrich-Heine-University. All patients gave written informed consent.

RESULTS

Patients' Characteristics

A total of 57 patients (21 females; 36 males) were enrolled in the study, 47 (82.5%) of whom were infected with Omicron variant BA.1 and 10 (17.5%) with Omicron variant BA.2 (Table 1). No symptoms were present in 21 of 57 (36.8%) patients, while the rest had symptoms consistent with early COVID-19. The median time from onset of symptoms to administration of sotrovimab was 3 days (interquartile range, 1–3.3). All participants were in the early phase of COVID-19 when sotrovimab was administered; 2 of them required low levels of oxygen supplementation for reasons unrelated to COVID-19. Forty-two of 57 patients (73.7%) received at least 3 doses of SARS-CoV-2 vaccine in accordance with the recommendations of the Standing Committee on Vaccination (Supplementary Table 1). The median time span since the last vaccination was 3 months (range, 1–5). Two patients died from causes unrelated to COVID-19: 1 from stage IV malignant melanoma, the other from complications of acute lymphoblastic leukemia. In total, 5 patients could not be monitored because they either died (malignant melanoma) or did not present to follow-up (n = 4).

Patients were grouped into immunocompetent (n = 14) and immunodeficient (n = 43). Immunodeficiency mostly comprised SOT, stem cell transplantation (SCT), active hematologic malignancies, and autoimmune diseases. The full spectrum of diseases is presented in Table 1. Immunosuppressive

Table 1. Baseline Characteristics of Patients Grouped by Immunodeficiency

Variable	n
Total	57
Gender	
Male	36
Female	21
Groups	
Immunocompetent	14
Immunodeficient	43
Solid organ transplantation	
Kidney	18
Heart	2
Heart + kidney	1
Heart + lung	1
Kidney + pancreas	1
Stem cell transplantation	
Allogeneic	5
Autologous	2
Leukemia	
Acute lymphoblastic leukemia	2
AML[a]	2
AML + CMML	1
CMML	1
Lymphoma	
Diffuse large B-cell lymphoma	1
T-cell lymphoma[a]	1
AL amyloidosis/smoldering multiple myeloma[a]	1
Other malignancies	
Stage IV malignant melanoma and stage IV non-small cell lung cancer[b]	1
Common variable immune deficiency	1
Autoimmune diseases	
Cryoglobulinemic vasculitis	1
p-ANCA vasculitis	1
Rheumatoid arthritis	1
Systemic lupus erythematosus	1
Ulcerative colitis	1
Liver cirrhosis Child–Pugh A[c]	1
Liver fibrosis with portal hypertension[c]	1

Abbreviations: ANCA, anti-neutrophil cytoplasmatic antibody; AML, acute myeloblastic leukemia; AL, amyloid; CMML, chronic myelomonocytic leukemia.

[a]Patients with previous allogeneic (2) and autologous (1) stem cell transplantation and malignancy relapse.

[b]Dexamethasone therapy for cerebral metastases.

[c]Patients with liver fibrosis/cirrhosis had a concurrent autoimmune disease.

medication was given to 39 of 43 patients (90%) classified as immunodeficient (Supplementary Table 1).

Prolonged Viral Shedding in Immunodeficient COVID-19 Patients Infected With an Omicron Variant and Treated With Sotrovimab

We analyzed the kinetics of viral clearance after the first positive SARS-CoV-2 PCR test and after sotrovimab administration in immunocompetent and immunodeficient patients (Figure 1). All but 1 of the immunocompetent patients had a viral load (VL) below 10^6 copies/mL at day 14, while 21 of 43 (48.8%) immunodeficient patients had prolonged viral shedding at this time point ($P = .011$). Moreover, even on day 21, 12 of 43 (27.9%) patients with immunodeficiency had not achieved a VL $<10^6$ copies/mL. The only immunocompetent patient who still had a VL $>10^6$ copies/mL on day 14 was lost to follow-up and therefore considered for analysis as having a VL $>10^6$ copies/mL on day 21 (patient 37; Supplementary Table 2). A higher proportion of patients who presented without COVID-related symptoms had prolonged viral shedding after days 14 and 21 (Supplementary Table 4).

Of note, 6 of 43 (13.9%) immunodeficient patients were infected with the BA.2 Omicron variant, characterized by higher levels of in vitro resistance of sotrovimab compared with Omicron BA.1. Twenty-nine of 43 (67.4%) immunodeficient patients received additional therapy with remdesivir at baseline (Supplementary Table 2). However, in the subgroup analysis, no significant association was found regarding the occurrence of prolonged viral shedding and the following factors: Omicron variant, remdesivir administration, number of vaccinations, and months since last vaccination or time between symptom onset and sotrovimab infusion (Table 2). The only risk factor identified for prolonged viral shedding was immunodeficiency ($r = 0.329$; $P = .016$).

Initial nonresponders, defined as patients whose symptoms either worsened despite sotrovimab administration and required hospitalization (patients 30, 34) or who experienced a viral rebound during the observation period (days 14–21: patients 8, 9, 10, 16; >21 days: patients 3, 26, 54), received further antiviral therapy. All patients who showed a slow but steady decline in SARS-CoV-2 viral load (VL) did not receive further antiviral therapy, and 5 patients were lost to follow-up. In all 10 patients re-treated with additional antiviral drugs, the virus was subsequently eliminated (Supplementary Table 2).

Taken together, these results show that immunocompromised patients have a substantial rate of prolonged viral shedding, even after administration of sotrovimab, which was the standard therapy for patients infected with SARS-CoV-2 at high risk for disease progression at the time of enrollment.

Emergence and Characterization of Escape Mutations in Omicron Variant of Concern After Use of Sotrovimab

Noting the prolonged viral shedding in immunocompromised patients after sotrovimab administration, we then performed whole-genome nanopore sequencing of all available viral samples with VL $>10^6$ copies/mL. Samples with detected resistance mutations were further analyzed with quantitative Illumina sequencing with spike amino acid coverage averaging 98.5% (range, 91.6%–100%; sample overview table on online data repository server, see Data availability section). This analysis revealed that mutations at spike protein residues associated with resistance to sotrovimab occurred in 14 of 57 patients (24.6%). No selection of escape mutations was observed in the

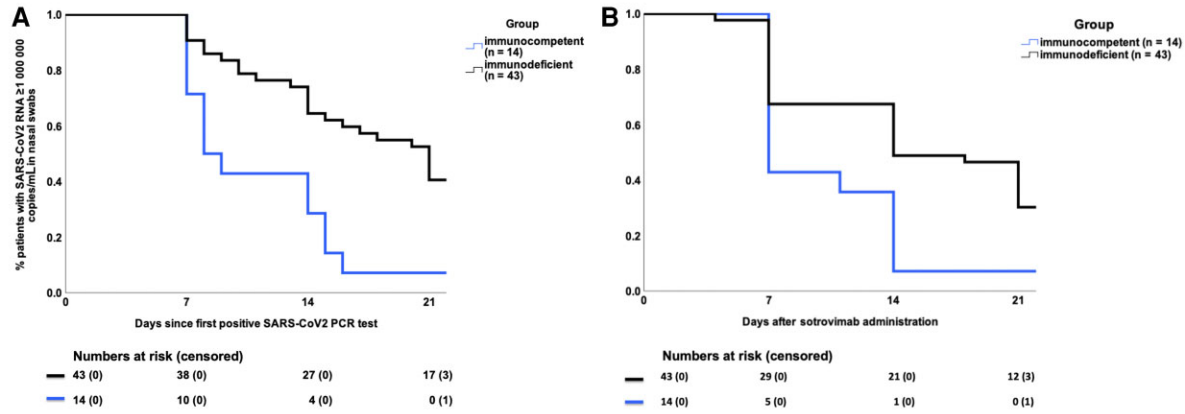


Figure 1. Patients with persistent viral replication ($\geq 10^6$ copies/mL) after sotrovimab administration. *A,* Prolonged viral shedding by day 21 after the first positive SARS-CoV-2 PCR test according to immunocompetence. *B,* Prolonged viral shedding by day 21 after sotrovimab administration in immunocompetent patients and patients with immunodeficiency. Numbers at risk are patients with a viral load $\geq 10^6$ copies/mL; censored are patients lost to follow-up (1 patient was first lost to follow-up on day 28 and was included in numbers at risk). Abbreviations: PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

immunocompetent patients, only in immunodeficient patients (14 of 43, 32.6%), most of whom had prolonged viral shedding. This group comprised 6 patients with SOT; 2 allogeneic SCT recipients; 2 patients with active hematologic malignancy who were receiving chemotherapy; 1 patient each with cryoglobulinemic vasculitis, systemic lupus erythematosus, and liver cirrhosis (Child–Pugh class A), each of whom received additional immunomodulatory therapies; and 1 patient with common variable immunodeficiency (Supplementary Table 2).

While no variants with reduced susceptibility to sotrovimab were detected at baseline confirmed by Illumina sequence analysis, 5 patients had sotrovimab-resistant variants by day 7, whereas most escape mutations occurred between day 7 and day 14. Details of the quantitative analysis of sotrovimab resistance mutations performed by Illumina sequencing on SARS-CoV-2 samples with evidence of immune escape in nanopore sequencing are shown in Figure 2. The resistance mutations that appeared first were detected exclusively at positions 337 or 340 in the spike protein, predominantly featuring the mutations P337S (n = 8), E340K (n = 9), and E340D (n = 5). In addition, amino acid substitutions P337H/L/R and E340A/V were found during our observation period of up to 28 days (Supplementary Table 3). During the observation period, not only an increase in escape variants (eg, patients 2, 31, 53) but also a change in the frequency of mutated variants were observed, for example, patient 10, E340D (d21) to E340K (d28) and patient 53, E340V (d7) to E340D (d14; Figure 2, Supplementary Table 3).

The sotrovimab-specific escape mutations (P337S, E340D/K/V) detected on day 7 were characterized in the BA.1 and BA.2 Omicron background using a pseudovirus neutralization assay (Figure 3). While in the B.1 background (a common lineage early in 2020 [14]), only E340K und E340D were associated with reduced neutralization by sotrovimab (IC_{50} (half maximal inhibitory concentration), >100 µg/mL and IC_{50}, 0.162 µg/mL, respectively), all other detected mutations completely abrogated neutralization by sotrovimab in both the BA.1 and BA.2 backgrounds (IC_{50}, >100 µg/mL).

To characterize the risk factors for the selection of escape mutations, correlation analysis was performed (Table 2). This analysis revealed that 2 factors correlated with the emergence of resistance mutations: immunodeficiency (r = 0.305, $P = .021$) and days until VL $<10^6$ SARS-CoV-2 RNA copies/mL achieved after sotrovimab administration (r = 0.322, $P = .019$). In detail, patients with emergence of mutations had significantly delayed time to viral clearance (mean, 28.2; standard deviation [SD], 16.2 days) compared with those without mutations (mean, 12.9; SD, 9.9 days; odds ratio, 5.04; 95% confidence interval, 1.29–18.3). In addition, for patients with tacrolimus therapy, higher tacrolimus levels at baseline positively correlated with the emergence of escape mutations (r = 0.523, $P = .015$). In immunodeficient patients, administration of remdesivir in combination with the corresponding duration correlated negatively with the occurrence of resistance mutations against sotrovimab (r = −0.392, $P = .009$). Most patients with selection of sotrovimab-specific escape mutations (13 of 14, 92.8%) were infected with the BA.1 variant; however, only 6 of 43 immunodeficient patients were infected with BA.2.

Together, these findings suggest that sotrovimab monotherapy in immunocompromised patients is associated with the risk of de novo development of specific mutations that lead to immune escape.

DISCUSSION

To our knowledge, this is one of the few studies to report the frequent emergence of escape mutations after sotrovimab

Table 2. Bivariate Correlation Among Clinical Parameters, Duration Until Viral Load <10^6 copies/mL, and Escape Mutations

Parameter	VL <10^6 Copies/mL Since First Positive Polymerase Chain Reaction Test (d)	VL <10^6 Copies/mL Since Sotrovimab Administration (d)	Mutations Day 7 (0 = None, 1 = Mutation)	Mutations Day 14 (0 = None, 1 = Mutation)	Mutations overall (0 = None, 1 = Mutation)
	Correlation Coefficient (r) P Value				
Remdesivir therapy at baseline (0 = 0, 1 = 3, and 2 = 5 d)					
Immunocompetent	−0.355	−0.224	NA	NA	NA
	0.234	0.462	NA	NA	NA
Immunodeficient	0.057	−0.036	**−0.372**	−0.261	**−0.392**
	0.726	0.827	**0.015**	0.099	**0.009**
Omicron variant (0 = BA.1,1 = BA.2)					
Immunocompetent	0.068	0.207	NA	NA	NA
	0.824	0.498	NA	NA	NA
Immunodeficient	0.032	0.107	−0.150	−0.095	−0.137
	0.844	0.510	0.343	0.555	0.383
Time since last vaccination (mo)					
Immunocompetent	0.073	0.080	NA	NA	NA
	0.822	0.805	NA	NA	NA
Immunodeficient	0.298	0.241	0.011	0.179	0.217
	0.109	0.199	0.953	0.345	0.240
Number of vaccinations					
Immunocompetent	0.408	0.496	NA	NA	NA
	0.167	0.085	NA	NA	NA
Immunodeficient	0.041	0.046	0.117	−0.125	−0.104
	0.804	0.780	0.467	0.422	0.512
Immunodeficiency (0 = immunocompetent, 1 = immunodeficient)	**0.329**	0.208	**0.320**	**0.275**	**0.305**
	0.016	0.135	**0.015**	**0.042**	**0.021**
Viral clearance after sotrovimab administration (d)	NA	NA	0.258	**0.401**	**0.322**
	NA	NA	0.062	**0.004**	**0.019**
Tacrolimus levels at baseline (ng/mL)	0.349	0.275	0.161	**0.451**	**0.523**
	0.132	0.240	0.486	**0.046**	**0.015**
Days since first symptoms (number of pairs = 35[a])	0.075	−0.251	0.090	−0.144	−0.10
	0.669	0.146	0.600	0.417	0.955

Significant correlations appear in bold.

Abbreviations: NA, not applicable; VL, viral load.

[a]One patient was lost to follow-up and not included in this analysis.

treatment in a predominantly immunodeficient cohort of patients infected with Omicron variants.

Previous publications showed a decreased severity of SARS-CoV-2 disease with the Omicron variant [15]. Consistent with this, all patients in our high-risk cohort had uncomplicated disease throughout the follow-up period and there was no SARS-CoV-2–related mortality. Due to the observational nature of our study, it remains unclear whether the clinical course might have been less favorable in some patients without early antiviral therapy. When the BA.1 and BA.2 Omicron variants were compared in terms of prolonged viral shedding after sotrovimab administration, there was no significant difference found in our cohort. At this point, however, it must be emphasized that BA.2 was underrepresented in our study cohort compared with BA.1 (17.5% vs 82.5%, respectively). In our pseudovirus neutralization assays (Figure 3), as well as in other studies, a reduced neutralization activity of sotrovimab against BA.2 was described [16, 17]. These data led the US Food and Drug Administration to revoke the approval of sotrovimab for patients infected with BA.2 in April 2022 [18].

A unique feature of our cohort is the large number of immunodeficient patients, almost half of whom were patients with SOT, resulting in a higher risk of prolonged viral shedding, therefore potentially promoting the emergence of highly mutated viruses [19–21]. In this context, a higher baseline tacrolimus serum level was associated with the selection of escape mutations in our study, which highlights the importance of considering treatment adjustments of immunosuppressive medication during SARS-CoV-2 infection.

In our cohort, all but 1 of the immunocompetent patients (13 of 14, 92.9%) were below the defined viral threshold of 10^6 SARS-CoV-2 RNA copies/mL at day 14 and no selection of resistant variants to sotrovimab was detected. In contrast,

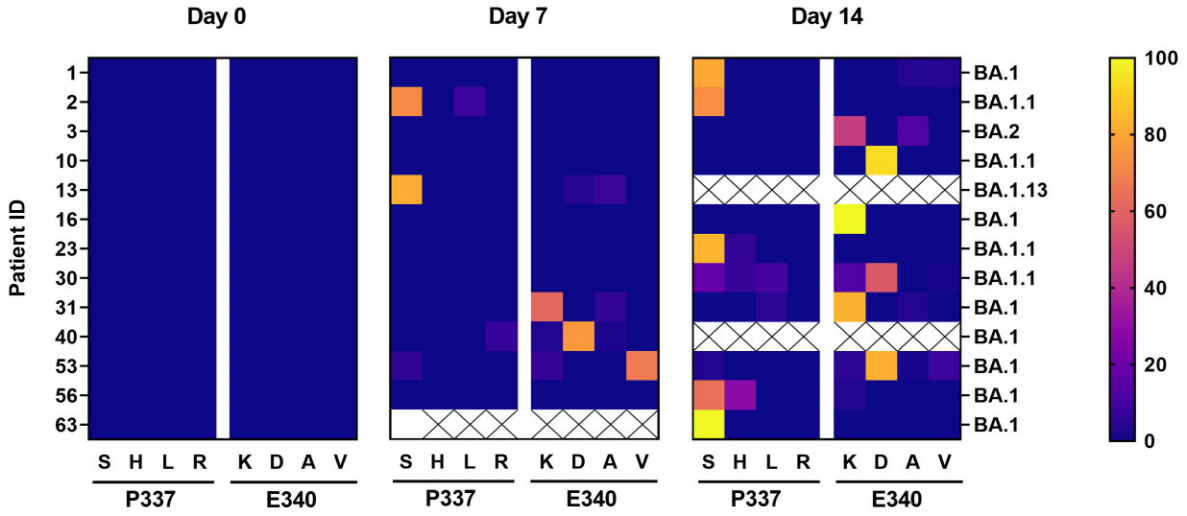


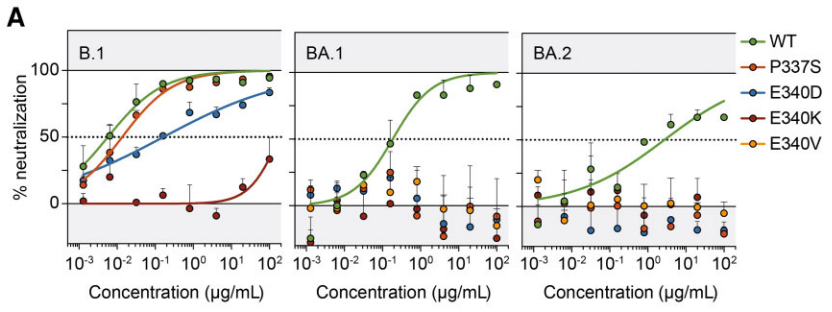
Figure 2. Prevalence and evolution of escape mutations in the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) after sotrovimab treatment. Detected amino acid exchanges in the spike protein at positions 337 and 340 on day 0, day 7, and day 14 after sotrovimab administration. The frequency of reads in % is indicated by the color scale. The determined patient-related SARS-CoV-2 variant is shown. Only patients with detected mutations after sotrovimab treatment are indicated. Patients selecting a spike protein mutation after day 14 are not included in this figure (patient 51).

sotrovimab escape mutations were detected in 32.6% of immunodeficient patients who predominantly experienced prolonged periods of viral replication. Similarly, treatment with other mAbs or antiviral agents (such as remdesivir) is also reported to promote the selection of viral mutations, particularly in immunosuppressed patients [10, 22–24].

Sotrovimab-specific resistance mutations were first described in an Australian cohort of patients infected with the Delta variant [25]. Genome sequencing of samples from the COMET-ICE trial detected sotrovimab escape mutations in 20 patients, of which P337L, E340A, and E340K showed reduced susceptibility to sotrovimab in pseudotyped viral-like particles (>100-fold change in EC_{50} (half maximal effective concentration) value) [3, 18]. In the study published by Rockett et al, 8 of 100 patients developed 1 of the following mutations, E340A/K/V or P337L, combined with the E340 mutation that occurred 6–13 days after sotrovimab administration [25]. While P337L and the E340A were selected primarily in the Delta variant of concern, other amino acids were selected in the Omicron variants at the same positions, predominantly P337S/R and E340D/K, as reported in other recent studies [11, 12].

In our longitudinal study, after detection of the sotrovimab-specific escape mutations P337S/L/R and E340A/D/K/V at day 7, an additional variant was detected during our observation period (P337H). Moreover, changes in frequency of different escape variants over time were observed, as described in infections with the Delta variant, presumably indicating ongoing viral evolution [25].

In the in vitro analyses carried out in our study, the pseudovirus neutralization assays confirmed that both sotrovimab mutations,



B

Pseudovirus neutralization IC_{50} (µg/mL)

Mutation	Variant B.1	BA.1	BA.2
WT	0.006	0.186	2.707
P337S	0.014	>100	>100
E340D	0.162	>100	>100
E340K	>100	>100	>100
E340V	n.d.	>100	>100

Figure 3. Neutralization of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike mutants by sotrovimab. *A,* SARS-CoV-2 variant-specific pseudoviruses harboring mutations that emerged after sotrovimab treatment were analyzed in sotrovimab neutralization assays. All samples were tested in duplicate. Symbols and bars indicate mean and standard deviation, respectively. The determined IC_{50} values are shown in (*B*). Abbreviation: IC_{50}, half maximal inhibitory concentration.

E340K and E340V, which were also selected in Delta, and mutations P337S and E340D, newly described in the Omicron context, completely abrogated the neutralization activity of sotrovimab. In the B.1 background, on the other hand, a strongly reduced neutralization activity could only be observed for E340K, whereas the E340D mutation reduced the neutralization activity of sotrovimab to a much lesser extent. These data clearly show that not only the escape mutation itself but also the broader genetic background of the spike protein influence the impact of a specific escape mutation on mAb efficacy, as has been observed in several efficacy studies for mAbs [17, 26].

In a previous small cohort study conducted before the Omicron era, we found that the E484K mutation occurred with bamlanivimab monotherapy in 83% of patients and in a major portion of the viral population in the respective patients [10]. In contrast, in our study, the frequency of sotrovimab-resistant viral variants was lower in most patients and showed a very heterogeneous mutation spectrum [27, 28].

Our study has limitations that should be considered for future studies. First, the relatively small cohort made subgroup analysis difficult. Second, the quantitative analysis with Illumina sequencing was performed only in patients in whom spike protein mutations were detected in nanopore sequencing. Therefore, the diversity of viral quasispecies cannot be compared to patients without detection of mutations in nanopore sequencing. However, failure to account for possible viral minorities with spike protein mutations in this group seems unlikely, as these were not detected in the patients with emerging sotrovimab resistance mutations.

There is growing evidence to support the hypothesis that new SARS-CoV-2 variants preferentially occur in immunocompromised patients with persistent SARS-CoV-2 infection. Since some of these variants may be more transmissible or may have better immune escape, this potentially has significant implications for individual medical care and public health. In immunocompromised patients, prolonged viral shedding must therefore be considered with respect to infection control. Given the available data, administration of a single mAb or single antiviral drug should be avoided in immunocompromised patients because of the risk of emergent mutations. In our study, we demonstrated that the presence and length of remdesivir therapy at baseline was associated with a reduced emergence of escape mutations in immunodeficient patients. In addition, a second remdesivir administration over a longer period of 10 days and combination antiviral therapy resulted in a sustained decrease in viral load in the vast majority of patients with persistently high nasopharyngeal VL, and viral shedding was successfully terminated in those patients.

In summary, combination therapies with at least 2 mAbs or other antivirals, such as remdesivir, molnupiravir, and nirmatrelvir/ritonavir, should be considered when treating immunodeficient patients with SARS-CoV-2 infection. These results also highlight the importance of careful monitoring and the need to conduct dedicated clinical trials to establish the optimal treatment strategy for this patient population. This is especially true at this stage of the pandemic since, with the availability of vaccines that prevent severe disease courses for most patients, immunodeficient patients represent one of the most vulnerable and severely affected patient groups.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. S. G., N. L., A. K., T. L., and B.-E. O. J. were responsible for conceptualization and supervised the study. S. G., N. L., A. K., H. G., A. W., A. T. D., C. L., C. F., S. K., J. V., T. S., A. Z., T. F., J. B., H.-M. O., F. K., J. T., T. L., and B.-E. O. J. contributed to the investigation and data curation. S. G., N. L., A. K., B.-E. O. J., H. G., and J. T. conducted the formal analysis. S. G., B.-E. O. J., T. L., N. L., A. K., J. T., H. G., and F. K. were responsible for methodology, data validation, and visualization. S.G., N. L., A. K., H. G., J. T., T. L., and B.-E. O. J. contributed to the original draft. All authors critically revised the manuscript and approved the final version. Additionally, S. K., J. V. P., T. P. S., and A. Z. were instrumental in patient management, as well as the coordination of outpatient care for the study participants. A. T. and M. D. coordinated the Illumina sequencing and performed the bioinformatic analysis of the sequencing data.

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Data availability. Raw data are generated at the University Hospital Düsseldorf and in cooperation with the University Hospital Cologne. Derived or additional data that support the findings of this study can be provided by the corresponding author upon reasonable request. All Nanopore and Illumina sequence data can be found on the Open Science Framework server https://osf.io/q7js6/?view_only=59884b79343e449ea9f7103f99c118a9.

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Convalescent plasma treatment for SARS-CoV-2 infected high-risk patients: a matched pair analysis to the LEOSS cohort

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Establishing the optimal treatment for COVID-19 patients remains challenging. Specifically, immunocompromised and pre-diseased patients are at high risk for severe disease course and face limited therapeutic options. Convalescent plasma (CP) has been considered as therapeutic approach, but reliable data are lacking, especially for high-risk patients. We performed a retrospective analysis of 55 hospitalized COVID-19 patients from University Hospital Duesseldorf (UKD) at high risk for disease progression, in a substantial proportion due to immunosuppression from cancer, solid organ transplantation, autoimmune disease, dialysis. A matched-pairs analysis (1:4) was performed with 220 patients from the Lean European Open Survey on SARS-CoV-2-infected Patients (LEOSS) who were treated or not treated with CP. Both cohorts had high mortality (UKD 41.8%, LEOSS 34.1%). A matched-pairs analysis showed no significant effect on mortality. CP administration before the formation of pulmonary infiltrates showed the lowest mortality in both cohorts (10%), whereas mortality in the complicated phase was 27.8%. CP administration during the critical phase revealed the highest mortality: UKD 60.9%, LEOSS 48.3%. In our cohort of COVID-19 patients with severe comorbidities CP did not significantly reduce mortality in a retrospective matched-pairs analysis.

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However, our data supports the concept that a reduction in mortality is achievable by early CP administration.

Abbreviations

SARS	Severe acute respiratory syndrome
ARDS	Acute respiratory distress syndrome
HIV	Human immunodeficiency virus
FDA	U.S. food and drug administration
eGFR	Estimated glomerular filtration rate
CP	Convalescent plasma
UKD	University hospital duesseldorf
LEOSS	Lean european open survey on SARS-CoV-2 infected patients
ISARIC-4C	Coronavirus clinical characterisation consortium

In December 2019, the novel corona virus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) triggered a global pandemic that continues to this day, resulting in millions of deaths from severe pneumonia with acute respiratory distress syndrome (ARDS) and multi-organ failure. The widespread impact of the virus includes a severely strained health system and a global socio-economic crisis, as well as long-term health limitations following coronavirus disease 2019 (COVID-19). By 29 July 2022, more than 572 million cases of SARS-CoV-2 infection and more than 6 million deaths from the virus have been recorded[1].

Although most patients have only mild symptoms (81%), 14% develop a severe course of COVID-19 and 5% ARDS[2]. Patients at increased risk for severe disease progression include dialysis patients, patients with cardiovascular diseases or immunosuppression, e.g., due to previous solid organ transplantation, stem cell transplantation, autoimmune disease, treatment of active malignancy or uncontrolled human immunodeficiency virus infection (HIV). Morbidity and mortality have been observed to be significantly increased in the above patients[3–5].

At the onset of the pandemic, there were no evidence-based therapeutic options for COVID-19; to date, although several treatment options have been investigated and approved, e.g., by the U.S. Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA), therapeutic options remain unsatisfactory for many patients. For example, antiviral treatment with remdesivir is not approved when estimated glomerular filtration rate (eGFR) is below 30 ml/min[6]. In light of this, the FDA published a recommendation for the use of convalescent plasma (CP) for the treatment of COVID-19 in August 2020[7]. Data on monoclonal antibodies such as bamlanivimab or the combination casirivimab/imdevimab only became available toward the end of 2020 and showed good efficacy in the early disease stage of COVID-19[8].

Convalescent plasma has been used during pandemics caused by influenza (1918)[9], Severe Acute Respiratory Syndrome associated Corona-Virus SARS-CoV (2003) and influenza H1N1 (2009) and is reported to have resulted in lower mortality, but the data is not very reliable[10]. Early transfusion of CP with high antibody titres is also a recommended and routine treatment for other high consequence diseases like Argentine haemorrhagic fever[11]. The underlying concept appears to be particularly promising for immunocompromised patients who have a delayed or severely reduced immune response and, after administration of CP, might be able to control the infection earlier and thus avoid disease progression[12,13]. Against this background, CP was already transfused at the beginning of the pandemic[13–16], but always with some restraint, because of the lack of clinical data on efficacy and safety in the different phases of COVID-19 and emerging concerns that CP may promote variant selection[14,17].

Klassen et al. performed a meta-analysis from 32 studies and 96 case series or case reports published between January 2020 to January 2021 that compared mortality of COVID-19 patients who received CP to control groups[18]. The aggregate mortality rate was lower in the transfused group than in the control groups. A major determinant was the timing of CP administration: early transfusion (within 3 days of hospital admission) with high antibody titers resulted in lower mortality[18]. Some case series report successful treatment with CP of immunocompromised SARS-CoV-2 infected patients[19–23]. Randomized controlled trials of investigational drugs usually exclude patients with severe comorbidities such as organ transplantation, active cancer, or end-stage renal disease with dialysis. However, in a pandemic, all people are affected, and data and studies on the treatment of vulnerable patient groups are especially needed.

This is a retrospective analysis of our highly selected cohort of COVID-19 patients who were hospitalized at an academic tertiary centre in Germany (UKD, University Hospital Düsseldorf) between February 2020 and February 2021 and received at least one CP. In our cohort, a significant part of our patients was immunocompromised either due to prior organ transplantation, active malignancy, or were at high risk for severe disease progression due to dialysis dependence, cardiovascular disease or advanced age. In addition, we performed a matched-pairs analysis with our cohort and two subsets of patients from the LEOSS cohort (Lean European Open Survey on SARS-CoV-2 infected patients) who did or did not receive CP.

Results

University Hospital Düsseldorf Cohort (UKD). In total, 55 patients who received CP during the observation period were included in the study. The median age was 68 (range 25–100), the sex distribution was 34 men to 21 women. The total intra-hospital mortality was 41.8% (23/55) and the expected mortality according to the Coronavirus Clinical Characterisation Consortium (ISARIC-4C) Score was 26% (Range 0.3–59.1%).

UKD patient characteristics, comorbidities, received therapies as well as mortality for each subgroup are presented in Tables 1 and 2.

Variable	Nr patients		Mortality (n/% out of category)	
	LEOSS	UKD	LEOSS	UKD
Total	156	55	46	23
Age				
18–25 years	3	1	0	0
26–35 years	6	1	0	0
36–45 years	12	5	2 (16.7)	0
46–55 years	32	3	6 (18.8)	3 (100)
56–65 years	36	13	11 (30.1)	4 (30.7)
66–75 years	36	15	14 (38.9)	10 (66.7)
76–85 years	25	9	11 (44)	3 (33.3)
> 85 years	6	8	2 (33.3)	3 (37.5)
Gender				
Male	119	34	40 (33.6)	16 (47)
Female	37	21	6 (16.7)	7 (33.3)
BMI				
18,5–24,9 kg/m2	35	18	7 (20)	9 (50)
25–29,9 kg/m2	47	18	17 (36.2)	7 (38.9)
30–34,9 kg/m2	31	8	11 (35.5)	2 (25)
> 34,9 kg/m2	14	5	5 (35.7)	3 (60)
Unknown	29	6	6 (20.7)	2 (33.3)
Stage at diagnosis				
Uncomplicated phase	81	14	17 (21)	3 (21.4)
Complicated Phase	45	18	15 (33.3)	6 (33.3)
Critical illness	29	23	14 (48.3)	14 (60.8)
Unknown	1		0	
Duration inpatient stay				
≥ 14 days	119	36	35 (29.4)	14 (38.9)
< 14 days	36	19	10 (27.8)	9 (47.4)
Missing	1		1 (100)	
Duration ICU stay				
< 3	31	2	5 (16.1)	1 (50)
3–15	37	20	15 (40.5)	12 (60)
> 15 days	77	14	26 (33.8)	7 (50)
Missing	11		0	
None		19		3 (15.8)

Table 1. Characteristics of LEOSS and UKD patients with CP treatment.

As previously mentioned, UKD patients were divided into the three subgroups defined by the LEOSS study group, according to the disease stage at first CP administration. Additionally, we stratified patients according to the level of respiratory support. In detail, 14 patients required no oxygen supplementation at first CP administration, 18 had low-flow oxygen requirement, 14 were receiving high-flow oxygen therapy or non-invasive ventilation (HF/NIV), 5 were intubated (IMV) and 4 received extracorporeal membrane oxygenation (ECMO) support. Patients received a median of 3 CPs (range, 1–14), with a mean of 7 days (range, 0–59) after symptom onset.

Eight patients each from the subgroups without or with low-flow oxygen therapy, six patients with non-invasive ventilation, and two ECMO patients remained stable or improved subsequent to CP administration.

In the patients in whom the disease progressed after CP administration, the disease progressed as follows: Of 6 patients without supplemental oxygen, three required low-flow oxygen and three died; of 10 patients with low-flow oxygen, five required non-invasive ventilation and five died; of eight patients with non-invasive ventilation, one was intubated and seven died; of five intubated patients, one required ECMO support and four died; the other two ECMO patients died. Accordingly, intrahospital mortality was stratified by respiratory status at the time of CP administration: 21.4% (no supplemental oxygen; 3/14), 27.8% (low-flow supplemental oxygen; 5/18), 50% (high-flow supplemental oxygen/NIV; 7/14), 80% (IMV 4/5), and 50% (ECMO 2/4). Only one documented adverse event (low fever, chills) was observed after plasma administration, and a clear association could not be established.

Survival analysis. Eighteen patients received CP therapy in the first 3 days and 21 eight or more days after onset of symptoms. Dividing the patients into four subgroups according to days after onset of symptoms at

Variable	LEOSS	UKD	LEOSS	UKD
	156	55	46	23
Total	Yes/total available values			
Comorbidities				
Myocardial infarction	11/153	1	5 (45.5)	0
Chronic heart failure	4/153	3	2 (50)	3 (100)
Coronary Artery disease	23/153	3	15 (65.2)	2 (66.7)
Chronic pulmonary disease	11/154	2	8 (72.7)	0
Malignancies				
Leukemia	9/154	0	2 (22.2)	
Lymphoma	15/153	1	4 (26.6)	0
Solid tumor	10/149	4	6 (60)	4 (100)
Previous stem cell transplantation	4/149	2	1 (25)	1 (50)
SCID	n/a	1	n/a	0
Solid organ transplantation	14/153	8	5 (35.7)	2 (25)
Heart	3	1	0	0
Kidney	11	7	5 (45.5)	2 (28.6)
Diabetes mellitus				
No complications	25/155	0	9 (36)	
Complications	15/153	1	10 (71.4)	0
Acute Kidney injury	20/150	5	7 (35)	1 (20)
Chronic kidney disease	36/151	44	16 (44.4)	21 (47.7)
Dialysis	5/150	13	3 (60)	7 (53.8)
New renal dialysis during CR	29/110	0	21 (72.4)	0
Autoimmune disease	n/a	8		4 (50)
Vasculitis	n/a	4		4 (100)
HIV (CD4 > 500 / µl)	n/a	1		0
Immunosuppressive therapy	34/152	17	11 (32.3)	7 (41.2)
New O2 supplementation during CO	89/153	6	25 (28)	3 (50)
Prior O2 (during UC)	1/152			
Thrombo-embolic complications				
(TVT, PE, abdominal thrombosis)	23/153	0	7 (30.4)	0
Other therapies				
Remdesivir	39/151	11	10 (25.6)	2 (18.2)
Steroids High	60/153	44	21 (35)	16 (36.4)
Tocilizumab	7/141	4	5 (71.4)	2 (50)

Table 2. Characteristics of LEOSS and UKD patients with CP treatment.

first CP administration: 0–3, 4–5, 6–7 and 8 ≥ days, we observe there is no significant difference in mortality (log-rank: 0.72) among these subgroups (The Kaplan–Meier curves are presented in Fig. 1). Out of the patients presenting in the first 3 days after onset of symptoms ($n = 18$), as documented in the patient records, only 7 were classified as uncomplicated, the rest being either in the complicated ($n = 3$) or critical phase ($n = 8$).

Patients receiving the first CP/CPs during the uncomplicated phase had higher chances of survival vs. those in the critical illness phase (log-rank = 0.007). Furthermore, patients with better kidney function and non-dialysis patients had a higher chance of survival but the difference was not statistically significant (log-rank = 0.458 and 0.224 respectively). In summary, 14/23 (60.9%) patients who received first CP/CPs in the critical phase died, compared to 6/18 (33.3%) who received CP in the complicated phase and 3/14 (21.4%) who received CP in the uncomplicated phase. The mortality in the dialysis subgroup was 53.8% (7/13) vs 38% (16/42) in the non-dialysis subgroup.

Body mass index (BMI) and the presence of immunodeficiency had no significant influence on mortality (log-rank = 0.994 and 0.767 resp.) in this cohort. Immunodeficiency was defined as the presence of following comorbidities: solid organ transplantation (heart, kidney), active malignancies, autoimmune disease, HIV, immunosuppressive therapy or corticosteroid therapy for other pathologies (COPD). Five patients had a combination of the above comorbidities. Out of the four patients with ANCA vasculitis, two patients had concurrently an active malignancy. Three patients with vasculitis had received B-cell depleting agents or chemotherapy in the months before SARS-CoV-2 diagnosis. The remaining patient with vasculitis was also a kidney transplant patient and had an untreated carcinoma.

None of the patients with an ISARIC-4C score ($n = 5$) under 3 died vs. 16/27 patients with a very high score (15–21 points) (log rank = 0.23). The ISARIC-4C score for patients receiving first CPs during the uncomplicated

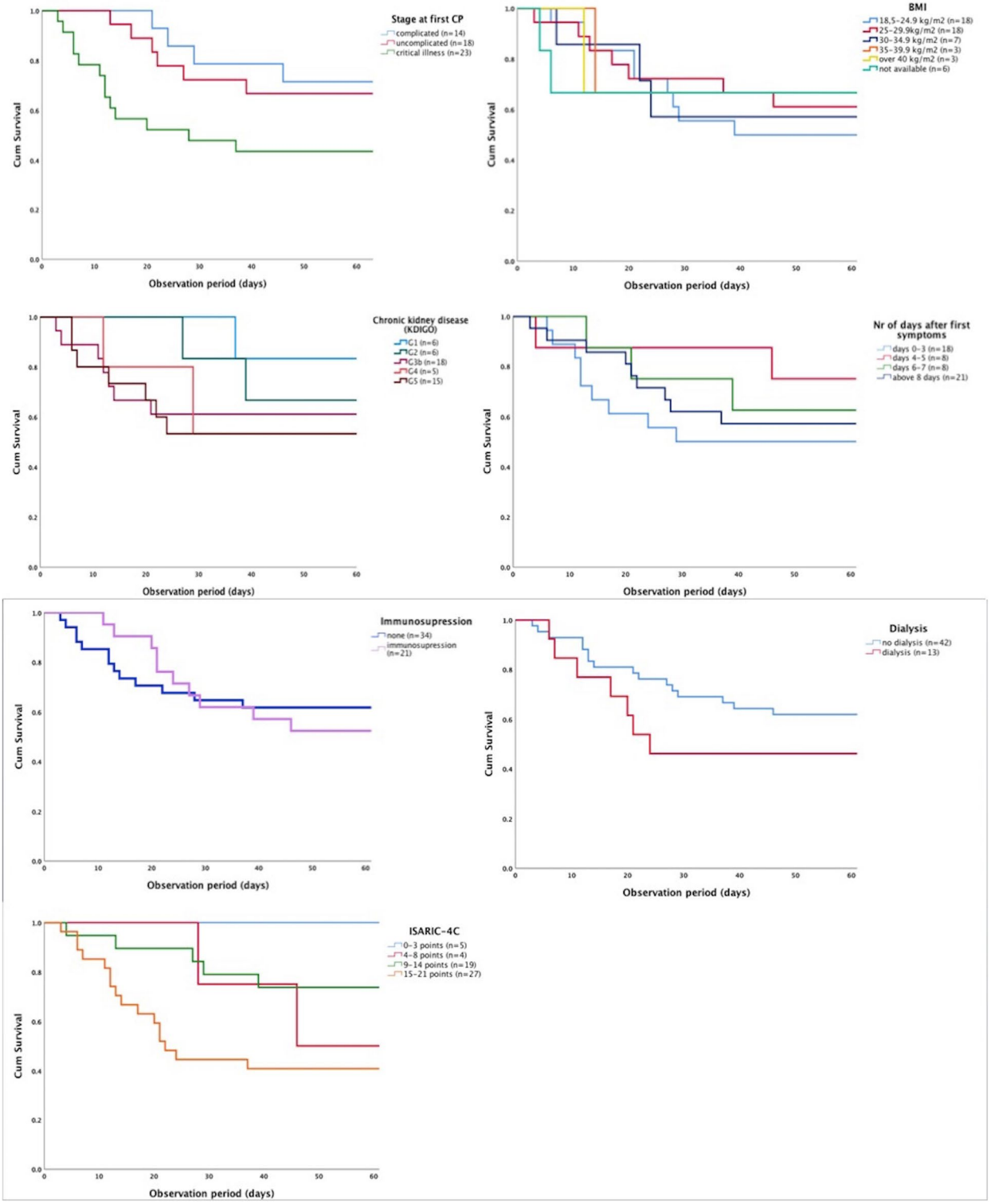

Figure 1. Kaplan–Meier survival plots for various parameters. *CP* convalescent plasma, *BMI* body mass index, *KDIGO* Kidney Disease Improving Global Outcomes, *ISARIC-4C* Coronavirus Clinical Characterisation Consortium.

phase ranged from 0.3 to 40.1%. Out of the five patients with ISARIC-4C score under 3, only two patients did not have other risk factors such as reduced kidney function, immunodeficiency or advanced age.

There were only ten patients who did not have lung infiltrates at administration of first CP/CPs. The mortality rate in this small subgroup was 10% (1/10).

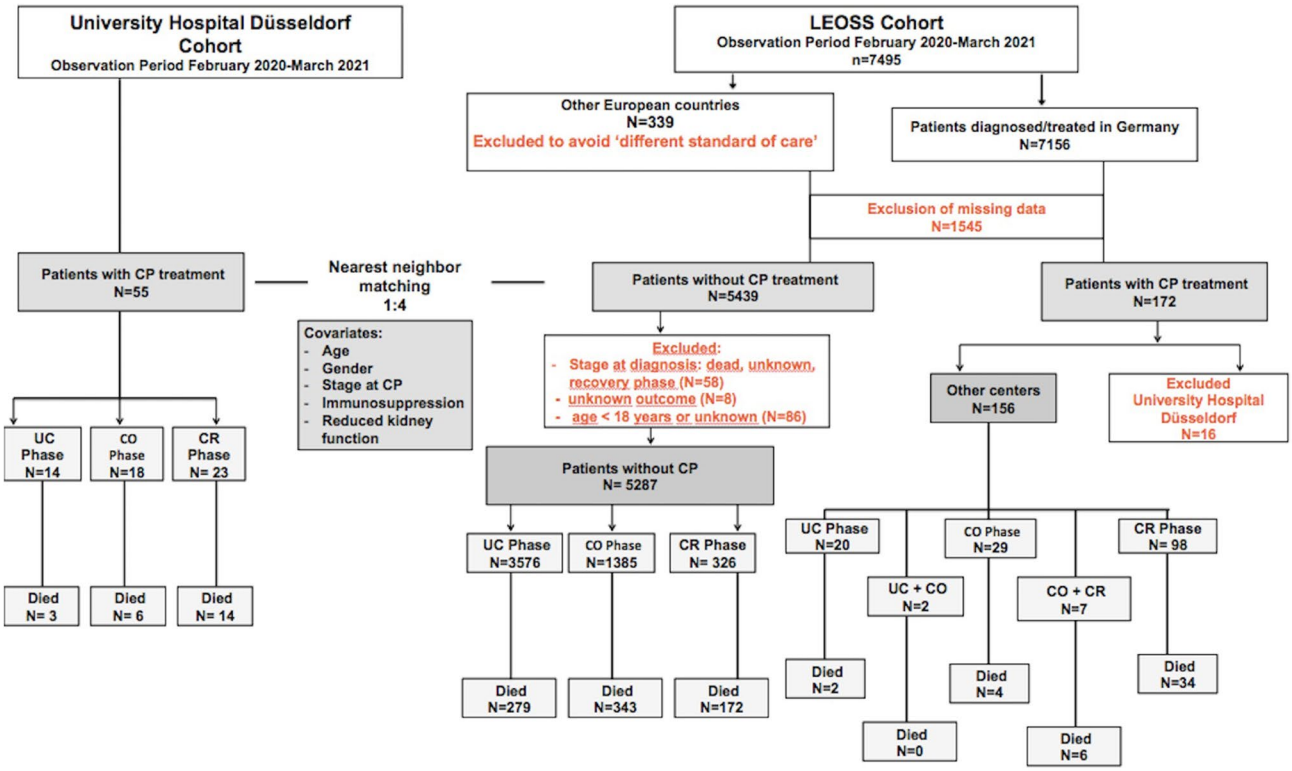

Figure 2. Study design and study population. *N* number, *CP* convalescent plasma, *UC* uncomplicated, *CO* complicated, *CR* critical.

Evolution of SARS-CoV-2 antibodies after transfusion of convalescent plasma (UKD). With the exception of two patients who received three CPs the same day, no more than two CPs were administered at once. There were 23 patients with a single CP/CPs administration out of which six died (mortality: 26%) and 32 patients who received multiple CP/CPs administration. The mortality in the latter subgroup was 53% (17/32) ($p = 0.054$; OR 0.69; CI 95% 0.47–1.02). In total patients received a median of three CPs (range 1–14).

There was a statistically significant increase in titre after the first CP/CPs administration but afterwards there is little variation in antibody titre (Fig. S1a). There is a significant effect of time on antibody titre, especially between baseline and values two, three and five. In regard to the final outcome, patients who died had a lower antibody titre at value two and four time points (Fig. S1b). The four patients with ANCA vasculitis, who had previously received chemotherapy with B-cell depleting agents or were currently on immunosuppressive therapy did not achieve a significant antibody titre (< 5) after the first CP administration, and this titre rapidly decreased, or no antibodies were detected in follow-up measurements. Patients who presented 8 or more days after onset of symptoms had higher antibody levels at baseline that those presenting in the first 7 days (Fig. S1c).

LEOSS cohort: patients with CP therapy. Out of the 156 patients who received CP therapy, 98 patients were in the critical phase. The COVID-19 associated mortality rate for these patients was 34.7%. Two patients received CPs both in the uncomplicated and complicated phase, none died. Seven patients received CPs in the complicated and critical phase, out of which 6 died (Fig. 2). For patients who received CPs in the uncomplicated phase the mortality rate was 10% (2/20); in the complicated phase: 27.8% (10/36). The total mortality rate for patients who received CP therapy was 29.5%.

Patient's characteristics, comorbidities and therapies are presented in Tables 1 and 2.

Factors associated with mortality, length of hospital and ICU stay for UKD and LEOSS cohort. In the UKD cohort the factors introduced in the regression model were stage at CP, reduced kidney function, immunodeficiency and age ($R^2 = 0.294$). Dialysis was analysed separately to avoid multicollinearity ($R^2 = 0.018$) (Table 3). In the binary logistic regression analysis, the factors associated with mortality in the UKD cohort were the disease stage at first CP administration and immunodeficiency.

In a multinomial logistic regression analysis (data not shown in Tables 3, 4, 5), patients receiving the first CP therapy during the uncomplicated phase had a 3.6 higher chance of survival in the UKD cohort (95% Confidence Intervall (CI) for Odds Ratio (OR) 1.02–13.14, $p = 0.046$). Since only a small number of patients was included in subgroups (age, kidney function, BMI), there was no significant association between a specific subgroup and mortality.

According to the strength of association, the factors introduced in the regression model for the LEOSS cohort were the stage at first CP administration, dialysis, immunodeficiency and age ($R^2 = 0.220$). Reduced kidney

Demographics	UKD Cohort		LEOSS CP cohort	
	OR	95% CI for OR	OR	95% CI for OR
Stage at first CP	6.2**	1.87–20.5	1.92	0.96- 3.82
Reduced kidney function	1.7	0.98–3.07	0.98	0.41–2.39
Age	1.5	0.77–3.4	1.7**	1.22–2.32
Dialysis	1.8	0.54–6.65	12.09***	4.42–33.07
Immunosuppression	8.16*	1.35–49	0.63	0.27–1.49

Table 3. Binary logistic regression to identify risk factors for death.

Demographics	UKD Cohort		LEOSS CP Cohort	
	OR	95% CI forOR	OR	95% CI forOR
	Longer hospital stay			
Stage at first CP				
UC	1.65	0.42–6.46	1.1	0.47–2.59
CO	5.00*	1.44–17.24	1.6	0.80–3.07
CR	1.09	0.48–2.47	7.9***	4.22–14.8
Reduced kidney function	0.75	0.48–1.19	2.39	0.89–6.43
Immunosuppression	2.28	0.57–8.14	0.94	0.37–2.38
Age	1.01	0.77- 1.78	1.09	0.83–1.44

Table 4. Binary and multinomial logistic (stage at CP) regression to identify risk factors for longer hospital stay (≥ 14 days).

Demographics	UKD Cohort		LEOSS CP Cohort	
	OR	95% CI for OR	OR	95% CI for OR
ICU stay 3–15 days				
Immunosuppression	1.46	0.39–5.51	1.69	0.47–6.02
Age	0.98	0.95–1.03	0.96	0.65–1.44
Dialysis	1.6	0.37–6.92	1.13	0.33–3.89
ICU stay > 15 days				
Immunosuppression	1.52	0.35–6.63	1.27	0.33–4.96
Age	0.98	0.94–1.02	0.76	0.49–1.18
Dialysis	1.02	0.19–5.53	1.58	0.41–6.17

Table 5. Multinomial logistic regression to identify risk factors for longer ICU stay (3–15 and > 15 days). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; *UKD* University Hospital Düsseldorf, *OR* odds ratio, *CI* confidence interval, *UC* uncomplicated, *CO* complicated, *CR* critical phase.

function was analysed separately. Among LEOSS patients receiving CP treatment, factors associated with death were dialysis and age and to a lesser extent stage at first CP. In a multinomial logistic regression analysis, patients receiving the first CP therapy during the uncomplicated phase had an 8.4 higher chance of survival in the LEOSS cohort (95% CI for OR 1.96–36.8, $p = 0.04$). The OR and confidence intervals CI for ORs are presented in the Tables 3, 4, 5.

Most patients, both in the UKD and LEOSS cohort had a long hospital stay (≥ 14 days): 36/55 and 119/156 respectively and median/long ICU stay (3–15 days or above 15 days).

Matched-pairs analysis of UKD CP cohort vs. LEOSS cohort without CP. The propensity score matching was performed using five variables: age, gender, stage at CP, immunosuppression and presence of reduced renal function. Four controls were matched to one case. There was no difference in survival between the UKD and LEOSS matched cases. Patient in the UKD cohort had longer hospital and ICU stays (Table 6).

Discussion

Treating COVID-19 patients with complex and severe comorbidities, such as previous organ transplantation, immunosuppressive diseases or medications is still a challenge.

	UKD (cases)	LEOSS (controls)	*p*-value
Total nr	**55**	**220**	
Survival	Died/Total (%)	Died/Total (%)	0.345
	23/55 (41.8)	75/220 (34.1)	
Hospital stay			**0.031**
< 4 days	2 (3.6)	31 (14.1)	
4–13 days	18 (32.7)	86 (39.1)	
≥ 14 days	35 (63.7)	103 (46.8)	
ICU stay			**< 0.001**
None	19 (34.5)	46 (20.1)	
< 3 days	2 (3.6)	82 (37.3)	
3–15 days	20 (36.4)	42 (19.1)	
≥ 15 days	14 (25.5)	38 (17.3)	

Table 6. Outcome comparison in the UKD-LEOSS matched pairs. *For 11 patients in the LEOSS controls cohort, data regarding ICU stay was not available. They were not included in analysis. Significant values are in [bold].

This retrospective analysis presents a highly selected cohort of 55 patients with diverse but severe and complex underlying diseases who required treatment at a tertiary academic centre. Often, these patients with severe comorbidities are excluded from trials, especially prospective or randomized trials, e.g. randomized trial from Sekine et al.[24].

21 of 55 patients were immunocompromised due to previous solid organ transplantation, active malignancy, autoimmune disease or HIV infection. The remaining patients had a variety of other risk factors, most of which also affect immune function and influence disease progression and mortality. One patient was a young woman with a SCID syndrome (severe combined immunodeficiency), the case is also separately presented by Keitel et al.[19]. Another patient was infected by HIV-1 that does not necessarily lead to a severe disease course; this depends on the treatment status and concomitant diseases, but he suffered also from histiocytosis X[25,26].

Common comorbidities present in the patients treated with CP from the LEOSS cohort were immunodeficiency (solid cancer, solid organ transplantation, hematologic cancer) and reduced renal function. Performing a matched-pairs analysis was a challenging task because the 55 patients in the UKD cohort had complex pre-existing conditions that were difficult to account for in matching. This is one limitation of our study.

Our retrospective analysis showed an in-hospital mortality of 41.8% in our UKD cohort and a mortality of 29.5% in the LEOSS cohort of patients who received CP treatment. In the matched-pairs analysis there was no significant difference between the UKD cohort and matched controls from the LEOSS cohort without CP treatment. The mortality in our cohort is higher than the in-hospital mortality of COVID-19 patients in Germany of 22% reported by Karagiannidis et al. in September 2020[27]. The literature search revealed an increased mortality rate in immunocompromised patients with COVID-19. Martinez-Urbistando et al. showed that illnesses causing immunodeficiency are an independent risk factor for increased mortality[5]. Mehta et al. studied mortality rates in COVID-19 patients with active cancer and found a higher overall mortality rate of 28%; 25% in solid cancers and 37% in haematological cancer[28]. A meta-analysis from Belsky et al., reviewed the disease course of immunodeficient patients infected by SARS-CoV-2 and found that solid organ transplant patients, who suffer from multiple comorbidities, are more likely to require intensive care, which is a surrogate for mortality[4]. E.g., in our cohort 7 patients with previous kidney transplantation had a mortality rate of 28.6%, in accordance to our results, Alberici et al., Yilmaz et al. and Akalin et al. found mortality rates of 28%, 23% and 25% in kidney transplant recipients with COVID-19[29–31]. However, the mortality rate in our cohort was lower than in LEOSS patients with prior renal transplantation and CP therapy (45.5%).

On the other hand, a compromised immune response to the pathogen due to immunosuppression may also protect against the organ destroying cytokine storm caused by SARS-COV-2 and lead to a favourable outcome[32,33]. But the study of Minotti et al. included mostly children or adults with rare immunodeficiencies in contrast to our cohort[33]. Regarding comorbidities and age, observational cohorts from Yilmaz et al., Alberici et al., Mehta et al. and Belsky et al. are much more comparable to our cohort[4,28,30,31]. But they reported smaller case series and all patients had the same underlying disease. Nevertheless, the overall mortality rate was still higher in our cohort, assuming due to the complex comorbidities.

In our study, patients receiving repeated CP transfusions had higher mortality rates, which might be explained by the fact that repeated CPs were given more frequently to severely ill patients who did not improve clinically and were less likely to be given to stable or improved patients.

A meta-analysis by Klassen et al.[18] showed positive effects regarding mortality and morbidity when transfusion of CP is performed in early disease stage. Mortality was 10% in patients in our cohort who received plasma without radiologically detectable pulmonary infiltrates, presumably because CP administration occurred early in the disease. In the LEOSS cohort, mortality was similar at 10% when CP was transfused in the uncomplicated phase. CP was administered in the UKD cohort on average 7 days after onset of symptoms, only 18 out of 55 patients received it within 3 days after onset of symptoms but there was no significant difference in mortality rate. A diagnosis in the critical phase of disease and thus late CP therapy resulted in a mortality rate of 60.8% in our

cohort. This can be explained partly by the high risk of death due to severe comorbidities in this selected group of patients. However, in the late phase of COVID-19, the SARS-CoV-2 virus plays only a minor pathophysiological role compared to the hyperactivation of the immune system, so that a primarily antiviral intended therapy such as CP could also be expected to be of little benefit.

Another influencing factor is the antibody titre of the CP. Joyner et al. were able to demonstrate that mortality and morbidity are only reduced if the CP have an antibody titre of at least 1:2560 (SARS-CoV-2 spike subunit 1 protein)[18,34]. Joyner et al. could also show that a lower risk of death after transfusions with high antibody titres was present only in the group of patients who were not mechanically ventilated[34]. Other studies support these findings: In CP recipients treated within 72 h of symptom onset who had high antibody titres (SARS-CoV-2 S IgG titres > 1:3200), the relative risk reduction was 73.3%, compared with recipients with antibody titres of less than 1:3200, for whom the risk reduction was only 31.4%[35]. Similar results were obtained by Salazar et al., who found a significant reduction in 28-day mortality when CP was administered within 72 h of hospital admission and the anti-IgG to spike protein titre was ≥ 1:1350[36]. Sekine et al. found no significant difference in 28-day improvement after CP transfusion, but transfusion was performed late in the disease stage when 0_2 supplementation was already required and at least 10 days after symptom onset, confirmed by detection of neutralizing antibodies[24].

Regarding the use of CP in immunocompromised patients with severe COVID-19, Gupta et al. reported 10 kidney transplanted patients with severe COVID-19 according to the WHO Interim Guidance[37], 9 of whom recovered completely[38]. Transfusion of CP in our high-risk cohort at an early stage of disease resulted in a low mortality rate of 10% and, in a larger cohort, may also have shown a significant benefit in reducing mortality.

Matched-pairs analysis showed a significant longer stay in ICU and in-hospital compared to the LEOSS cohort that has not received CP. The selection of patients in the UKD cohort can in part explain the significant longer stay in ICU and in hospital caused by a higher awareness to this patient group by expecting complications, including those that may not occur directly as a result of COVID-19. In addition, patients with haematological diseases needed more time for viral clearance as also described by Avanzato et al. and Mira et al.[12,13].

However, it must also be considered that CP administration could have a negative impact on overall survival. Systematic reviews and meta-analyses of a total of more than 10,000 patients showed no significant negative effect in terms of mortality, use of mechanical ventilation, clinical improvement or clinical deterioration[39].The observation in our cohort that no serious adverse events, e.g., allergic reactions, were observed is supported by other publications that consider transfusion of CP to be a safe and a low-risk procedure[40].

The ISARIC-4C score is also a good predictor of mortality in this cohort of high-risk patients. Accordingly, 46 of 55 patients were classified as high-risk (more than 9 points). All patients with an ISARIC-4C score under 3 survived and 16 of 27 patients with a very high score (15–21points) died. It is noteworthy that most of our patients had a high ISARIC-4C score, although this did not take into account the immunodeficiency and impaired renal function that were present in a large proportion of our patients. Also, young patients with severe comorbidities or rare life-limiting diseases usually achieve only a low score, so that their individual risk is not adequately reflected by the score[19,41]. In our view, these factors explain the higher in-hospital mortality in our cohort compared with that predicted by the ISARIC-4C score; in contrast, the association between a high and very high score and the outcome is clearly evident in our cohort (OR 4.07, 95% CI 1.14–14.6).

There are few reports of virus development in severely immunosuppressed patients, e.g., with deficiency of both B and T cells[14,17]. On the one hand, especially in the absence of specific monoclonal antibodies until the beginning of 2021, the indication for CP therapy was obvious in these patients due to their impaired own immune response and their risk profile. However, it should be taken into account that especially in the context of immunodeficiency and high-replicative viral variants, viral evolution after therapy with CP and monoclonal antibodies, in particular bamlanivimab, has been reported[17,41,42].

The analysis is limited because of several factors. Since the study was performed at an academic tertiary centre, many patients were referred from secondary and tertiary hospitals, so some patient data on symptom onset and timing of SARS-CoV-2 infection were missing. Defining of onset of symptoms has not been exactly defined at the beginning of the pandemic. Therefore, in some cases, the timing of symptom onset was apparently assessed in the emergency department based on shortness of breath or clinical worsening, rather than with regard to early, mild symptoms such as sore throat, headache, or fever. Furthermore, antibody titres of the CPs were not routinely tested, so positive effects by high antibody titres in CP may not be documented. Because of the lack of a prospective study protocol, our results have many other limitations since we cannot account for multiple possible confounders or aspects such as immortal time bias. Further, another factor to mention is the rather small size of the cohorts, so the results should be interpreted within this context. In addition, the diversity of comorbidities in the UKD cohort makes it difficult to extrapolate results to the general population.

Larger cohorts would be needed to better assess the impact of CP therapy.

We hypothesize that any potential beneficial impact of CP therapy on morbidity and mortality in this highly selected group of patients is masked by the fact that in most cases CP was administered at an advanced stage of the disease.

Available data suggest that CP administration, especially when given early, holds promise for more rapid clearance of the virus and amelioration of the severity of the disease course. Considering that CP administration is not associated with high risk based on available data, CP administration should be considered as an early available and promising option for future viral pandemics or viral variants until specific therapies become available. This is especially true for vulnerable patient populations at high risk for severe disease progression or with contraindications to alternative treatment options, as represented by the patient population in our cohort.

Methods

Patient cohort. Retrospective cohort study of 55 patients with diagnosed SARS-CoV-2 infection (defined as detection of SARS-CoV-2 RNA from naso-pharyngeal secretions) admitted to the infectious diseases unit or intensive care unit (ICU) at the UKD between February 2020 and 25th February 2021, who received CP from cured SARS-CoV-2 donors within the framework of an individual treatment option after informed consent. None of the patients had received a vaccination against SARS-CoV-2.

The main objectives were to describe the pattern of CP use and to analyse in-hospital mortality, length of hospital stay, and ICU stay. Second, we aimed to describe the evolution of SARS-CoV-2 antibody titres after CP administration.

Matched pairs analysis to LEOSS cohort. The Lean European Open Survey on SARS-CoV-2 infected patients (LEOSS) is an ongoing multicentric study collecting epidemiological and clinical information on SARS-CoV-2 disease.

We aimed to compare demographical parameters and clinical outcomes between our cohort and LEOSS patients receiving CP.

Furthermore, in order to evaluate if CP influences our primary outcomes, we performed a matched-pairs analysis using as cases our cohort of CP patients and as controls, LEOSS patients without CP therapy.

For this purpose, LEOSS patients were divided into two groups: patients without CP therapy and patients with CP therapy. To avoid statistical bias, LEOSS patients diagnosed and treated in countries other than Germany and patients with missing data were excluded, as described in Fig. 2.

Patients were matched based on age, sex and confounding factors influencing the outcome (disease stage at first administration of CP, reduced renal function and immunosuppression).

SARS-CoV-2 stages of disease. Patients in our cohort were divided into three subgroups according to the disease stage at first administration of CP. The disease stage definitions were retrieved from the LEOSS cohort: uncomplicated, complicated and critical phase. The characteristics of each phase have been defined in previous publications[43].

Confirmation of SARS-CoV-2 infection. *Isolation of viral genomic material and SARS-CoV-2 quantification.* Respiratory samples from nasopharyngeal swabs were used for total nucleic acid extraction using the EZ1 Virus Mini Kit v2.0 on an EZ1 Advanced XL (Qiagen, Germany) according to manufacturers' instructions. SARS-CoV-2 was detected as previously described by Corman and colleagues[44] with a plasmid-standard for quantification[45], by the cobas® SARS-CoV-2 test on the cobas®6800 system (c6800, Roche Diagnostics), or by the SARS-CoV-2 test on the NeuMoDx™ platform (NDX, Qiagen).

Detection of SARS-CoV-2 antibodies. SARS-CoV-2 antibodies were detected using the Elecsys® anti-SARS-CoV-2 (Cobas, Roche Diagnostics, Mannheim, Germany), which uses a protein representing the nucleocapsid (N) antigen in a double-antigen sandwich assay format. The assay was performed according to the manufacturer's instructions and has a sensitivity of 85.3% 7–13 days after confirmed diagnosis (positive PCR) and 99.5 at more the 14 days after diagnosis[46]. Testing of SARS-CoV-2 anti-Spike (S) antibodies was only available after introduction of the SARS-CoV2 vaccines in early 2021. The testing of neutralizing antibodies was not routinely available during the study period.

SARS-CoV-2 antibodies were measured at baseline, either hospital admission or before administration of CP and after administration of CP. For patients receiving multiple plasmas, repeated measures were available. After CP administration, the available values were grouped in intervals of days after baseline to facilitate statistical analysis: 0 to 5 days, 6 to 10 days, 11 to 15 days, 16 to 20 days and 21 to 25 days after baseline.

Convalescent plasma. *Collecting plasma.* CP was collected from patients with confirmed SARS-CoV-2 infection. All plasmas were collected in the Institute for Transfusion Medicine, University Hospital Düsseldorf according to the following criteria. The convalescent plasma donors donated 650 ml, 750 ml or 850 ml of plasma. Plasmapheresis was performed according to Standard Operation Procedure of the Institute for Transfusion Medicine. One plasma unit had a volume of 190 ml to 350 ml, depending on body weight of the donor. The production of convalescent plasma was subject to the same criteria as plasma products for transfusion purposes (approval according to Arneimittelgesetz (AMG), paragraph 13). As described in the permission of the blood donation services high sensitive PCR for hepatitis virus A, B, C, E, West nile virus according to the recommendation of the Paul Ehrlich Institute and HIV was performed. The donors for CP had to meet the following criteria in accordance with the guidance of the European Union on collection of COVID-19 CP: Informed written consent to donate plasma; previous infection with SARS-CoV-2 as documented by either a positive PCR (from nasal or nasopharyngeal swab, bronchoalveolar lavage or stool or a past medical history suggestive of COVID-19 and presence of anti-SARS-CoV-2 antibodies with a documented date of first positive test; proven clearance of SARS-CoV-2 from nasopharyngeal mucosa by one negative PCR result from nasal swabs or nasopharyngeal swabs and an interval of at least 4 weeks since resolution of SARS-CoV-2 associated symptoms or an interval of at least 4 weeks since resolution of symptoms of SARS-CoV-2 infection. Time and types of symptoms were documented as well as time of resolution, as well as most severe clinical status according to 9 point ordinal WHO scale; no residual severe organ dysfunction due to COVID-19; negative test for antibodies against HLA class I, class II and HNA-antigens in female donors with a history of pregnancy; Anti-SARS-CoV-2 antibodies detectable in a neutralization assay (NT) titer of > 1:160 (measured at Institute for Virology, University Duesseldorf).

Transfusion of convalescent plasma. Patients received 1–2/3 units of AB0-matched CP, dependent on bodyweight and depending on the volume status. CP was transfused over 30 to 60 min. During transfusion, patients were monitored, at least by continuous measurement of oxygen saturation. All patients or their legal representatives gave written informed consent to the transfusion of CP.

Statistical analysis. Data were analysed using SPSS Statistics for Mac OS version 25.0 (IBM Corp., Armonk, USA). Simple frequencies, description and survival analysis were performed. A non-parametric Mann–Whitney test was used to assess differences in antibody titre after plasma administration. A two-way analysis of variance (ANOVA) was used to test associations between time, primary outcome and antibody titre. Bivariate correlations were used to determine associations between different parameters. Binary and multinomial logistic regression models were used to determine predictors of major outcomes. Odds ratios and 95% CI for odds ratio were calculated to assess the strength of association and statistical significance. Significant outcome predictors in both cases and controls groups were introduced as covariates in a case–control matching model. The case–control matching was performed using propensity score (nearest-neighbour 1:4) matching with R statistic[47,48]. Differences in outcome between matched cases and controls were assessed using an unpaired t-test. Differences were considered statistically significant at two-tailed $p < 0.05$.

Variable definition. For the logistic regression and variance analyses some variables had to be regrouped. In the UKD cohort, immunosuppression was defined as the presence of following comorbidities: solid organ transplantation (heart, kidney), active malignancies, autoimmune disease, HIV, immunosuppressive therapy or cortisone therapy for other pathologies (for example COPD). Acute kidney injury (AKI) and chronic kidney disease (CKD) were regrouped as a single variable entitled reduced kidney function.

In the LEOSS cohort receiving CP the final outcome was regrouped as binary (dead/alive); malignancies/organ transplantations/stem cell transplantation/ other immunosuppressive therapies were grouped together as Immunosuppression, much like in the UKD cohort. Prior and new dialysis were grouped as a single variable named dialysis.

Declaration of accordance to relevant guidelines and regulation. We can confirm that all methods were carried out in accordance with the relevant guidelines, treatment recommendations, and regulations available at the time of use of convalescent plasma in the cohorts presented.

Ethical considerations. The ethical committee of the Heinrich-Heine-University Duesseldorf approved the retrospective analysis of the cohort (Study number: 2021-1385). The investigator board of Lean European Open Survey on SARS-CoV-2 infected patients (LEOSS) approved the matched-pair-analysis. Approval for LEOSS was obtained by the applicable local ethics committees of all participating centers and registered at the German Clinical Trials Register (DRKS, No. S00021145).

Data availability

The datasets generated and analysed are available from the corresponding author on reasonable request. Datas of the LEOSS cohort are available from the LEOSS study group.

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Author contributions

N.F.F., S.G., T.L., B.J., T.F. and E.B. initiated this retrospective analysis. N.F.F. managed the study group and wrote this manuscript together with B.J. and S.G. S.G. performed the statistical analysis of the data. M.L., S.G., N.F.F., M.S. produced the metadata. M.L. and S.G. scrubbed and maintained research data. N.L. performed and provided material and laboratory for the swabs. J.F. and E.B. provided and analysed the C.P. The medical treatment was provided by N.F.F., S.G., M.L., T.F., H.M.O., T.L. und B.J. H.M.O., E.B. and T.L. critically revised this manuscript. C.R., D.W., J.S., K.W., C.H., R.S., M.W., A.R., J.S., N.I., M.S., L.P., J.J.V., F.H. as members of the LEOSS study groups provided data of the patients of the LEOSS cohort and revised critically the manuscript. All authors read and approved critically the manuscript.

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Competing interests
The authors declare no competing interests.

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ORIGINAL PAPER

All-cause mortality and disease progression in SARS-CoV-2-infected patients with or without antibiotic therapy: an analysis of the LEOSS cohort

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Abstract

Purpose Reported antibiotic use in coronavirus disease 2019 (COVID-19) is far higher than the actual rate of reported bacterial co- and superinfection. A better understanding of antibiotic therapy in COVID-19 is necessary.

Methods 6457 SARS-CoV-2-infected cases, documented from March 18, 2020, until February 16, 2021, in the LEOSS cohort were analyzed. As primary endpoint, the correlation between any antibiotic treatment and all-cause mortality/progression to the next more advanced phase of disease was calculated for adult patients in the complicated phase of disease and procalcitonin (PCT) ≤ 0.5 ng/ml. The analysis took the confounders gender, age, and comorbidities into account.

Results Three thousand, six hundred twenty-seven cases matched all inclusion criteria for analyses. For the primary endpoint, antibiotic treatment was not correlated with lower all-cause mortality or progression to the next more advanced (critical) phase ($n = 996$) (both $p > 0.05$). For the secondary endpoints, patients in the uncomplicated phase ($n = 1195$), regardless of PCT level, had no lower all-cause mortality and did not progress less to the next more advanced (complicated) phase when treated with antibiotics ($p > 0.05$). Patients in the complicated phase with PCT > 0.5 ng/ml and antibiotic treatment ($n = 286$) had a significantly increased all-cause mortality ($p = 0.029$) but no significantly different probability of progression to the critical phase ($p > 0.05$).

Conclusion In this cohort, antibiotics in SARS-CoV-2-infected patients were not associated with positive effects on all-cause mortality or disease progression. Additional studies are needed. Advice of local antibiotic stewardship- (ABS-) teams and local educational campaigns should be sought to improve rational antibiotic use in COVID-19 patients.

Keywords COVID-19 · Antibiotics · Antibiotic stewardship · Procalcitonin · LEOSS

Introduction

Coronavirus disease 2019 (COVID-19) resulting from infection with severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), first described in Wuhan, China, in late 2019, has become a global pandemic. The role of bacterial superinfections, their influence on the clinical course, and the appropriate use of antibiotics in a primarily viral respiratory disease are becoming increasingly important in this context [1]. In respiratory viral infections such as influenza, bacterial superinfections can lead to higher morbidity and mortality and require timely diagnosis and initiation of antibiotic therapy (ABT) [2]. Publications report bacterial co- and superinfection rates of less than 10% in COVID-19 patients [2, 3], while the percentage of systemic ABT prescribed was over 60% [2]. This discrepancy is also well documented for other viral diseases such as influenza [4, 5], and international and national campaigns on antibiotic stewardship (ABS) intensively address the consequences for hospitals for more than a decade. ABS aims to sustainably

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reduce the development of antibiotic resistance by creating awareness for rational antibiotic use and optimized antibiotic therapy strategies [6]. The core elements of ABS are reviewing the indication of ABT and optimizing its duration, dosage, and substance selection based on validated clinical criteria and biomarkers [7].

Possible consequences of untreated bacterial co- and superinfections and diagnostic uncertainties confront medical staff with complex decisions regarding ABT initiation, especially in severely affected patients. In a meta-analysis published in 2021, Langfort et al. summarize that there is currently insufficient evidence to support the widespread use of empiric ABT in hospitalized COVID-19 patients [2, 8]. The World Health Organization (WHO) does not recommend initiating ABT for uncomplicated courses of SARS-CoV-2 infection but recommends therapy for moderate to severe courses of illness and clinical suspicion of bacterial co- or superinfection [9, 10]. Especially for patients in the complicated phase of the disease, it is of crucial importance to name contraindications for and effects of ABT on treatment outcomes to provide physicians with decision-making strategies while global COVID-19 case rates stay high [11]. Procalcitonin (PCT) is a validated serological marker for differentiating between bacterial and non-bacterial acute respiratory tract infections. Bacterial infections enhance its production and release from extrathyroidal sources into the circulation and low PCT indicates a lower likelihood for bacterial infection [12–14]. First studies investigated PCT's relevance for SARS-CoV-2-infected patients [15]. A better understanding of antibiotic therapy guided by (low) PCT in COVID-19, especially for complicated patients, would be beneficial. This study focuses on the association of ABT and the outcomes all-cause mortality and clinical worsening in patients in a complicated phase of COVID-19 and low PCT values.

Method

Study design

This study uses data from the multicenter Lean European Open Survey on SARS-CoV-2-Infected Patients (LEOSS) cohort established in March 2020 (DRKS, No. S00021145, https://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00021145). Cases between March 18, 2020, and February 16, 2021, were included, if they were ≥ 18 years, information on ABT was available, and a minimum observation period of 3 days (≥ 72 h) was reached. In addition to censored cases, those without a documented treatment outcome were excluded (see Fig. 1). PCT was dichotomized by a threshold commonly used for lower respiratory diseases [16, 17] of 0.5 ng/ml (≤ 0.5 ng/ml [PCT↓] and > 0.5 ng/ml [PCT↑]). The clinical outcomes considered in this study were all-cause mortality (yes/no) and progression to the next advanced phase of the disease (yes/no) in the LEOSS schema (see next section and Fig. 2), each until the end of the acute phase of SARS-CoV-2 infection (e.g., recovery, or death).

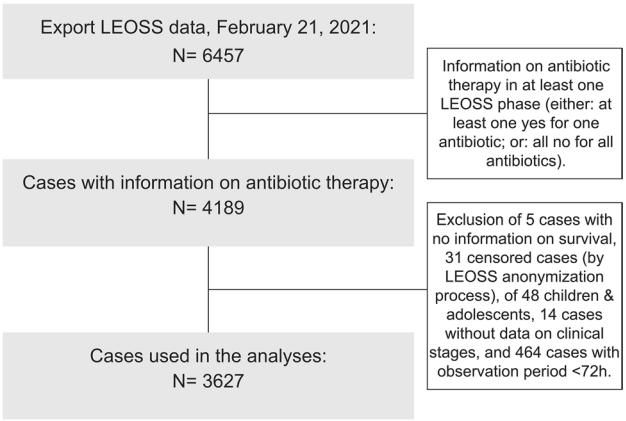

Fig. 1 Flowchart showing the inclusion criteria for the analysis. LEOSS: Lean European Open Survey on SARS-CoV-2-Infected Patients

The primary endpoint of this study was the effect of ABT, defined as any antibiotic agent received irrespective of dose or duration, on all-cause mortality and progression to the critical phase in patients in the complicated phase with low PCT values (PCT↓). Secondary endpoints were the effects of ABT on all-cause mortality and progression to the next advanced phase in patients in the complicated phase with PCT↑ and patients in the uncomplicated phase with PCT↓ and PCT↑.

Possible confounders were chosen after literature review and availability in the data set, resulting in the inclusion of gender, age, and comorbidity state as Charlson comorbidity Index (CCI) [18–24]. We calculated the CCI instead of individual comorbidities to sustain high case numbers for conclusive statistical analysis, [25]. The dataset included binary information on all relevant diseases to calculate the CCI (see also Table 1) [28]. CCI strata of 0–2, 3–4, and > 4 were chosen, reflecting a low, increased, or high comorbidity state, respectively. Where possible, body-mass-index (BMI) and quick Sepsis Related Organ Failure Assessment (qSOFA) were considered. Due to the insufficient data on bacterial superinfections, those were not included in the analysis.

LEOSS cohort

The LEOSS cohort was initiated to identify independent predictors of outcome in patients diagnosed with SARS-CoV-2 and performs no follow-ups. LEOSS collects data from health care records of any outpatients or inpatients

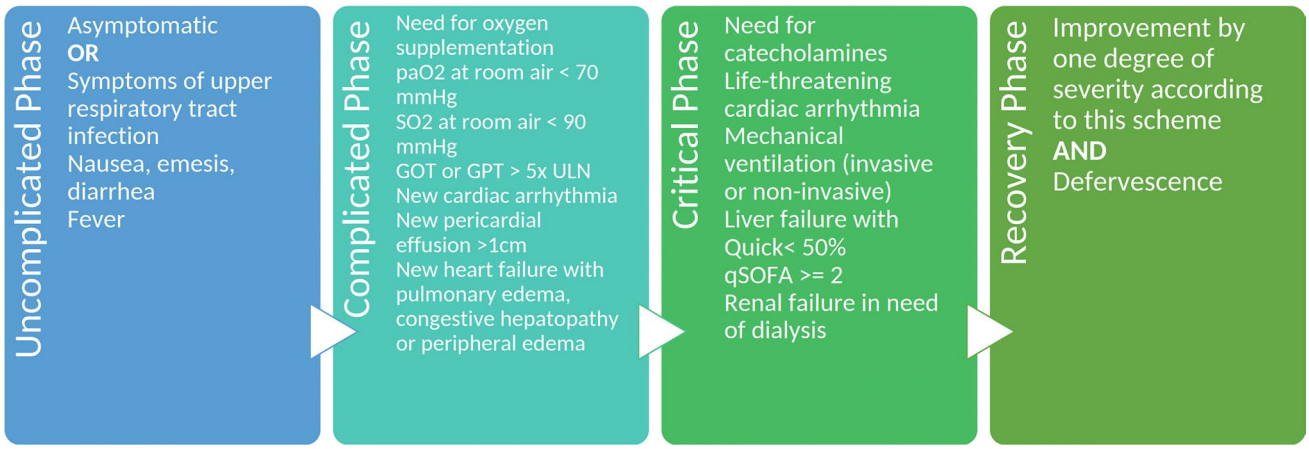

Fig. 2 Clinical symptoms and characteristics defining the different phases (uncomplicated [UC], complicated [CO], critical [CR] and recovery) in the LEOSS cohort. The alternative endpoint "death" is not displayed in this figure

with confirmed SARS-CoV-2 infection (either via positive reverse transcriptase-polymerase chain reaction [rtPCR] or rapid antigen test) and completed acute treatment at participating university hospitals, non-university hospitals, and practices [26]. As of March 18, 2021, 133 active study sites with valid ethical votes from 12 European countries are documenting. Study centers outside Germany documented approximately 5% of the cases, the non-university sector approximately 45%. The study protocol excludes pregnant women.

The LEOSS cohort defines three clinical phases of COVID-19 (uncomplicated, complicated, critical) and two outcome phases (recovery and death; see Fig. 2 for details):

- Clinical phases
  - Uncomplicated (UC) phase: oligo-/asymptomatic
  - Complicated (CO) phase: oxygenation or equivalent clinical deterioration
  - Critical (CR) phase: life-sustaining measures
- Outcome phases
  - Recovery: clinical improvement/discharge
  - Death: from COVID-19; from other cause

Depending on the course of the disease, patients moved through multiple phases or skipped up to two clinical phases. Patients can appear in several subgroups, e.g., in both the UC- and the CO-phase. A patient cannot move back to a previous clinical phase.

LEOSS collects an extensive anonymous dataset and provides individual anonymized LEOSS Scientific Use Files (SUFs) for analyses [27]. Anonymization is mainly achieved by summarizing the values of variables into categories. Information on therapy, diagnostics, and interventions is aggregated over each phase. Usually, only one value that deviates the most from the normal range is documented. The electronic case report form (eCRF) enforces binary documentation of therapies and interventions [26]. Due to anonymity and retrospective documentation, inclusion was performed without explicit written consent.

Statistical analysis

First, all available cases in LEOSS that met the inclusion criteria were characterized by descriptive statistics and analyzed for the influence of the risk factors (age, gender CCI, BMI, and qSOFA) at baseline on clinical outcomes using univariate and multivariate models in an exploratory way. For the primary endpoint, the effect of ABT on clinical outcomes in the CO-phase with PCT↓ was tested in univariate and multivariate models, in the latter case adjusted for age, gender, and CCI in. qSOFA / SOFA and BMI had to be omitted due to too many missing values. A missingness analysis was performed for patients in the CO phase (see Table 5 in the Appendix). It included a comparison of the group of patients with complete information on PCT and ABT against the group of patients with incomplete information on PCT and ABT (i.e., at least one missing value in PCT or ABT) concerning the two clinical outcomes and the risk factors age, gender, CCI, BMI and qSOFA at the time of admission. For the secondary endpoints, patients in the CO-phase with PCT↑ and patients in the UC-phase with PCT↓ or PCT↑ were studied using the same clinical outcomes, influence factors and statistical analyses as for the primary endpoint.

The univariate analyses and the missingness analysis tested the association of individual variables for

Table 1 Baseline characteristics of patients included in the analyses by clinical phase (a) and Influence of risk factors on all-cause mortality and entry into critical (CR)-phase in all COVID-19 patients included in the analysis (b)

a. Baseline characteristics of patients included in the analyses by clinical phase. As patients move through clinical phases, the sum of the phases is higher than the total. Differences of cases to the respective total population in each column account for missing or unknown values

	Total	Uncomplicated phase	Complicated phase	Critical phase
Included cases	3627	82.6% (2995/3627)	51.0% (1850/3627)	20.2% (731/3627)
Age				
18–25 years	3.0% (111/3627)	3.5% (104/2995)	0.7% (13/1850)	0.5% (9/731)
26–35 years	6.8% (248/3627)	7.9% (237/2995)	2.8% (52/1850)	0.9% (17/731)
36–45 years	8.9% (324/3627)	9.7% (290/2995)	5.9% (109/1850)	2.1% (39/731)
46–55 years	15.8% (574/3627)	16.6% (498/2995)	15.2% (281/1850)	6.1% (113/731)
56–65 years	18.8% (681/3627)	18.4% (552/2995)	18.8% (348/1850)	9.5% (176/731)
66–75 years	17.1% (621/3627)	16.4% (491/2995)	19.2% (355/1850)	9.3% (172/731)
76–85 years	21.1% (766/3627)	19.7% (590/2995)	27.0% (499/1850)	8.8% (163/731)
> 85 years	8.3% (302/3627)	7.8% (233/2995)	10.4% (193/1850)	2.3% (42/731)
Gender				
Male	58,0% (2107/3627)	57.4% (1719/2995)	60.8% (1124/1850)	73.5% (537/731)
Female	42.0% (1520/3627)	42.6% (1276/2995)	39.2% (726/1850)	26.5% (194/731)
Body mass index (kg/m^2)				
< 18.5	2.2% (52/2293)	2.2% (42/1920)	2.0% (23/1178)	1.4% (7/513)
18.5–24.9	30.4% (697/2293)	32.3% (620/1920)	27.8% (328/1178)	21.4% (110/513)
25–29.9	36.0% (825/2293)	36.3% (696/1920)	34.9% (411/1178)	38.6% (198/513)
30–34.9	19.8% (455/2293)	19.2% (368/1920)	23.0% (271/1178)	21.1% (108/513)
≥ 35	11.5% (264/2293)	10.1% (194/1920)	12.3% (145/1178)	17.5% (90/513)
Comorbidities (as included in the Charlson Comorbidity Index)				
Acute myocardial infarction	5.8% (201/3495)	5.5% (160/2910)	6.6% (117/1767)	6.4% (44/687)
Congestive heart failure	8.8% (307/3493)	8.1% (235/2911)	10.6% (187/1768)	11.7% (80/686)
Peripheral vascular disease	4.4% (154/3491)	4.1% (120/2907)	5.8% (102/1765)	5.4% (37/684)
Cerebral vascular disease	9.1% (320/3509)	8.3% (242/2922)	11.8% (210/1780)	8.6% (59/685)
Dementia	8.9% (313/3501)	8.0% (234/2917)	12.1% (215/1773)	6.6% (45/683)
Pulmonary disease	3.8% (132/3503)	3.4% (98/2916)	4.6% (82/1777)	6.6% (45/681)
Connective tissue disease	0.5% (16/3502)	0.5% (16/2917)	0.3% (5/1776)	0.6% (4/682)
Peptic ulcer disease	1.5% (53/3497)	1.3% (38/2910)	1.6% (28/1772)	2.8% (19/681)
Liver disease	2.0% (69/3503)	1.9% (56/2918)	2.4% (42/1779)	2.7% (19/684)
Diabetes	15.4% (542/3528)	13.8% (403/2927)	18.1% (326/1797)	21.6% (151/698)
Diabetes with complications	7.9% (277/3509)	6.8% (198/2919)	10.1% (181/1784)	11.2% (77/684)
Hemiplegia or paraplegia	1.9% (68/3504)	1.6% (48/2917)	2.4% (42/1775)	2.6% (18/684)
Renal disease	14.9% (524/3519)	13.7% (401/2925)	17.7% (316/1786)	18.9% (130/689)
Cancer (solid tumor)	2.4% (84/3499)	2.3% (66/2914)	2.7% (48/1775)	1.9% (13/681)
Cancer (leukemia)	7.9% (278/3499)	7.9% (229/2916)	9.6% (171/1775)	8.3% (57/685)
Cancer (lymphoma)	1.6% (55/3498)	1.6% (47/2913)	2.0% (36/1776)	2.0% (14/683)
Metastatic cancer	1.0% (36/3500)	1.0% (29/2914)	1.5% (27/1778)	1.3% (9/684)
Severe liver disease	0.9% (31/3501)	1.0% (25/2916)	0.6% (11/1777)	0.9% (6/683)
HIV disease	Censored*	Censored*	Censored*	Censored*

Table 1 (continued)

b. Influence of risk factors on all-cause mortality and entry into critical (CR)-phase in all COVID-19 patients included in the analysis (see Methods section for inclusion criteria)

$n=3627$	All-cause mortality (death from any cause incl. COVID-19) $n=508$			Entry into CR-phase $n=731$		
	n (%)	Univariate	Multivariate OR [CI]	*n* (%)	Univariate	Multivariate OR [CI]
Gender						
M ($n=2107$, 58%)	336 (16%)		Ref	537 (25%)		Ref
F ($n=1520$, 42%)	172 (11%)		0.6 [0.5–0.7]	194 (13%)		0.43 [0.36–0.52]
NA ($n=0$)						
p value		< 0.001	< 0.001		< 0.001	< 0.001
Age						
18–55 ($n=1257$, 35%)	38 (3%)		Ref	178 (14%)		Ref
56–75 ($n=1302$, 36%)	177 (14%)		4.2 [2.9–6.1]	348 (27%)		2.2 [1.8–2.7]
> 75 ($n=1068$, 29%)	293 (27%)		9.0 [6.3–13.0]	205 (19%)		1.6 [1.2–1.9]
NA ($n=0$)						
p value		< 0.001	< 0.001		< 0.001	< 0.001
Charlson-Comorbidty-Index						
0–2 ($n=2826$, 78%)	267 (9%)		Ref	547 (19%)		Ref
3–4 ($n=471$, 13%)	137 (29%)		2.3 [1.8–3.0]	108 (23%)		
> 4 ($n=330$, 9%)	104 (32%)		2.5 [1.9–3.3]	76 (23%)		
NA ($n=0$)						
p value		< 0.001	< 0.001		0.077	*n*.s
Baseline-qSOFA						
0–1 ($n=1590$, 92%)	206 (13%)		Too many missing values	292 (18% CI 16.5–20.4)		Too many missing values
2–3 ($n=132$, 8%)	27 (20%)			36 (27% CI 19.9–35.7)		
NA ($n=1905$)						
p value		0.023			0.015	
Body-Mass-Index						
< 30 ($n=1574$, 69%)	203 (13%)		Too many missing values	315 (20%)		Too many missing values
> = 30 ($n=719$, 31%)	96 (13%)			198 (28%)		
NA ($n=1334$)						
p value		0.79			< 0.001	

m male, *f* female, age in years, *NA* missing values, *p value p* value for the univariate or multivariate analysis; for the multivariate logistic regression analysis the influence variables gender, age, CCI were included (after backward selection for $p > 0.05$), *n.s.* not significant in multivariate analysis, *CI* 95% confidence interval, *OR* odds ratio, *Ref* reference category, *qSOFA* quick Sequential Organ Failure Assessment

*Censored by LEOSS anonymization pipeline. Read more in the article "Design and evaluation of a data anonymization pipeline to promote Open Science on COVID-19" by Jakob et. al.

significance using the Fisher exact test. The multivariate analyses used a logistic regression model. Our model selection used backward selection with a cut-off value of 0.05 for the *p* value. For this purpose, we compared the model with and without the influencing variable under consideration with the *anova* command. We calculated the odds ratios with the 95% confidence interval for the significant influence variables in the multivariate analysis. All analyses used a two-sided significance level of $p=0.05$. The statistics program R, version 4.0.3. [29], was used for all analyses.

Results

Overview

Of all patients documented in the LEOSS registry at the time of our analysis $n=6457$ data on antibiotic use in at least one

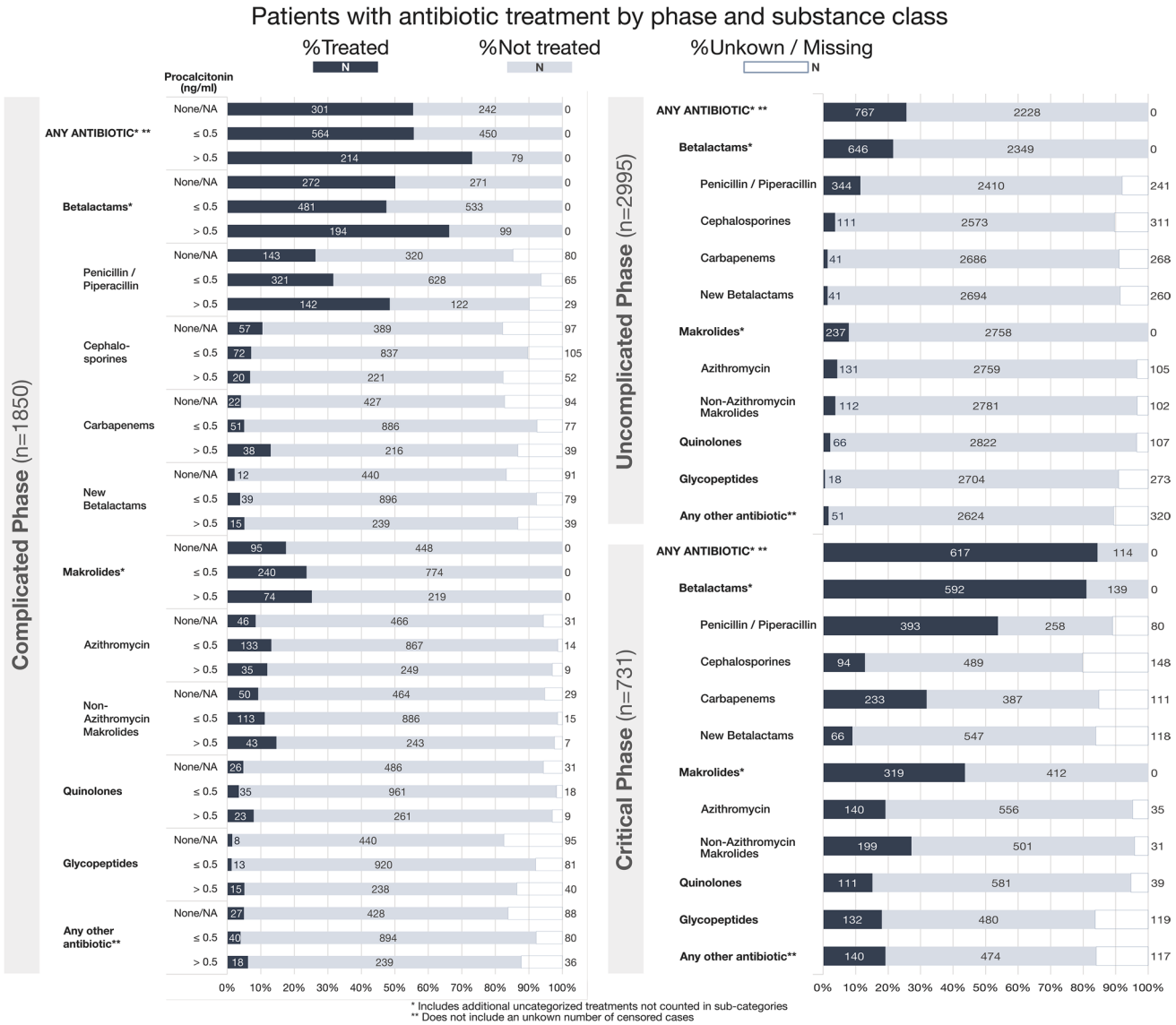

Fig. 3 Illustration of antibiotic treatment by clinical phase and antibiotic class. Complicated phase patients are additionally stratified by procalcitonin. Relative percentages are indicated visually by the length of the boxes. Absolute numbers are printed into or next to the respective box. Some antibiotic groups include additional cases not counted in the subgroups due to the documentation process (e.g., "Betalactams", includes "Penicillin/ Piperacillin", "Cephalosporins", "Carbapenems". "New betalactams" and Betalactams where the exact type is not specified). A patient can both show up in multiple phases (disease progression) and in multiple antibiotic classes (e.g., multiple antibiotic classes used for treatment), but will be counted once only for a given antibiotic class in a phase (e.g., multiple Betalactam treatments of the same subgroup will be counted as one)

clinical phase were available in 4189 cases. Five cases had to be removed due to lack of information on survival and another 31 cases due to censored variables, 48 cases due to age < 18 years, 14 cases due to missing data on clinical stages, and 464 cases due to an observation period < 72 h. The results below refer to the remaining 3627 cases (see Fig. 1). Of those 3627 patients, 1024 had missing information on PCT across all phases. Table 1 a summarizes the patient characteristics of the cohort. In Fig. 3, relative and absolute ABT is illustrated for both clinical stage and antibiotic class—for CO-phase patients additionally broken down by PCT levels. In the UC-phase 25.6% (767/2995) received any ABT, 58.3% (1079/1850) and 84.4% (617/731) in the CO-phase and CR-phase, respectively.

Of the 3627 patients included, 508 (14.0%) died. Seven hundred thirty-one (20.2%) reached the CR-phase. In the multivariate analysis of the total study population, male gender (female: OR 0.6 [0.5–0.7], $p < 0.001$; reference male), advanced age (age 56–75: OR 4.2 [2.9–6.1]; age > 75: OR 9.0 [6.3–13.0], $p < 0.001$; reference age 18–55) and a high CCI (CCI 3–4: OR 2.3 [1.8–3.0];

CCI > 4: OR 2.5 [1.9–3.3], $p < 0.001$; reference CCI 0–2) were significantly associated with a higher all-cause mortality (see Table 1 b). In particular, age > 75 compared to the reference group (18–55 years) showed significantly increased death rates. For entering the CR-phase, cases with male gender (female: OR 0.43 [0.36–0.52], $p < 0.001$; reference male) and higher age (age 56–75: OR 2.2 [1.8–2.7]; age > 75: OR 1.6 [1.2–1.9], $p < 0.001$; reference age 18–55) showed a significantly increased probability. The CCI did not correlate with a higher chance of entering the CR-phase. In the univariate analysis, a baseline qSOFA of > 1 also showed a significantly higher risk of death ($p = 0.023$) but no significant association for entering the CR-phase. A BMI of > 30, on the other hand, was a significant risk factor only for entry into the CR-phase ($p < 0.001$) and not for higher all-cause mortality. With too many missing values, we did not include BMI and qSOFA in the multivariate analyses.

Antibiotic treatment in the clinical phases stratified by PCT levels

Primary endpoint: antibiotic therapy in CO-phase patients with PCT levels ≤ 0.5 ng/ml

60.4% (602/996) of CO-phase PCT↓ patients received ABT, 39.6% (394/996) did not. In the multivariate analysis, ABT had no significant association with all-cause mortality or entry into the next more advanced stage (CR-phase) ($p > 0.05$) when adjusting for the possible confounders gender, age, and CCI (see Table 2). Female gender was significantly associated with lower all-cause mortality (OR 0.7 [0.4–1.0], $p = 0.039$; reference male) and less frequent entry into the CR-phase (OR 0.5 [0.4–0.7], $p < 0.001$, reference male). Higher age showed a significant association with a strong increase in all-cause mortality (age 56–75: OR 4.6 [1.8–12.1]; age > 75: OR 13.0 [5.1–33.4], $p < 0.001$; reference age 18–55); the same applies to an increased CCI (CCI 3–4: OR 2.5 [1.6–3.9]; CCI > 4: OR 2.7 [1.5–4.6], $p < 0.001$;

Table 2 Influence of risk factors on all-cause mortality and progression into the next more advanced stage (critical [CR]-phase) in COVID-19 patients in the complicated (CO)-phase with procalcitonin levels ≤ 0.5 ng/ml (PCT↓)

$n = 996$	All-cause mortality (death from any cause incl. COVID-19) $n = 138$			Progression into next more advanced stage (CR-phase) $n = 185$		
	n (%)	Univariate	Multivariate OR [CI]	*n* (%)	Univariate	Multivariate OR [CI]
Antibiotic treatment						
Yes ($n = 602$, 60%)	99 (16%)		Ref	121 (20%)		Ref
No ($n = 394$, 40%)	39 (10%)			64 (16%)		
NA ($n = 0$)						
p value		0.0036	n.s		0.13	n.s
Gender						
M ($n = 588$, 59%)	85 (14%)		Ref	131 (22%)		Ref
F ($n = 408$, 41%)	53 (13%)		0.7 [0.4–1.0]	54 (13%)		0.5 [0.4–0.7]
NA ($n = 0$)						
p value		0.58	0.039		< 0.001	< 0.001
Age						
18–55 ($n = 259$, 26%)	5 (2%)		Ref	45 (17%)		Ref
56–75 ($n = 373$, 37%)	37 (10%)		4.6 [1.8–12.1]	78 (21%)		
> 75 ($n = 364$, 37%)	96 (26%)		13.0 [5.1–33.4]	62 (17%)		
NA ($n = 0$)						
p value		< 0.001	0.001		0.35	n.s
Charlson Comorbidity Index						
0–2 ($n = 768$, 77%)	69 (9%)		Ref	133 (17%)		Ref
3–4 ($n = 144$, 14%)	42 (29%)		2.5 [1.6–3.9]	34 (24%)		
> 4 ($n = 84$, 8%)	27 (32%)		2.7 [1.5–4.6]	18 (21%)		
NA ($n = 0$)						
P value		< 0.001	< 0.001		0.15	n.s

m male, *f* female, age in years, *NA* missing values, *p value p* value for the univariate or multivariate analysis; for the multivariate logistic regression analysis the influence variables gender, age, CCI were included (after backward selection for $p > 0.05$), *n.s.* not significant in the multivariate analysis, *CI* 95% confidence interval, *OR* odds ratio, *Ref* reference category

reference CCI 0–2). Age and CCI were not associated with an increased probability of entering the CR-phase ($p > 0.05$).

65% of administered antibiotics in this patient population were Betalactam antibiotics; approximately a quarter of the cases received Macrolides; Quinolones were used in 4% of the cases (data not shown). The missingness analysis of patients in the CO-phase showed significant differences between patients with and without missing data of ABT and PCT with respect to progression into the CR-phase ($p < 0.001$) but not for age, gender, ABT, CCI strata, BMI, and baseline qSOFA ($p > 0.05$, see Table 6 in the Appendix).

Secondary analyses: Antibiotic therapy in other clinical constellations

In the UC-phase PCT↓ subgroup, 38.1% (399/1045) of patients received ABT, and 61.9% (646/1045) did not. Here, age and CCI (all $p < 0.001$), but not ABT and gender (both $p > 0.05$), were significantly associated with increased all-cause mortality in the multivariate analysis (see Table 3 in the Appendix). The entry of this subgroup into the CO-phase was significantly associated with ABT and age (both $p < 0.001$), but not with an increased CCI ($p > 0.05$).

In the UC-phase PCT↑, 69.3% (104/150) of patients received systemic antibiotic therapy, and 30.7% (46/150) did not (see Table 4 in the Appendix). All-cause mortality and entry into the CO-phase were increased for age ($p < 0.001$, $p = 0.020$, respectively). Analyses for gender, ABT, and CCI strata yielded no significant associations for either all-cause mortality or entry in the CO-phase ($p > 0.05$).

In the CO-phase PCT↑ subgroup, antibiotics were prescribed in 85.3% (244/286) of patients and not prescribed in 14.7% (42/286) (see Table 5 in the Appendix). Patients with ABT or age > 55 years had a significantly increased risk of death from any cause ($p = 0.029$, $p < 0.001$, respectively). Male gender was the only parameter that showed a statistically significant difference in this subgroup for entry into the CR-phase ($p = 0.0034$).

Discussion

In this cohort of SARS-CoV-2-infected patients with documented information on ABT, established risk factors such as male gender, patient age > 55 years, and CCI ≥ 3 were significantly associated with all-cause mortality. Similar results have been reported before [18–24]. For the primary endpoint, CO-phase patients with PCT↓, no significant correlation between antibiotic treatment and all-cause mortality or progression to the critical phase was seen.

This study's additional subgroup analyses found similar results, in line with WHO's recommendations [10]. For neither the primary nor the secondary endpoints a significant benefit of ABT could be demonstrated. CO-phase PCT↑ patients with ABT had increased all-cause mortality, UC-phase PCT↓ patients with ABT had a higher likelihood to enter the complicated phase. For both, we highly suspect a worsening clinical course to trigger ABT, with the former being the driving factor for increased mortality/progression and the latter being an intervention of uncertain benefit or harm. The clinical state of the patient probably is a classical confounder. Unfortunately, in our cohort we do not have a clinical severity score (e.g., SOFA) available.

Surprisingly, the CCI was no significant risk factor for progression into a more advanced phase in any of the analyses, but was associated with all-cause mortality in both UC- and CO-phase patients with PCT↓. Palliative care concepts for multimorbid patients could be a possible explanatory hypothesis here. Difficulties with this outcome are also reflected by a significant difference between patients in the missingness analysis for the complicated phase.

SARS-CoV-2 infections are frequently co-treated with antibiotics in the LEOSS cohort, regardless of the respective phase. International publications report similarly high rates of antibiotic prescriptions [30, 31]. The antibiotics administered mainly matched the empirical antibiotics recommended in guidelines for community-acquired or nosocomial pneumonia [32, 33]. The proportion of antibiotics with *Pseudomonas*- or *Methicillin-resistant Staphylococcus aureus* (MRSA) activity was comparatively low compared to a study from South Korea [34].

Strength of this study

To the best of our knowledge, our analysis is the largest evaluation of antibiotic therapy effects on mortality and disease progression in a German SARS-CoV-2-infected population. Data collection took place at > 100 recruiting sites with an intersectoral recruitment approach across university hospitals, non-university hospitals, and primary care practices. Anonymous recruitment allowed for broad inclusion of patients reducing selection bias [35]. The study population's characteristics seem to be representative of German [18, 36] and international cohorts of hospitalized COVID-19 patients [19–24, 30]. The study population includes cases from the first and second waves of the COVID-19 pandemic in Germany. Our analysis includes established risk factors and is stratified by typical PCT thresholds for lower respiratory infections [16, 17]. We stratified patients according to their clinical phase to obtain more robust results.

Limitations

As a retrospective, non-randomized analysis, some limitations need to be considered when assessing our results. The analyzed patient population did not include pregnant women and individuals < 18 years. We excluded pediatric cases due to low case numbers and the broad heterogeneity of this patient collective ranging from neonate to young adult. Our data did not provide reasons for the initiation of ABT, and high-quality superinfection data was not available. Our analyses thus assume

the administration of antibiotics in the context of COVID-19 (co-)therapy and suspected bacterial superinfection. However, reasons for antibiotic therapy could often be independent of a SARS-CoV-2 infection, e.g., typical infections such as urinary tract infections or catheter-associated infections [37, 38]. The LEOSS cohort potentially contains numerous patients who were not primarily hospitalized because of COVID-19 but instead had a SARS-CoV-2 infection as a secondary diagnosis (e.g., asymptomatic coinfection or nosocomial infection).

LEOSS' study design introduces further limitations. First, LEOSS has no dedicated review process of the data beyond automated plausibility checks and queries for implausible cases. Second, there is no follow-up after the acute course. Hence, we could not include higher re-hospitalization rates or post-discharge effects in our analyses and endpoints are limited to the end of the acute infection (e.g., until discharge or recovery). Third, the analysis could not include essential information about repetition, course, period, and dosage of antibiotic therapies or microbiological or radiological diagnostics and the relationship between events within a phase due to anonymous data acquisition. For example, early discontinuation of an antibiotic prescription that low PCT levels might trigger cannot be observed in our dataset and thus is not accounted for in the analyses.

Finally, although our analysis considers many covariates, additional risk factors are described in the literature, e.g., socioeconomic or genetic factors [39, 40], that were not taken into account. These variables were either not present or insufficiently documented as for the BMI or SOFA scores. Given that the clinical presentation is probably the essential factor for physicians' initial assessment for or against ABT, the lack of a marker for clinical presentation, such as the SOFA, is probably the most substantial limitation of our analysis.

Conclusion

In summary, the data and analyses of ABT in SARS-CoV-2-infected patients presented here do not demonstrate a correlation of ABT with lower all-cause mortality or protection from progression to the next more advanced phase of disease for uncomplicated or complicated patients irrespective of PCT levels. The limitations of our available cohort data demand further comprehensive studies, such as the German National Pandemic Cohort Network (NAPKON). Antibiotic-resistant bacteria are another severe and global pandemic and many authors already called for conscious ABS activities in times of COVID-19 [1, 4, 34, 41, 42]. The involvement of local ABS-teams or ABS-commissioned physicians in the decision process for or against antibiotic therapy in COVID-19 patients, in addition to educational campaigns focused on rational use of antibiotics, remains of crucial importance [41].

Appendix

Table 3 Influence of risk factors on all-cause mortality and progression into the next more advanced stage (complicated [CO]-phase) in COVID-19 patients in the uncomplicated (UC)-phase with procalcitonin levels ≤ 0.5 ng/ml (PCT↓)

$n = 1045$	All-cause mortality (death from any cause incl. COVID-19) $n = 50$	Progression into next more advanced stage (CO-phase) $n = 287$
Antibiotic treatment		
Yes ($n = 399$, 38%)	25 (6% CI 4.1–9.1)	136 (34% CI 29.4–39.0)
No ($n = 646$, 62%)	25 (4% CI 2.5–5.7)	151 (23% CI 20.2–26.8)
p (p-mult)	0.10 (*n.s.*)	< 0.001 (< 0.001)
Gender		
M ($n = 565$, 54%)	28 (5% CI 3.3–7.1)	158 (28% CI 24.3–31.9)
F ($n = 480$, 46%)	22 (5% CI 2.9–6.9)	129 (27% CI 23.0–31.1)
p (p-mult)	0.88 (n.s.)	0.73 (n.s.)
Age		
18–55 ($n = 412$, 39%)	1 (0.2% CI 0.006–1.3)	70 (17% CI 13.5–21.0)
56–75 ($n = 361$, 35%)	15 (4% CI 2.3–6.8)	104 (29% CI 24.2–33.8)
> 75 ($n = 272$, 26%)	34 (13% CI 8.8–17.0)	113 (42% CI 35.6–47.7)
p (p-mult)	< 0.001 (< 0.001)	< 0.001 (< 0.001)
Charlson Comorbidity Index		
0–2 ($n = 851$, 81%)	20 (2% CI 1.4–3.6)	213 (25% CI 22.2–28.1)
3–4 ($n = 107$, 10%)	17 (16% CI 9.5–24.2)	47 (44% CI 34.3–53.9)
> 4 ($n = 87$, 8%)	13 (15% CI 8.2–24.2)	27 (31% CI 21.5–41.9)
p (p-mult)	< 0.001 (< 0.001)	< 0.001 (n.s.)

m male, *f* female, age in years, *p* *p* value of the univariate analysis, *p-mult* *p* value for the multivariate logistic regression analysis with the multiple influence variables antibiotics, gender, age, CCI (after backward selection for $p > 0.05$), *n.s.* not significant in the multivariate analysis, *CI* 95% confidence interval

Table 4 Influence of risk factors on all-cause mortality and progression into the next more advanced stage (complicated [CO]-phase) in COVID-19 patients in the uncomplicated (UC)-phase with procalcitonin levels > 0.5 ng/ml (PCT↑)

$n = 150$	All-cause mortality (death from any cause incl. COVID-19) $n = 25$	Progression into next more advanced stage (CO-phase) $n = 55$
Antibiotic treatment		
Yes ($n = 104$, 69%)	20 (19% CI 12.2–28.1)	42 (40% CI 30.9–50.5)
No ($n = 46$, 31%)	5 (11% CI 3.6–23.6)	13 (28% CI 16.0–43.5)
p (p-mult)	0.24 (n.s.)	0.20 (n.s.)
Gender		
M ($n = 95$, 63%)	18 (19% CI 11.6–28.3)	37 (39% CI 29.1–49.5)
F ($n = 55$, 37%)	7 (13% CI 5.3–24.5)	18 (33% CI 20.7–46.7)
p (p-mult)	0.37 (n.s.)	0.49 (n.s.)
Age		
18–55 ($n = 52$, 35%)	3 (6% CI 1.2–15.9)	12 (23% CI 12.5–36.8)
56–75 ($n = 61$, 41%)	7 (11% CI 4.7–22.2)	24 (39% CI 27.1–52.7)
> 75 ($n = 37$, 25%)	15 (41% CI 24.8–57.9)	19 (51% CI 34.4–68.1)
p (p-mult)	< 0.001 (< 0.001)	0.020 (0.020)
Charlson Comorbidity Index		
0–2 ($n = 94$, 63%)	12 (13% CI 6.8–21.2)	34 (36% CI 26.5–46.7)
3–4 ($n = 30$, 20%)	6 (20% CI 7.7–28.6)	12 (40% CI 22.7–59.4)
> 4 ($n = 26$, 17%)	7 (27% CI 11.6–47.8)	9 (35% CI 17.2–55.7)
p (p-mult)	0.19 (n.s.)	0.91 (n.s.)

m male, *f* female, age in years, *p p* value of the univariate analysis, *p-mult p* value for the multivariate logistic regression analysis with the multiple influence variables antibiotics, gender, age, CCI (after backward selection for $p > 0.05$), *n.s.* not significant in the multivariate analysis, *CI* 95% confidence interval

Table 5 Influence of risk factors on all-cause mortality and progression into the next more advanced stage (critical [CR]-phase) in COVID-19 patients in the complicated (CO)-phase with procalcitonin levels > 0.5 ng/ml (PCT↑)

$n = 286$	All-cause mortality (death from any cause incl. COVID-19) $n = 109$	Progression into next more advanced stage (CR-phase) $n = 83$
Antibiotic treatment		
Yes ($n = 244$, 85%)	98 (40% CI 34.0–46.7)	70 (29% CI 23.1–34.8)
No ($n = 42$, 15%)	11 (26% CI 13.9–42.0)	13 (31% CI 17.6–47.1)
p (p-mult)	0.089 (0.029)	0.85 (n.s.)
Gender		
M ($n = 195$, 68%)	75 (38% CI 31.6–46.1)	67 (34% CI 27.7–41.5)
F ($n = 91$, 32%)	34 (37% CI 27.4–48.1)	16 (18% CI 10.4–27.0)
p (p-mult)	0.90 (n.s.)	0.0034 (0.0034)
Age		
18–55 ($n = 50$, 17%)	7 (14% CI 5.8–26.7)	17 (34% CI 21.2–48.8)
56–75 ($n = 104$, 36%)	26 (25% CI 17.0–34.4)	35 (34% CI 24.7–43.6)
> 75 ($n = 132$, 46%)	76 (58% CI 48.7–66.1)	31 (23% CI 16.5–31.6)
p (p-mult)	< 0.001 (< 0.001)	0.16 (n.s.)
Charlson Comorbidity Score		
0–2 ($n = 166$, 58%)	49 (30% CI 22.7–37.1)	55 (33% CI 26.0–40.8)
3–4 ($n = 65$, 23%)	31 (48% CI 35.1–60.5)	14 (22% CI 12.3–33.5)
> 4 ($n = 55$, 19%)	29 (53% CI 38.8–66.3)	14 (25% CI 14.7–39.0)
p (p-mult)	0.0017 (n.s.)	0.19 (n.s.)

m male, *f* female, age given in years, *p value p* value of the univariate analysis, *p-mult p* value for the multivariate logistic regression analysis with the multiple influence variables antibiotics, gender, age, CCI (after backward selection for $p > 0.05$), *n.s.* not significant in the multivariate analysis, *CI* 95% confidence interval

Table 6 Missing analysis for patients in the complicated (CO)-phase comparing complete and incomplete information on antibiotic treatment (ABT) and procalcitonin (PCT)

	ABT and PCT in CO available $n=1282$	ABT or PCT in CO not available $n=703$	p value comparison complete vs. missing data
Death from any cause yes / no (% death)	247/1035 (19% CI 17.1–21.5)	138/565 (20% CI 16.8–22.8)	0.86
Entry into CR-phase yes / no (% entry)	268/1014 (21% CI 18.7–23.2)	200/503 (28% CI 25.1–31.9)	< 0.001
Gender: m/f (% male)	783/499 (61% CI 58.3–63.8)	426/277 (61% CI 56.9–64.2)	0.85
Age groups (%)	18–55: 309 (24% CI 21.8–26.5) 56–75: 477 (37% CI 34.6–39.9) > 75: 496 (39% CI 36.0–41.4)	18–55: 176 (25% CI 21.9–28.4) 56–75: 287 (41% CI 37.2–44.6) > 75: 240 (34% CI 30.6–37.8)	0.12
CCI groups (%)	0–2: 934 (73% CI 70.3–75.3) 3–4: 209 (16% CI 14.3–18.4) > 4: 139 (11% CI 9.2–12.7)	0–2: 515 (73% CI 69.8–76.5) 3–4: 116 (17% CI 13.8–19.5) > 4: 72 (10% CI 8.1–12.7)	0.92
qSOFA Baseline (%)	0–1: 606 (91% CI 88.3–92.8) 2–3: 62 (9% CI 7.2–11.7) NA: 614	0–1: 308 (91% CI 87.3–93.7) 2–3: 31 (9% CI 6.3–12.7) NA: 364	0.25
BMI Baseline (%)	< 30: 523 (64% CI 61.0–67.7) > = 30: 289 (36% CI 32.3–39.0) NA: 470	< 30: 280 (66% CI 61.2–70.4) > = 30: 145 (34% CI 29.6–38.8) NA: 278	0.40

m male, *f* female, age in years, *NA* missing values, *CI* 95% -confidence interval

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Availability of data and material A scientific use file (SUF) can be requested from the LEOSS analysis team via www.leoss.net.

Code availability Upon reasonable request from corresponding author.

Declarations

Conflict of interest The authors do not disclose any conflicts of interest.

Ethical approval LEOSS has received ethics approval at all participating study sites.

Consent to participate Patient consent was waived as the study was based on a Scientific Use File (SUF) generated from the Lean European Open Survey on SARS-CoV-2-Infected Patients (LEOSS) registry.

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