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Individualisierte Konzepte der Impfprävention und antiviralen Therapie bei Infektionen mit SARS-CoV-2

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Subgruppenanalysen in Risikopopulationen

Habilitationsschrift
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Il faut avoir l'esprit doux et le cœur doux.

Jacques Maritain

Die folgenden Originalarbeiten sind Grundlage der vorliegenden kumulativen Habilitationsschrift zum Thema „Individualisierte Konzepte der Impfprävention und antiviralen Therapie bei Infektionen mit SARS-CoV-2 – Subgruppenanalysen in Hochrisikopopulationen“:

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Inhaltsverzeichnis

1 Einleitung

1.1 Eine gesundheitspolitische Einordnung der SARS-CoV-2-Pandemie

Aufgrund der rapiden weltweiten Ausbreitung von Infektionen mit dem *severe acute respiratory syndrome coronarvirus type 2* (SARS-CoV-2), ursächlicher Erreger von *coronavirus disease 2019* (COVID-19), rief der Generaldirektor der *World Health Organization* (WHO) am 20. März 2020 die SARS-CoV-2-Pandemie aus[1]. Die Pandemie wirkte sich weltweit auf das Gesundheitswesen, die Gesellschaft, die Wirtschaft und das tägliche Leben der Menschen aus und stellte eine der ersten großen Herausforderungen des 21. Jahrhunderts dar. Bis heute (Stand: 29.03.2025) wurden mehr als 777,5 Millionen Fälle von COVID-19 und über sieben Millionen assoziierte Todesfälle von der WHO registriert[2]. Zwischen 2019 und 2021 kam es pandemiebedingt zu einer Verringerung der weltweiten Lebenserwartung von anderthalb Jahren[3].

Regierungen und Gesundheitssysteme wurden vor enorme Herausforderungen gestellt. Die pandemische Ausbreitung des Virus erforderte schnelle gesundheitspolitische Entscheidungen, um Infektionsketten zu durchbrechen, die Gesundheitsversorgung sicherzustellen und die negativen Auswirkungen auf die Bevölkerung zu minimieren. Lockdowns, *social distancing*, Maskenpflicht und andere Maßnahmen wurden in vielen Ländern eingeführt, um die Verbreitung des Virus zu verlangsamen. Medizinische Ressourcen wurden für die Versorgung von PatientInnen mit COVID-19 priorisiert genutzt, was zu Einschränkungen bei der Verfügbarkeit von diagnostischen Verfahren und therapeutischen Maßnahmen für andere Krankheiten führte. Infektiologische Sicherheitsbedenken bewirkten, dass elektive Untersuchungen und Eingriffe verschoben wurden. Dies hatte aufgrund von Diagnose- und Behandlungsverzögerungen für PatientInnen mit chronischen und/oder insbesondere hämato-logischen/onkologischen Erkrankungen weitreichende Folgen[4]. Das Spannungsfeld zwischen dem Schutz vulnerabler Gruppen und dem Risiko einer daraus resultierenden medizinischen Unterversorgung anderer Patientengruppen spiegelt die Komplexität der Pandemiebewältigung für das Gesundheitswesen wider.

Die Pandemie führte weltweit aber auch zu einer beispiellosen Kooperation in der Wissenschaft und im Gesundheitswesen zur Erforschung und Bekämpfung von COVID-19. Im Dezember 2019 fielen 41 Fälle einer neuartigen Pneumonie in der chinesischen Stadt Wuhan auf[5]. Retrospektiv markierte dieser Ausbruch den Beginn der SARS-CoV-2-Pandemie, die Anfang

2020 Deutschland erreichen sollte. Für Europa erfolgte die Empfehlung zur Zulassung eines ersten antiviralen Therapeutikums gegen SARS-CoV-2 bereits im Juni sowie eine Empfehlung zur Zulassung eines mRNA-basierten Impfstoffs im Dezember des Jahres 2020[6,7].

Anhaltende Herausforderungen für Wissenschaft und Gesundheitswesen stellen die virale Evolution mit dem Aufkommen neuer Virusvarianten und das Post COVID-19-Syndrom, also die klinischen Langzeitfolgen von Infektionen mit SARS-CoV-2, dar. Über das komplexe Krankheitsbild tauschten sich betroffene PatientInnen pandemiebedingt zunächst über soziale Netzwerke aus[8], bevor die WHO auf die Problematik im Sinne eines postinfektiösen/-viralen Syndroms erstmals im August 2020 hinwies[9,10].

1.2 Klinischer und virologischer Verlauf von COVID-19

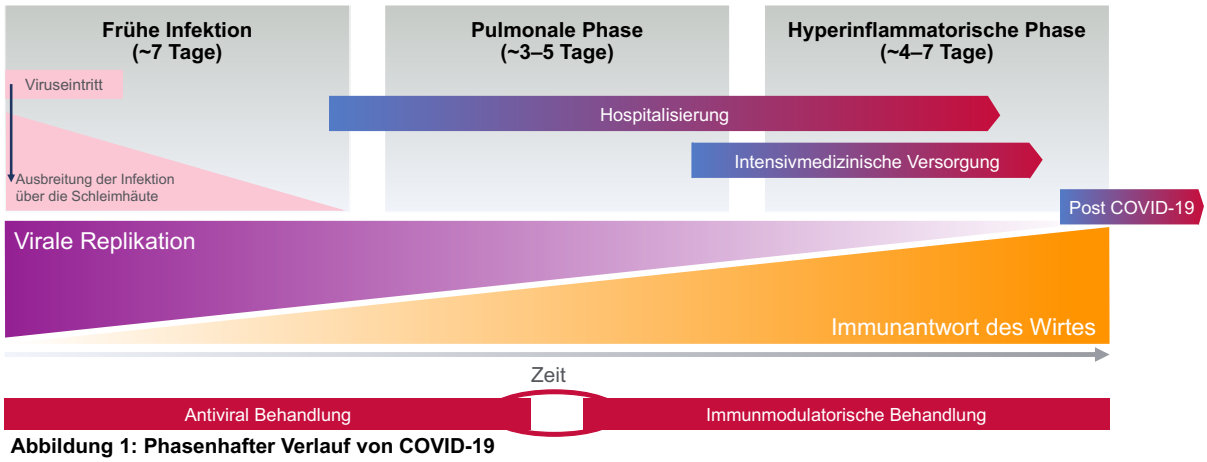

Abbildung 1: Phasenhafter Verlauf von COVID-19

Modifiziert nach Siddiqi, H.K., and Mehra, M.R. (2020). COVID-19 illness in native and immunosuppressed states: A clinical–therapeutic staging proposal. The Journal of Heart and Lung Transplantation *39*, 405–407. 10.1016/j.healun.2020.03.012.[11]

Der klassische klinische Verlauf von COVID-19 ist phasenhaft (vgl. Abbildung 1). Zu Beginn steht die virale Replikation und Ausbreitung von SARS-CoV-2 im Körper im Vordergrund (Frühe Infektion). SARS-CoV-2 kann mit Hilfe des *spike* Proteins über die ACE-Rezeptoren (ACE: *angiotensin converting enzyme 2*), die ubiquitär im Körper vorhanden sind, in Zellen eindringen. Dies führt zur Beteiligung verschiedener Organsysteme, was die Symptomvielfalt von COVID-19 erklärt. Da die Lunge eine besonders hohe Dichte an ACE-Rezeptoren aufweist, ist diese besonders exponiert, was sich klinisch als Pneumonie manifestieren kann (Pulmonale Phase). Bei einer überschießenden Immunantwort des Wirtes kann es nachfolgend zu einer überschießenden Inflammation mit kritischer Morbidität kommen (Hyperinflammatorische Phase). Entsprechend steht therapeutisch zunächst eine antivirale und im Verlauf der Infektion eher eine immunmodulatorische Therapie im Vordergrund. Nicht alle PatientInnen durchlaufen

jede Phase und die klinische Entwicklung kann von milden Symptomen bis zum Tod variieren[11]. Beschwerden, die mit einer SARS-CoV-2-Infektion in Verbindung gebracht werden, dabei länger als drei Monate persistieren und nicht durch eine andere Ursache erklärbar sind, werden dem Post COVID-19-Syndrom subsummiert[12]. Es fasst einen heterogenen Symptomkomplex multifaktorieller Ätiologie zusammen, wobei ein persistierendes SARS-CoV-2-Reservoir, Reaktivierungen latenter Herpesviren, autoimmune Prozesse sowie Gewebeschäden und -dysfunktionen durch Hypoxämie und/oder Mikrothromben im Vordergrund stehen[13,14]. Eine interdisziplinäre Behandlung der betroffenen PatientInnen ist somit notwendig.

Tabelle 1: *Ordinal Clinical Severity Scale/* WHO-Ordinalskala

Patientenstatus	**Deskriptoren**	**Score**
Uninfiziert	Kein klinischer oder virologischer Nachweis einer Infektion	0
Ambulant	Keine Aktivitätseinschränkungen	1
	Aktivitätseinschränkungen	2
Hospitalisiert	Keine Sauerstofftherapie	3
	Sauerstoff per Maske oder Nasensbrille	4
	Nicht-invasive Beatmung oder *high-flow* Sauerstofftherapie	5
	Intubation und invasive Beatmung	6
	Invasive Beatmung und zusätzliche Organunterstützung einschließlich ECMO	7
Tot	Tod	8

Modifiziert nach Rubio-Rivas, M., Mora-Luján, J.M., Formiga, F., Arévalo-Cañas, C., Lebrón Ramos, J.M., Villalba García, M.V., Fonseca Aizpuru, E.M., Díez-Manglano, J., Arnalich Fernández, F., Romero Cabrera, J.L., et al. (2022). WHO Ordinal Scale and Inflammation Risk Categories in COVID-19. Comparative Study of the Severity Scales. J Gen Intern Med *37*, 1980–1987. 10.1007/s11606-022-07511-7.[15]

Abkürzung: ECMO = *extracorporeal membrane oxygenation (deutsch:* Extrakorporale Membranoxygenierung)

Um den klinischen Krankheitsverlauf von COVID-19 standardisiert beurteilen zu können, hat die WHO die *Ordinal Clinical Severity Scale* (im Weiteren: WHO-Ordinalskala) entwickelt (vgl. Tabelle 1). Diese Skala umfasst neun Stufen, die den Schweregrad der Erkrankung anhand des klinischen Verlaufs und der Inanspruchnahme von Ressourcen des Gesundheitswesens beurteilen, beginnend von keinem Hinweis auf eine Infektion (Score: 0) bis zum Tod (Score: 8). Die WHO-Ordinalskala ermöglicht eine Klassifizierung des Krankheitsverlaufs und hilft dabei, die Behandlung und das Management von PatientInnen mit COVID-19 zu standardisieren. Ein wichtiger Schwellenwert auf der Skala ist der Übergang von einer leichten (Score: 1-3) zu einer mittelschweren Erkrankung (Score: 4), der durch den Beginn einer unterstützenden Sauerstofftherapie definiert ist. Darüber hinaus kennzeichnet der Einsatz mechanischer Beatmung das Fortschreiten zu einer schweren Erkrankung (Score: 5-7)[15].

Neben dem klinischen muss auch der virologische Verlauf von COVID-19 berücksichtigt werden. Bei Nachweis von >10^6 SARS-CoV-2-Kopien/ml mittels Polymerasekettenreaktion (PCR: *polymerase chain reaction*) aus einem nasopharyngealen Abstrich wird allgemeinhin von Infektiosität ausgegangen, da *in vitro* Daten eine Korrelation zwischen einer entsprechend hohen Viruslast und der viralen Kultivierbarkeit sowie der damit einhergehenden Kontagiosität belegt haben[16]. Insbesondere bei PatientInnen mit systemischer Immundefizienz kann es trotz Verbesserung des klinischen Zustandes zu langen Nachweisen infektiologisch formal relevanter Mengen von SARS-CoV-2 kommen mit dem Risiko der Übertragung auf andere PatientInnen und/oder das medizinische Fachpersonal[17–24]. Dabei korreliert die Dauer eines relevanten SARS-CoV-2 Nachweises mittels PCR auch mit der Wahrscheinlichkeit des Auftretens eines Post COVID-19-Syndroms[25].

In der vorliegenden Arbeit ist der Nachweis von >10^6 SARS-CoV-2-Kopien/ml über mindestens 21 Tage mittels PCR als prolongierter virologischer Verlauf definiert[26,27]. Auch wenn nur im Falle einer positiven Viruskultur eine relevante Infektiosität dieser Patientengruppe besteht, sind PatientInnen mit prolongiertem Nachweis von SARS-CoV-2 mittels PCR im täglichen Leben, einschließlich des Zugangs zum medizinischen System, weiterhin eingeschränkt. In der Folge kann die Behandlung anderer therapie- oder kontrollbedürftiger Erkrankungen beeinträchtigt sein[4].

1.3 Risikofaktoren für schwere klinische und/oder prolongierte virologische Verläufe von COVID-19

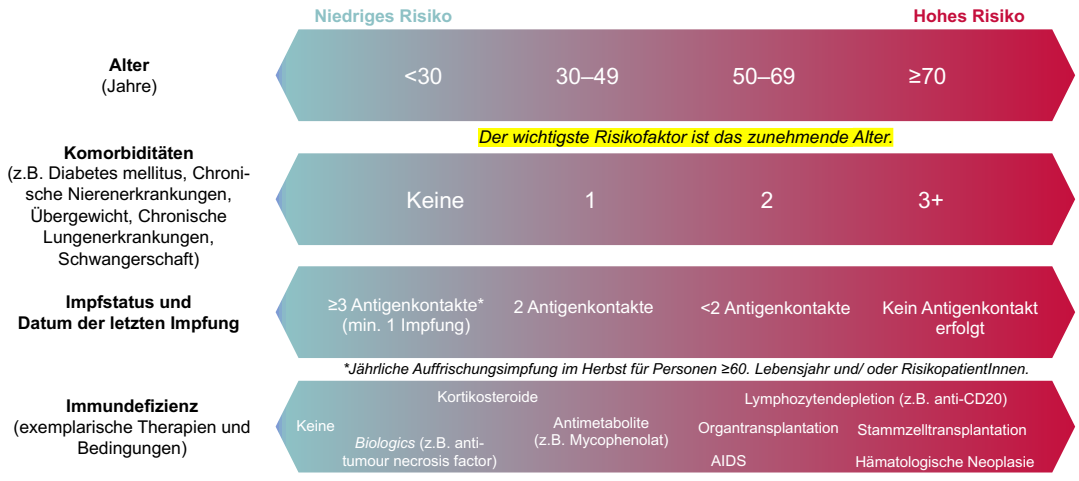

Abbildung 2: COVID-19 Risikokontinuum

Modifiziert nach www.health.state.mn.us/diseases/coronavirus/hcp/conditions.html. (Zugriff Oktober 2023).

Immungeschwächte PatientInnen weisen – trotz aktuell breitem immunologischem Schutz der Gesamtbevölkerung durch SARS-CoV-2-Impfungen und/oder durchgemachte Infektionen – weiterhin ein erhöhtes Risiko für schwere Verläufe von COVID-19 auf (vgl. Abbildung 2). In Folge der Immundefizienz ist die protektive Immunantwort auf Impfungen und/oder durchgemachte Infektionen eingeschränkt[28].

Zunehmendes Alter (≥50. Lebensjahr) stellt den zentralen Risikofaktor für einen schweren klinischen Verlauf von Infektionen mit SARS-CoV-2 dar. Ältere PatientInnen haben aufgrund einer fortschreitenden Immunseneszenz und einer höheren Wahrscheinlichkeit für Komorbiditäten ein weniger effektives Immunsystem[29]. Zu den prädisponierenden Grunderkrankungen zählen arterielle Hypertonie, Diabetes mellitus, Adipositas, interstitielle und chronisch-obstruktive Lungenerkrankungen, Thrombophilien, chronische Nieren- und Lebererkrankungen sowie solide Neoplasien unter Therapie. Im Vergleich zur saisonalen Influenza war das Risiko schwerer Krankheitsverläufe bei PatientInnen mit COVID-19 höher, wobei PatientInnen mit COVID-19 im Vergleich jünger waren und weniger Komorbiditäten aufwiesen als PatientInnen mit Influenza[30,31]. Die einzelnen genannten Erkrankungen haben einen stark divergierenden immunsuppressiven Effekt. Dabei ist nicht nur die Komorbidität *per se*, sondern auch deren kumulative Anzahl im Sinne einer Potenzierung des immunsuppressiven Ausmaßes entscheidend. In der Folge befindet sich das Immunsystem in einem chronisch pro-inflammatorischen Status mit jedoch persistierend niedrig-gradiger Aktivierung des angeborenen Immunsystems[32]. Differenzierte Daten zu einzelnen Subgruppen sind derzeit limitiert und schränken individualisierte Empfehlungsmöglichkeiten ein. Daher werden Daten einzelner Subgruppen auf vergleichbare und/oder übergeordnete Gruppen transferiert. Vornehmlich ältere, multimorbide PatientInnen mit einem hohen Risiko für einen schweren klinischen und einem geringen Risiko für einen prolongierten virologischen Verlauf von COVID-19 haben eine Indikation für eine antivirale Monotherapie im Falle einer Infektion mit SARS-CoV-2. Ein solches Kollektiv wurde in eine Studie aufgenommen, in der antivirale Monotherapien als therapeutische Intervention anhand der WHO-Ordinalskala klinisch evaluiert wurden und die Teil der vorliegenden Habilitationsschrift ist (vgl. Kapitel 1.8.2 und 2.4)[33].

Von einer systemischen Immundefizienz wird in dieser Habilitationsschrift bei PatientInnen mit angeborenen oder erworbenen Immundefekten, mit hämatologischen Neoplasien, mit Autoimmunerkrankungen und/oder nach Transplantation solider Organe oder von Stammzellen mit Indikation einer systemischen immunsuppressiven Therapie einschließlich Lymphozyten-depletierender Therapien ausgegangen. Eine systemische Immundefizienz schränkt Immun-

antworten auf Infektionen und/oder Impfungen so stark ein, dass ein hohes Risiko für einen prolongierten virologischen Verlauf von COVID-19 besteht (vgl. Kapitel 1.2), der neben dem potenziell schweren klinischen Verlauf therapeutisch berücksichtigt werden sollte. Um prolongierten Virusnachweisen entgegenzuwirken, haben wir Kombinationen aus direkt antiviralen Wirkstoffen (DAAs: *direct-acting antivirals*) und/oder neutralisierenden monoklonalen Antikörpern (mABs: *monoclonal antibodies*) als therapeutische Intervention in der Erstlinie im Falle einer Infektion mit SARS-CoV-2 in einem Patientenkollektiv mit einer hohen Prävalenz für die genannten immunsupprimierenden Grunderkrankungen und/oder Therapien in einer weiteren Arbeit betrachtet (vgl. Kapitel 1.8.2 und 2.5)[27].

1.4 COVID-19-Impfempfehlungen der Ständigen Impfkommission (Stand: 23.01.2025)

Im Dezember 2023 hatten laut WHO 67% der Weltbevölkerung zumindest eine vollständige Primärserie eines COVID-19-Impfstoffs und 32% zusätzlich mindestens eine Auffrischungsimpfung erhalten. Im globalen Vergleich waren dabei deutliche regionale Unterschiede zu beobachten[34]. Geschätzt konnten weltweit 19,8 Millionen Todesfälle, die mit COVID-19 assoziiert gewesen wären, durch Impfungen verhindert werden[35].

Eine Basisimmunität gegen SARS-CoV-2 wird nach drei Antigenkontakten durch Impfungen und/oder Infektionen erreicht. Dabei scheint eine hybride Immunität, die durch eine Kombination von natürlicher Immunantwort nach einer Infektion und künstlicher Immunantwort durch Impfung erreicht wird, einer Schutzwirkung durch drei Impfungen ohne Infektionsereignis überlegen zu sein[36–39]. Auch bei alleinigen SARS-CoV-2-Infektionen ohne Impfung wird von einer geringeren Schutzwirkung als durch eine hybride Immunität ausgegangen[40].

Die ExpertInnen der Ständigen Impfkommission (STIKO) in Deutschland empfehlen derzeit allen Personen ab einem Alter von 18 Jahren eine Basisimmunität gegen SARS-CoV-2 zu erlangen, wobei zumindest ein Antigenkontakt durch Impfung erfolgen sollte. Für Patientengruppen mit hohem Risiko für schwere klinische und/oder prolongierte virologische Verläufe von COVID-19 (vgl. Kapitel 1.3) sowie deren unmittelbare Kontaktpersonen spricht sich die STIKO bis auf Weiteres ab einem Alter von sechs Monaten zusätzlich für jährliche Auffrischungsimpfungen im Herbst aus. Dafür sollen Impfstoffe mit aktueller SARS-CoV-2-Variantenanpassung gemäß den Vorgaben der WHO verwendet werden[41].

Bei PatientInnen mit ausgeprägter Immundefizienz kann laut STIKO eine serologische Untersuchung auf spezifische Immunglobuline G gegen das *spike* Protein von SARS-CoV-2 (SARS-CoV-2 anti-spike IgG) zur Evaluation der Impfansprechraten erfolgen[42]. Es ist jedoch kein Antikörpertiter definiert, bei dessen Erreichen von einem Schutz vor schweren Verläufen von COVID-19 ausgegangen werden kann. Gegen den SARS-CoV-2-Wildtyp wurden initial absolute Gesamttiter von 264,0 BUA/ml als effektiv diskutiert[43]. Wir konnten zeigen, dass nach Gabe von mABs die serologische Überprüfung der SARS-CoV-2-Impfantwort bei PatientInnen mit systemischer Immundefizienz falsch positiv sein kann, was entsprechend beachtet werden muss[44]. Außerdem wird durch Messungen von SARS-CoV-2 anti-spike IgG der prozentuale Anteil neutralisierender Antikörper (NABs: *neutralizing antibodies*), also die Antikörper, die SARS-CoV-2 tatsächlich spezifisch neutralisieren, noch nicht erfasst. Ein durch Antikörper neutralisiertes Virus kann menschliche Zellen nicht mehr infizieren. Studien haben zwar eine positive Korrelation zwischen dem Gesamttiter von SARS-CoV-2 anti-spike IgG und dem Anteil der NABs belegt[45], jedoch muss berücksichtigt werden, welche Virusvarianten tatsächlich neutralisiert werden (vgl. Kapitel 1.7). Zusätzlich zur humoralen Immunantwort müsste die zelluläre Immunantwort, in der Routinediagnostik beispielsweise über Messung SARS-CoV-2-spezifischer Interferon-Gamma-Spiegel, gesondert erfasst werden.

Für individualisierte Impfempfehlungen für die heterogene Patientengruppe mit einem weiterhin bestehenden Risiko für schwere klinische und/oder prolongierte virologische Verläufe (Kapitel 1.3) ist die aktuelle Studienlage zu begrenzt. In Abhängigkeit des Grades der Immunkompetenz ist von einer geringeren Immunogenität der Impfungen und/oder einem rascheren Schwinden der Immunität über die Zeit bei diesen PatientInnen auszugehen, wie es beispielhaft für PatientInnen mit soliden Neoplasien gezeigt wurde[46–49]. Dennoch ist der klinische Nutzen einer Impfung gegen SARS-CoV-2 in der Subgruppe der PatientInnen mit soliden Neoplasien trotz der reduzierten Immunantwort belegt. Es konnte für geimpfte PatientInnen eine Verringerung des Risikos von Durchbruchinfektionen, schwerer Verläufe und möglicher Folgeschäden verglichen mit ungeimpften PatientInnen gezeigt werden[50–57]. Bei PatientInnen mit Karzinomen unter Checkpoint-Inhibition konnte durch eine Impfung gegen die Influenza sogar ein Überlebensvorteil belegt werden[58]. Differenzierte Daten zum Impfansprechen bei einzelnen Tumorentitäten fehlen bisher. In drei Arbeiten einer longitudinalen SARS-CoV-2-Impfstudie, die in die vorliegende Habilitationsschrift eingegangen sind, lag der Fokus deshalb auf PatientInnen mit gastrointestinalen Karzinomen (vgl. Kapitel 1.8.1, 2.1, 2.2 und 2.3)[59–61]. Dabei können die Ergebnisse dieser Studien wiederum auf PatientInnen mit

anderen soliden Krebserkrankungen übertragbar sein. In Zusammenschau wird derzeit von einer reduzierten Schutzwirkung bei PatientInnen mit soliden Neoplasien im Vergleich zu gleichaltrigen, immunkompetenten Personen ausgegangen, sodass die seitens der STIKO empfohlenen jährlichen Auffrischungsimpfungen von den deutschen und amerikanischen Leitlinien für PatientInnen mit hämatologischen/onkologischen Neoplasien geteilt werden[62,63]. Durch die Auffrischungsimpfungen wird die Basisimmunität in diesen Patientengruppen aufrechterhalten und es wird auf neue Virusvarianten reagiert. Insbesondere bei Risikopatient-Innen, die den Empfehlungen nicht folgen, sollten antivirale Therapien eingesetzt werden.

1.5 Antivirale Therapien von COVID-19

Drei direkt wirksame DAAs haben sich als besonders wirksam bei SARS-CoV-2-Infektionen erwiesen: Das Nukleosidanalogon Remdesivir (RDV) hemmt die virale Replikation in der Phase der Frühen Infektion bei ungeimpften und geimpften PatientInnen und konnte dadurch Vorstellungen in der Notaufnahme, Hospitalisierungen und Todesfälle effektiv verhindern[64,65]. Zusätzlich wurde im Verlauf der Pandemie die Wirksamkeit von RDV in der Pulmonalen Phase bei COVID-19-Pneumonien mit *low-flow* Sauerstoffpflichtigkeit vor allem auch in Kombination mit dem immunmodulatorischen Dexamethason belegt[66,67]. In der Frühen Phase der Infektion war der Einsatz des geboosterten Proteaseinhibitors Nirmatrelvir/ Ritonavir (NIR/r) als alternatives DAA vergleichbar effektiv[68,69]. Auch die Gabe von Molnupiravir (MOL), das wie RDV die virale Replikation hemmt, wird in der Frühen Phase der Infektion auf Grundlage der *MOVe-Out* Studie durch die WHO empfohlen[70,71]. Für Europa hat die *European Medicines Agency* (EMA) eine bedingte Zulassung im Februar 2023 nach Re-Evaluation der Datenlage jedoch zurückgenommen[72]. Ergänzend zu den Daten bezüglich der kombinierten Endpunkte der Studien (Vorstellungen in der Notaufnahme, Hospitalisierungen und Todesfälle) fehlen fundierte Daten zum klinischen Verlauf unter antiviralen Monotherapien, die wir in unserer obenen genannten Studie nun erhoben haben (vgl. Kapitel 1.3, 1.8.2 und 2.4)[33].

Zahlreiche mABs respektive mAB-Kombinationen mit Wirksamkeit gegen verschiedene SARS-CoV-2-Varianten wurden in der Frühen Phase der Infektion therapeutisch und/oder als Präexpositionsprophylaxe eingesetzt. MABs neutralisieren das Virus und verringern somit die Schwere der Erkrankung. Gerade für HochrisikopatientInnen, die nach Impfung keine ausreichende Immunantwort ausgebildet hatten, waren diese Substanzen essenziell (vgl. Kapitel 1.4). Durch virale Evolution, insbesondere durch Veränderungen am *spike* Protein, verloren alle Antikörper im Verlauf der Pandemie im Gegensatz zu den DAAs bisher

sukzessive ihre Wirksamkeit[73] (vgl. Kapitel 1.6). Aktuell haben mABs daher keine wesentliche Bedeutung in der Therapie und/oder Prophylaxe von Infektionen mit SARS-CoV-2.
Grundsätzlich konnte ein Rückgang von Hospitalisierungen und der Mortalität bei PatientInnen mit systemischer Immundefizienz und entsprechend eingeschränkter Immunantwort nach Impfungen und/oder Infektionen mit SARS-CoV-2 unter antiviralen Monotherapien belegt werden[74–77]. Das Problem des prolongierten SARS-CoV-2-Nachweises bei diesen PatientInnen wurde durch antivirale Monotherapien jedoch nicht ausreichend gelöst. Auf Grundlage von *in vitro*- und Tiermodelldaten[78–82] wurden bei HochrisikopatientInnen mit systemischer Immundefizienz daher nicht nur Monotherapien, sondern auch Kombinationen der verschiedenen genannten Substanzen therapeutisch eingesetzt, um schweren klinischen Verläufen und insbesondere auch prolongierten Virusnachweisen zu entgegnen. In ersten Fallberichten und -serien zum Einsatz antiviraler Kombinationstherapien bei COVID-19 bei Menschnen wurden diese reaktiv eingesetzt, wenn es bereits zu einem prolongierten Verlauf der Infektion gekommen war[83–94]. Kombinationstherapien sind derzeit in Deutschland nur mit RDV und NIR/r sinnvoll möglich, da alle mABs wie dargestellt ihre Wirksamkeit verloren haben[95]. Für individualisierte Therapieempfehlungen sind die Daten bisher aber zu limitiert, sodass eine infektiologische Expertenmeinung eingeholt werden sollte. Es fehlen weiterhin größere Untersuchungen, ob durch den Einsatz antiviraler Kombinationstherapien bei PatientInnen mit systemischer Immundefizienz in der Erstlinie die Rate prolongierter Virusnachweise hätte reduziert werden können.

1.6 Virale Evolution in PatientInnen mit systemischer Immundefizienz

Die hohe Replikationsrate, pandemische Verbreitung und hohe Prävalenz von SARS-CoV-2 begünstigen das Auftreten neuer Virusvarianten durch Mutationen im viralen Genom. Dabei verändern besonders Mutationen im Bereich des *spike* Proteins die Pathogenität und Kontagiosität des Virus[96]. Zahlreiche Studien belegen, dass neue SARS-CoV-2-Varianten bevorzugt in PatientInnen mit prolongiertem Virusnachweis bei systemischer Immundefizienz entstanden sind[18,26,97,98]. Dabei waren antivirale Monotherapien mit DAAs oder mABs bei diesen PatientInnen insbesondere bezüglich der Viruselimination teils ineffektiv (vgl. Kapitel 1.5), was zu einem weiteren Mutationsdruck führte. Infolgedessen kam es auch zum Wirkverlust aller mABs wie unter 1.5 dargestellt[99–104].

Neue Virusvarianten haben erhebliche Auswirkungen sowohl auf die individuelle medizinische Versorgung als auch die öffentliche Gesundheit, weil sie die Kontrolle epidemischer Ausbrüche

erschweren. Immungeschwächte PatientInnen als Treiber der viralen Evolution müssen bei der Infektionskontrolle entsprechend berücksichtigt werden. Der Einsatz antiviraler Monotherapien bei PatientInnen mit systemischer Immundefizienz sollte kritisch abgewogen und Kombinationstherapieregime als alternatives Konzept in Erwägung gezogen werden. Aus unserer Sicht war der Einsatz von Kombinationstherapien in der Erstlinie bei einem Hochrisikopatientenkollektiv mit systemischer Immundefizienz sinnvoll, um prolongierte Verläufe *upfront* zu verhindern und somit konsekutiv den Mutationsdruck sowie mögliche Resistenzentwicklungen potenziell zu verringern. Bei diesem Ansatz haben wir uns an den therapeutischen Prinzipien anderer Infektionskrankheiten wie Infektionen mit dem *human immunodeficiency virus* (HIV) oder der Tuberkulose orientiert und unsere Studie entsprechend konzipiert[27] (vgl. Kapitel 1.3, 1.8.2 und 2.5). Im Vergleich dazu erschien das Konzept des reaktiven Einsatzes antiviraler Kombinationstherapien bei bereits eingetretenem prolongierten Virusnachweis[83–88] (vgl. Kapitel 1.5) weniger überzeugend.

1.7 Klinischer Verlauf von Infektionen mit den Omikron-Varianten von SARS-CoV-2

Neuere SARS-CoV-2-Virusvarianten einschließlich der Omikron-Varianten sind kontagiöser als der Wildtyp[105], die Mehrheit der Bevölkerung weist jedoch einen effektiven Immunschutz nach Impfungen und/oder Infektionen auf[106–109]. Dabei besteht trotz wildtypbasierter Impfungen zusätzlich ein Basisschutz vor schweren Verläufen bei Erstinfektionen mit den aktuell vorherrschenden Omikron-Varianten, was auf humorale und vornehmlich zelluläre Kreuzreaktivitäten zurückgeführt wird[110–116]. Darüber hinaus kann der klinische Verlauf bei HochrisikopatientInnen durch antivirale Medikamente günstig beeinflusst werden (vgl. Kapitel 1.5). Da Omikron-Varianten jedoch resistenter gegen die immunologische Neutralisation nach wildtypbasierten Impfungen und/oder Infektionen mit prä-Omikron-Varianten sind, werden weiterhin auch schwere Verläufe von COVID-19 beobachtet, sodass die Omikron-Varianten weiterhin mit einer erheblichen Morbidität und Mortalität in Verbindung gebracht werden[31,117–119]. Immunologisch gegenüber SASR-CoV-2 naive und auch PatientInnen mit systemischer Immundefizienz einschließlich PatientInnen mit hämatologischen und soliden Neoplasien und anzunehmender eingeschränkter Immunantwort auf SARS-CoV-2-Antigenkontakte sind weiterhin besonders gefährdet, einen schweren Verlauf von COVID-19 durchzumachen[120–123].

1.8 Methodischer Ansatz

1.8.1 Prospektive, longitudinale SARS-CoV-2-Impfstudie

Drei Arbeiten der vorliegenden kumulativen Habilitationsschrift gehen aus einer prospektiven, longitudinalen Beobachtungsstudie hervor. Ziel war es, die Impfantworten von PatientInnen mit gastrointestinalen Karzinomen im Langzeitverlauf zu beurteilen und Faktoren zu identifizieren, die die Impfantworten beeinflusst haben. PatientInnen, die in der Medizinischen Klinik und Poliklinik I des Universitätsklinikums Bonn mit einer aktiven Krebserkrankung gastroonkologisch therapiert wurden, konnten in die Studie aufgenommen werden (Referenzgruppe). PatientInnen in der Nachsorge, bei denen mindestens 12 Monate lang keine Tumoraktivität nachweisbar war und die seit mehr als 12 Monaten keine Erhaltungstherapie bekommen hatten, wurden als Kontrollgruppe mit vergleichbaren Risikofaktoren für die Karzinogenese, für schwere Verläufe von COVID-19 und/oder für eine gestörte Immunantwort auf Impfungen einbezogen. Initial wurde die humorale Immunantwort nach einer Primärimpf-serie (in der Regel zwei Impfungen mit mRNA-basierten Impfstoffen gegen SARS-CoV-2) ermittelt. Dabei wurden die totalen Spiegel des SARS-CoV-2 anti-spike IgGs als auch der Anteil der NABs gemessen. Speziell bei PatientInnen mit hepatobiliären Karzinomen fielen dabei besonders eingeschränkte humorale Impfantworten auf[59]. Nach erster, wildtypbasierter Auffrischungsimpfung und/oder einer Infektion mit SARS-CoV-2, also nach Erreichen der Basisimmunität gegen SARS-CoV-2, wurde im Weiteren daher bei PatientInnen mit hepatobiliären Karzinomen die Entwicklung der humoralen und zusätzlich die zelluläre Impfantwort analysiert. Neben der Immunogenität wurden etwaige Durchbruchinfektionen nach der Impfung sowie deren klinischen Verläufe dokumentiert und zur klinischen Bewertung des Impfansprechens herangezogen[60]. In der finalen Analyse wurde erneut bei PatientInnen mit unterschiedlichen gastrointestinalen Karzinomen untersucht, ob sich die Basisimmunität ohne weitere Antigenkontakte aufrechterhalten lässt und wie gut die PatientInnen vor schweren Verläufen mit neuen, also den Omikron-Varianten von SARS-CoV-2 geschützt sind. Ab-schließend wurde der Effekt eines ersten Omikron-Antigenkontaktes auf die SARS-CoV-2-Immunantwort untersucht[61] (vgl. Kapitel 2.1, 2.2, 2.3).

1.8.2 Retrospektive *Real-World Studies* zu antiviralen Therapien von COVID-19

Zwei weitere Arbeiten der vorliegenden kumulativen Habilitationsschrift sind als *Real-World Studies* durchgeführt worden. Randomisierte, kontrollierte Studien liefern Nachweise über

therapeutische Wirksamkeit und Sicherheit einer definierten Intervention, die gegen ein Placebo oder den Goldstandard verglichen wird. Dabei gibt es strenge Ein- und Ausschlusskriterien, wodurch ein Teil der PatientInnen der Routineversorgung nicht in den Kohorten repräsentiert ist. Eine Übertragbarkeit der so erhobenen Daten ist nicht *per se* gegeben. *Real-World Studies* betrachten die Intervention im Weiteren in der klinischen Praxis in einer repräsentativeren Patientenpopulation. Kontrollgruppen sind dafür teils schwer zu benennen und können fehlen. Die erhobenen Daten sind nicht diametral zu den Daten randomisierter, kontrollierter Studien zu betrachten, sondern sollten als vergleichende und ergänzende Wirksamkeitsdaten und als eine externe Kontrolle verstanden werden, die für mehr klinische Evidenz sorgen[124].

Real-World Daten werden auch zur Re-Evaluation sogenannter bedingter Marktzulassungen genutzt. Die EMA hat die Möglichkeit diese zu erteilen, wenn im Sinne der öffentlichen Gesundheit hierdurch auf einen ungedeckten medizinischen Bedarf eingegangen wird. Dafür können die normalerweise erforderlichen klinischen Daten weniger umfangreich sein, wenn das Risiko, das sich aus der Tatsache ergibt, dass noch zusätzliche Daten erforderlich wären, geringer eingeschätzt wird als der Nutzen einer bedingten Zulassung. In der Folge werden weitere Daten analysiert und die Zulassung stetig reevaluiert[125]. Vergleichbare Verfahren nutzt die amerikanische *Food and Drug Administration* (FDA) für sogenannte Notfallzulassungen[126]. Beide Behörden haben die Verfahren genutzt, um Impfungen und Therapeutika gegen SARS-CoV-2 zeitnah zur Pandemiebekämpfung zur Verfügung zu stellen.

In einer ersten retrospektiven Kohortenstudie haben wir Monotherapien mit RDV oder der mAB-Kombination Casirivimab/ Imdevimab (CVIV) zur Therapie von COVID-19 zwischen März 2020 und November 2021 in Bezug auf den weiteren klinischen Verlauf der Infektion anhand der WHO-Ordinalskala bewertet. In die Studie wurden vornehmlich ältere, multimorbide PatientInnen mit einem hohen Risiko für einen schweren klinischen, aber einem geringen Risiko für einen prolongierten virologischen Verlauf von COVID-19 mit Indikation zu einer antiviralen Monotherapie aufgenommen[33]. Die Medikamente wurden zunächst insbesondere aufgrund der begrenzten Ressourcen als individuelle Therapieversuche und somit nicht bei allen PatientInnen eingesetzt. Entsprechend konnten Kontrollgruppen gebildet werden, in die PatientInnen aufgenommen wurden, die bei vergleichbarer Krankheitsschwere lediglich den Therapiestandard, d.h. eine symptomatische Therapie, erhalten hatten. *Inverse probability weighting* wurde für das Matchen der Gruppen methodisch angewandt. Beobachtungen in der Behandlungsgruppe, die der erwarteten Beobachtung in der

Kontrollgruppe ähnlich waren, wurden mit Hilfe von *propensity scores* dadurch stärker gewichtet und umgekehrt. In der Folge war die Verwendung der gesamten Stichprobe möglich und PatientInnen, für die keine passende Übereinstimmung gefunden wurde, wurden nicht ausgeschlossen[127] (vgl. Kapitel 2.4).

Für eine zweite retrospektive Beobachtungsstudie wurde zwischen März 2022 und April 2023 der Erstlinieneinsatz von Kombinationstherapien aus DDAs und/oder mABs gegen COVID-19 hinsichtlich der zeitlichen Dauer des Virusnachweises und des klinischen Verlaufs analysiert. In diese Kohorte wurden nun PatientInnen mit systemischer Immundefizienz und dem Risiko von schweren klinischen und auch prolongierten virologischen Verläufen aufgenommen. Die Studie wurde multizentrisch an den vier Standorten des *Centre for Integrated Oncology Aachen, Bonn, Cologne, Düsseldorf* (CIO ABCD) durchgeführt. Zum Zeitpunkt der Datenerfassung wurden Infektionen mit SARS-CoV-2 bei diesen HochrisikopatientInnen regelhaft mit antiviralen Erstlinienkombinationstherapien in den Zentren behandelt. Einigen PatientInnen diese Option vorzuenthalten, war ethisch nicht vertretbar, sodass keine Kontrollgruppe gebildet werden konnte. Insbesondere aufgrund vorherrschender anderer Virusvarianten zu vorherigen Zeitpunkten wurde davon abgesehen, eine retrospektive Kontrollgruppe mit einer zu groß vermuteten Verzerrung zu konstruieren[27] (vgl. Kapitel 2.5).

1.9 Ziele der vorliegenden Arbeit

Die kontinuierliche Forschung zur Prävention und Behandlung von SARS-CoV-2-Infektionen und deren Langzeitfolgen sind bemerkenswerte Errungenschaften im Kampf gegen die Pandemie. Durch die zunehmende Immunität der Bevölkerung durch flächendeckende Impfungen und/oder durchgemachte Infektionen sowie die Bereitstellung von Therapeutika wurde gesamtgesellschaftlich ein Wendepunkt in der Pandemie erreicht. Beschränkungen des täglichen Lebens konnten in der Folge sukzessive zurückgenommen werden. Dennoch gibt es weiterhin PatientInnen, die durch schwere klinische und/oder prolongierte virologische Verläufe von COVID-19 gefährdet sind. Für diese heterogene Patientengruppe gibt es bisher nur wenige Subgruppenanalysen, die individualisierte Konzepte der Impfprävention und antiviralen Therapie bei Infektionen mit SARS-CoV-2 erlauben würden und die auf vergleichbare und/oder übergeordnete Gruppen übertragbar sein könnten. Hierzu kann die vorliegende kumulative Habilitationsschrift einen Beitrag leisten:

- Bei insgesamt begrenzten Daten zu Impfansprechraten von PatientInnen mit soliden Tumorerkrankungen[63,128,129] werden differenzierte Daten zur humoralen und zellulären Immunogenität sowie zu klinischen Parametern nach der SARS-CoV-2-Primärimpfserie, nach der ersten wildtypbasierten Auffrischungsimpfung und nach einem ersten Omikron-Antigenkontakt in der Subgruppe von PatientInnen mit gastrointestinalen Tumorerkrankungen präsentiert[59–61].
- Im Weiteren werden anhand der WHO-Ordinalskala klinische Aspekte herausgearbeitet, die zur Therapieentscheidung und -bewertung antiviraler Monotherapien im Rahmen der Routineversorgung von älteren, multimorbiden PatientInnen mit hohem Risiko für einen schweren klinischen, aber geringem Risiko für einen prolongierten virologischen Verlauf von COVID-19 herangezogen werden sollten, anstatt sich auf virologische Marker zu fokussieren[33].
- Abschließend werden Sicherheit und Wirksamkeit antiviraler Kombinationstherapien als Option in der Erstlinie für HochrisikopatientInnen mit systemischer Immundefizienz und entsprechend zusätzlich hohem Risiko prolongierter Virusnachweise bezüglich des klinischen Verlaufs und der Dauer des Virusnachweises bewertet[27].

2 Ergebnisse

2.1 Longitudinale SARS-CoV-2-Impfstudie bei PatientInnen mit gastrointestinalen Karzinomen – Effekt der Primärimpfserie

Monin, M.B.*, Gorny, J.G.*, Berger, M., Baier, L.I., Zhou, T., Mahn, R., Sadeghlar, F., Möhring, C., Boesecke, C., van Bremen, K., Rieke, G.J., Schlabe, S., Breitschwerdt, S., Marinova, M., Schmidt-Wolf, I.G.H., Strassburg, C.P., Eis-Hübinger, A.-M. Gonzalez-Carmona, M.A. (2023). Impaired immunogenicity after vaccination for SARS-CoV-2 in patients with gastrointestinal cancer: does tumor entity matter? J Gastrointest Oncol *14*.

*geteilte Erstautorenschaft

In dieser ersten Arbeit der prospektiven, longitudinalen Impfstudie wurde zwischen Januar 2021 und April 2022 die humorale Immunantwort nach einer SARS-CoV-2-Primärimpfserie bei PatientInnen mit verschiedenen gastrointestinalen Karzinomen untersucht. Ziel war es, einen Titer zu ermitteln, der mit einem Schutz vor schweren Verläufen von COVID-19 assoziiert war (vgl. Kapitel 1.8.1).

Insgesamt wurden 125 PatientInnen in die Studie aufgenommen, von denen 85 ein aktives gastrointestinales Karzinom unter Therapie hatten (Referenzgruppe). Vierzig PatientInnen befanden sich nach gastrointestinaler Krebserkrankung seit mindestens 12 Monaten in der Nachsorge (Kontrollgruppe); eine Erhaltungstherapie erfolgte bei diesen PatientInnen nicht.

Nach der Primärimpfserie waren die absoluten und relativen SARS-CoV-2 anti-spike IgG-Spiegel in der Referenzgruppe signifikant niedriger im Vergleich zur Kontrollgruppe. Dabei war vor allem auch die Kapazität zur Neutralisation des Wildtyps (beurteilt anhand des Anteils an Surrogat-NABs [sNABs]) reduziert. Es bestand eine positive Korrelation zwischen dem IgG-Gesamttiter und dem Anteil der sNABs mit einem Koeffizienten von 0,93. Zwischen Woche vier und Woche 12 nach der zweiten Impfung sank die Höhe der Titer in beiden Gruppen, wobei der Abfall in der Referenzgruppe – insbesondere bezüglich der sNABs – ausgeprägter war. Zwölf Wochen nach der zweiten Impfung lag der mittlere geschätzte SARS-CoV-2 anti-spike IgG-Gesamttiter bei den PatientInnen in der Kontrollgruppe bei 282,0 BAU/mL und der Anteil sNABs bei 85%. Dabei wiesen alle PatientInnen mit einem IgG-Wert ab 482,0 BAU/ml einen sNAB-Anteil von >85% auf. Bei nur einer mild verlaufenden

Infektion in der Kontrollgruppe wurde bei diesen Werten von einem klinischen Schutz vor schweren Verläufen von COVID-19 nach der Primärimpfserie ausgegangen. PatientInnen der Referenzgruppe erreichten den Titer von 482,0 BAU/ml signifikant seltener. Die genannten Unterschiede zwischen der Referenz- und der Kontrollgruppe waren besonders ausgeprägt bei PatientInnen mit hepatozellulären, pankreatikobiliären und/oder kolorektalen Karzinomen. In einer ergänzenden multivariablen Analyse konnten zusätzlich eine systemische Immundefizienz und tumorspezifische Therapien als Faktoren identifiziert werden, die zu signifikant niedrigeren Titern führten.
Zum Zeitpunkt der Studie hatte nur ein kleiner Teil der PatientInnen bereits eine erste Auffrischungsimpfung erhalten. Es zeichnete sich bereits ab, dass die Höhe der Titer durch eine dritte Impfung zwischen den Gruppen ausgeglichener sein würde.

Wir empfahlen für PatientInnen mit gastrointestinalen Karzinomen individualisierte Vorgehensweisen hinsichtlich weiterer Impfungen, antiviraler Therapien und/oder damals noch verfügbarer passiver Immunisierungen und dabei insbesondere den Tumortyp (hepatozellulär, pankreatikobiliär, kolorektal), tumorspezifische Therapien und eine etwaige Immundefizienz zu berücksichtigen.

Original Article

Impaired immunogenicity after vaccination for SARS-CoV-2 in patients with gastrointestinal cancer: does tumor entity matter?

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Background: SARS-CoV-2 immunogenicity in patients with gastrointestinal cancer (GI cancer) following second and third vaccination was analyzed.

Methods: A total of 125 patients under active anticancer therapy or in follow-up care were included in this prospective study. Seroprevalence of SARS-CoV-2 anti-spike and surrogate neutralization antibodies (NABs) was measured.

Results: Four weeks after second vaccination, adequate titers of SARS-CoV-2 anti-spike immunoglobulin G (IgG) [≥282.0 binding antibody units (BAU)/mL] were found in 62.2% of patients under treatment versus 96.3% of patients in follow-up care (P<0.01). Sufficient titers of SARS-CoV-2 surrogate NAB (≥85.0%) were found in 32.7% of patients under treatment versus 70.6% in follow-up care (P<0.01). Titers of SARS-CoV-2 anti-spike IgG were especially low in patients with colorectal cancer (CRC). For SARS-CoV-2 surrogate NAB, patients with hepatocellular carcinoma (HCC) and with pancreaticobiliary cancer showed the lowest titers (P<0.01). SARS-CoV-2 anti-spike IgG and SARS-CoV-2 surrogate NAB were associated with a correlation coefficient of 0.93. Reaching a titer of SARS-CoV-2 anti-spike IgG ≥482.0 BAU/mL, protective levels of SARS-CoV-2 surrogate NAB (≥85.0%) could be assumed. Following booster vaccination, all patients reached effective antibody titers.

Conclusions: Patients with active GI cancer showed impaired immunogenicity after second SARS-CoV-2 vaccination which was overcome by booster vaccination. Our findings were tumor-related and pronounced in patients with CRC and HCC. Waning immunity over time and antibody escape phenomena by variant of concern Omicron must be considered in these especially vulnerable patients.

Keywords: Vaccination for SARS-CoV-2; gastrointestinal cancer; SARS-CoV-2 immunogenicity; SARS-CoV-2 surrogate neutralization antibodies; vaccination failure

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Introduction

SARS-CoV-2 infected patients with cancer, especially under active cancer treatment, are facing higher rates of morbidity and mortality compared to healthy people (1,2). Both, the American Society for Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO) recommended to prioritize patients with cancer in vaccination campaigns (3,4). However, actively treated patients with cancer were excluded from trials analyzing efficacy of SARS-CoV-2 vaccines (5-9).

First data concerning immunogenicity to SARS-CoV-2 revealed reduced response rates to vaccination in patients with cancer. However, most of the studies focused on patients with hematological cancer or included different types of solid cancer without differentiating between tumor types (10-19). For the time being, differentiated data on patients with gastrointestinal (GI) cancer are sparse (20). Antibody titers were compared to people in control groups without any history of cancer who were considerably healthier and younger. Finally, only a small number of studies referred to neutralization antibodies (NABs) as being decisive for immune protection from symptomatic SARS-CoV-2 (12-19,21-23). No titer could be defined as being linked to protection from severe coronavirus disease 2019 (COVID-19). Especially liver dysfunction due to primary hepatobiliary tumors or secondary hepatic metastases from GI tumors as well as due to underlying liver steatosis, fibrosis and/ or cirrhosis is associated with immunodeficiency resulting in impaired immune responses to well-known vaccines (24-28). Taking all these information into consideration, a more detailed analysis of immunogenicity in patients with GI tumors is necessary for making recommendations concerning additional booster vaccinations in these patients.

Thus, in the present study, we provide novel data on response rates to basic and booster vaccination for SARS-CoV-2 in patients with GI cancer under anticancer treatment but also in follow-up care, offering robust evidence for recommendations on antibody assessment, individual booster vaccinations as well as on passive immunization and/or antiviral therapy in individual patients with GI cancer. We present this article in accordance with the STROBE reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1065/rc).

Highlight box

Key findings

- Impaired immunogenicity after second SARS-CoV-2 vaccination was overcome by booster vaccination.

What is known and what is new?

- Impaired immunogenicity in patients with several solid cancer types has already been described. Yet, differentiated data for patients with gastrointestinal cancer are missing.
- Patients with gastrointestinal cancer showed impaired immune responses follwing vaccination for SARS-CoV-2.

What is the implication, and what should change now?

- Booster vaccinations for SARS-CoV-2 should be recommended to patients with gastrointestinal cancer.
- In patients with gastrointestinal cancer being infected with SARS-CoV-2, antiviral therapy should be taken into consideration tp prevent severe COVID-19.

Methods

Study design

This is a prospective, observational, longitudinal study on the efficacy of vaccination for SARS-CoV-2 in patients with GI cancer treated at the Department of Internal Medicine I, gastroenterology oncology section at the University Hospital Bonn between January 2021 and April 2022. We focused on humoral immunogenicity for SARS-CoV-2 in patients with GI cancer.

Total antibody titers as well as titers of surrogate NAB were considered and titers probably linked to protection from severe COVID-19 were defined for our cohort of patients. Blood samples were drawn to analyze seroprevalence of SARS-CoV-2 anti-spike antibodies 4, 12 and 24 weeks after second vaccination for SARS-CoV-2. After 12 weeks, SARS-CoV-2 surrogate NAB were additionally measured. Four and 12 weeks after booster vaccination, we again assessed antibody titers. During the study period, we screened for infections with SARS-CoV-2. The study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the institutional review board of the Medical Faculty of the University of Bonn (No. 341/17 and 023/22). Written informed consent was obtained from all participating patients.

Patient characteristics and eligibility criteria

In descending order, patients with pancreaticobiliary neoplasms [PBN: cholangiocarcinoma (CCC), papillary carcinoma, gallbladder cancer (GBC), pancreas cancer],

hepatocellular cancer (HCC), colorectal cancer (CRC), neuroendocrine tumors (NETs), upper GI tract cancer [esophageal, gastro-esophageal junction (GEJC), gastric cancer], cancer of unknown primary (CUP) with most likely GI origin as well as gastrointestinal stromal tumors (GISTs) were included. Any kind of ongoing oncological treatment (chemotherapy, targeted therapy, immune checkpoint inhibition, local therapy, or combination of different therapeutic strategies) was eligible for inclusion. Patients being off treatment but having undergone oncological treatment within the past 12 months were also included in this group. Patients in follow-up care being at least 12 months without detectable tumor and having been off treatment >12 months were included as control group. These patients share comparable risk factors, both for cancer pathogenesis as well as for severe COVID-19 and for impaired immune responses to vaccinations. Relevant clinical information, especially regarding COVID-19 infections, patients' performance [Eastern Cooperative Oncology Group (ECOG) score] and disease status (local, tumor recurrence and metastatic), were obtained from standardized medical records. Increased immunosuppression was suspected in patients with a medical history of autoimmune disease or organ transplantation with concomitant therapy with corticosteroids, methotrexate or calcineurin inhibitors as well as in patients with uncontrolled human immunodeficiency virus (HIV) infections (*Table 1*).

All vaccines for SARS-CoV-2 approved in Germany could be administered (BNT162b2 by Pfizer BioNTech, AZD1222 by AstraZeneca, mRNA-1273 by Moderna, Ad26.COV2.S by Johnson & Johnson Janssen).

Assessment of SARS-CoV-2 antibodies

SARS-CoV-2 immunoglobulin G (IgG) II Quant chemiluminescent microparticle immunoassay (Abbott Laboratories, Chicago, USA) was used to quantify IgG antibodies against SARS-CoV-2 spike receptor-binding domain (SARS-CoV-2 anti-spike IgG). Values ≥7.1 binding antibody units per milliliter (BAU/mL) were evaluated as positive though no threshold for protection was defined. In our study, mean estimated SARS-CoV-2 anti-spike IgG was 282.0 BAU/mL in patients in follow-up care 12 weeks after second vaccination. Titers ≥282.0 BAU/mL were thus regarded as being associated with most likely protection from severe COVID-19 in our cohort as only one patient of this group had mild COVID-19 after two vaccinations.

To identify the portion of SARS-CoV-2 NABs in relation to all antibodies [%], a blocking enzyme-linked immunosorbent assay (ELISA) detection tool (cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit; GenScript, New Jersey, USA) was used. This test detects functional virus neutralization strongly correlating with live-cell neutralization (29,30). We thus report on SARS-CoV-2 surrogate NAB as we did not explicitly measure live-cell neutralization. Reaching values ≥30% was defined as positive with no threshold for protection. The mean estimated SARS-CoV-2 surrogate NAB titer 12 weeks after second vaccination was 84.81% in patients in follow-up care in our cohort. Therefore, titers ≥85.0% were again regarded as probably equivalent to protection from severe COVID-19.

Elecsys anti-SARS-CoV-2 chemiluminescent immunoassay (Roche) was used for qualitative detection of SARS-CoV-2 anti-nucleocapsid IgG in patients having undergone an infection with SARS-CoV-2 prior to or despite vaccination.

Statistical analysis

Statistical analysis was carried out using R version 4.1.1 (R Core Team 2021: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria). Descriptive analyses included the calculation of mean and interquartile range for continuous variables and frequencies (absolute and relative) for categorical variables. Association between levels of SARS-CoV-2 anti-spike IgG and SARS-CoV-2 surrogate NAB was analyzed using Spearman's rank correlation coefficient. Proportions of patients with effective SARS-CoV-2 anti-spike IgG and/or effective SARS-CoV-2 surrogate NAB were compared by chi-square tests. Difference in ($\log_{10}$ transformed) levels of SARS-CoV-2 anti-spike IgG in patients with and without effective SARS-CoV-2 surrogate NAB was examined by *t*-test.

Univariate linear mixed regression models were used to compare ($\log_{10}$ transformed) levels of SARS-CoV-2 anti-spike IgG (including measurements at 4 and 12 weeks) with respect to treatment status, tumor type, disease status, intention of treatment and type of treatment. Analogously, univariate linear regression models were applied to compare levels of SARS-CoV-2 surrogate NAB (measurements at 12 weeks) with respect to the five discussed influencing factors. Furthermore, multivariable regression analysis was performed to examine a possible effect of age, sex,

Table 1 Patient baseline characteristics

Patients	Under treatment (N=85)	Off treatment >1 year (N=40)	P
Age (years), median [IQR]	67 [61–75]	68 [60–72]	0.47
Sex, n (%)			0.12
Women	31 (36.5)	21 (52.5)	
Men	54 (63.5)	19 (47.5)	
Tumor types, n (%)			0.02
PBN	21 (24.5)	5 (12.5)	
CCC	9 (10.5)	1 (2.5)	
GBC	1 (1.0)	1 (2.5)	
Pancreas cancer	11 (13.0)	3 (7.5)	
HCC	20 (23.5)	7 (17.5)	
CRC	17 (20.0)	13 (32.5)	
NET	13 (15.0)	1 (2.5)	
GEJC	9 (11.0)	11 (27.5)	
Gastric cancer	4 (5.0)	6 (15.0)	
Oesophageal cancer	5 (6.0)	5 (12.5)	
Other	5 (6.0)	3 (7.5)	
CUP	3 (4.0)	2 (5.0)	
GIST	2 (2.0)	1 (2.5)	
Disease status, n (%)			<0.01
Local	32 (37.5)	34 (85.0)	
Tumor recurrence	10 (12.0)	0 (0.0)	
Metastatic	43 (50.5)	6 (15.0)	
Intention of treatment, n (%)			
Neoadjuvant	13 (15.0)		
Adjuvant	12 (14.0)		
Palliative	60 (71.0)		
Type of treatment, n (%)			
Chemotherapy[a]	32 (38.0)		
Targeted therapy[b]	14 (16.0)		
Immune checkpoint inhibition	4 (5.0)		
Local therapy[c]	12 (14.0)		
Combined therapy[d]	23 (27.0)		
Additional immunosuppression[e]	14 (16.5)	2 (5.0)	0.09
Performance status (ECOG score), n (%)			<0.01
0	36 (77.5)	31 (43.4)	
1	39 (22.5)	9 (47.0)	
2	8 (9.6)	0 (0.0)	

Table 1 *(continued)*

Table 1 *(continued)*

Patients	Under treatment (N=85)	Off treatment >1 year (N=40)	P
Risk factors for severe COVID-19, n (%)			
Age >65 years	66 (78.0)	29 (72.5)	0.65
BMI >30 kg/m^2	6 (7.0)	1 (2.5)	0.43
History of smoking	21 (25.0)	13 (32.5)	0.39
Hypertension	49 (58.0)	19 (47.5)	0.34
Chronic respiratory disease	6 (7.0)	8 (20.0)	0.06
Cardiovascular disease	13 (15.0)	9 (22.5)	0.33
Chronic kidney disease	7 (8.0)	1 (2.5)	0.43
Liver disease	23 (27.0)	5 (12.5)	0.11
Neurological disorder	5 (6.0)	2 (5.0)	1.00
Organ transplant	3 (3.5)	0 (0.0)	0.55
Autoimmune disease	5 (6.0)	1 (2.5)	0.66
Diabetes mellitus	21 (25.0)	5 (12.5)	0.16
History of SARS-CoV-2 infection prior to vaccination (self-report, serology or PCR testing), n (%)	4 (5.0)	1 (2.5)	1.0
History of SARS-CoV-2 infection after second vaccination, (self-report, serology or PCR testing), n (%)	4 (5.0)	1 (2.5)	1.0
History of SARS-CoV-2 infection after booster vaccination, (self-report, serology or PCR testing), n (%)	3 (4.0)	1 (2.5)	1.0
Vaccine, n (%)			0.86
BNT162b2 (Pfizer & BioNTech)	73 (86.0)	33 (82.5)	
AZD1222 (AstraZeneca)	6 (7.0)	3 (7.5)	
mRNA-1273 (Moderna)	2 (2.0)	2 (5.0)	
AZD1222 + BNT162b2/mRNA-1273	3 (4.0)	2 (5.0)	
Ad26.COV2.s (Johnson & Johnson, Janssen)	1 (1.0)	0 (0.0)	
Patients with SARS-CoV-2 anti-spike IgG ≥282.0 BAU/mL (effective titer)			
4 weeks after second vaccination	62.2% (46/74)	96.3% (26/27)	<0.01
12 weeks after second vaccination	39.3% (22/56)	60.0% (21/35)	0.09
4 weeks after booster vaccination	100.0% (27/27)	100.0% (17/17)	
Patients with SARS-CoV-2 surrogate NAB ≥85.0% (effective titer)			
12 weeks after vaccination	32.7% (17/52)	70.6% (24/34)	<0.01
4 weeks after booster vaccination	100.0% (22/22)	100.0% (17/17)	

Baseline characteristics were compared between treatment and control group using Student's *t*-test for continuous variables and Fisher's exact tests for the categorical variables. [a], mostly with combinations of 5-fluorouracil, platinum derivates, gemcitabine, taxane or irinotecan; [b], including multikinase inhibitors such as sorafenib, lenvatinib, cabozantinib, EGFR (epidermal growth factor receptor) antibodies or VEGF (vessel endothelial growth factor) antibodies; [c], including transarterial embolization, radioembolization or endobiliary/transcutaneous radiofrequency ablation and photodynamic therapy; [d], chemotherapy + targeted therapy or chemotherapy + local therapy; [e], co-medication with corticosteroids, methotrexate or calcineurin inhibitor or HIV-infection. IQR, interquartile range; PBN, pancreaticobiliary neoplasm; CCC, cholangiocellular cancer; GBC, gallbladder cancer; HCC, hepatocellular cancer; CRC, colorectal cancer; NET, neuroendocrine tumor; GEJC, gastro-oesophageal-junction cancer; CUP, carcinoma of unknown primary; GIST, gastrointestinal stromal tumor; ECOG, Eastern Cooperative Oncology Group; COVID-19, coronavirus disease 2019; BMI, body mass index; PCR, polymerase chain reaction; BAU, binding antibody units; NAB, neutralization antibody.

additional immunosuppression and history of SARS CoV-2 infection on SARS-CoV-2 antibodies, respectively.

Results

Patient baseline characteristics

A total of 125 patients with GI cancer were included, of whom 68.0% (N=85) were under treatment and 32.0% (N=40) in follow-up care. In patients under treatment, the most common tumor type was of the pancreato-hepatobiliary type (PBN with 24.5% and HCC with 23.5%) followed by CRC (20.0%). Related to the prognosis of the different GI cancers, patients in follow-up care mainly suffered from CRC (32.5%) followed by GEJC (27.5%) leading to a different distribution of tumor types between the groups (P=0.02). As expected, patients under treatment had more frequently a metastatic disease status than patients in the control group (50.5% *vs.* 15.0%; P<0.01). Patients in both groups had a low ECOG score between 0 and 2. No further significant differences between the groups were observed (*Table 1*).

Seropositivity for SARS-CoV-2 anti-spike IgG

Mean antibody concentrations of SARS-CoV-2 anti-spike IgG were significantly lower in patients of the treatment group [2.47 $\log_{10}$ BAU/mL; 95% confidence interval (CI): 2.29 to 2.64; P<0.01] than in patients in follow-up care (3.06 $\log_{10}$ BAU/mL; 95% CI: 2.79 to 3.32) four weeks after second vaccination (*Table 2* and *Figure 1*). SARS-CoV-2 anti-spike IgG titers already decreased at week 12 after second vaccination in patients under active treatment (2.11 $\log_{10}$ BAU/mL; 95% CI: 1.92 to 2.29; P=0.08), but also in patients in follow-up care (2.45 $\log_{10}$ BAU/mL; 95% CI: 2.20 to 2.71; P<0.01). The differences in mean antibody titers between treatment and control group decreased at week 12 compared to week four after second vaccination, independent of tumor type, disease status, type of treatment or intention of treatment, but was especially pronounced in patients with CRC (2.18 $\log_{10}$ BAU/mL; 95% CI: 1.74 to 2.61; P=0.01) (*Table 2* and *Figure 1*).

In self-report and/or polymerase chain reaction (PCR) testing, 1 infection with SARS-CoV-2 in patients in follow-up care (2.5%) versus 4 infections in patients in the treatment group (5.0%) were documented after two vaccinations (*Table 1*). Effective titers of SARS-CoV-2 anti-spike IgG probably linked to protection from severe COVID-19 were thus defined by values ≥2.45 $\log_{10}$ BAU/mL, i.e., ≥282.0 BAU/mL, the mean estimated titer in patients in follow-up care 12 weeks after second vaccination. A total of 62.2% (N=46/74) of patients reached effective titers despite active GI cancer and anticancer treatment 4 weeks after second vaccination. This response rate was significantly reduced compared to patients in follow-up care, who showed effective titers in 96.3% of patients (N=26/27; P<0.01) (*Table 1*). Compared to the titers after 4 weeks, 12 weeks after second vaccination adequate response rates concerning SARS-CoV-2 anti-spike IgG (≥282.0 BAU/mL) were significantly reduced to 39.3% (N=22/56; P=0.02) of patients under treatment versus to 60.0% (N=21/35; P<0.01) of patients in follow-up care. The difference between the groups decreased and was non-significant at this point of time (P=0.09) (*Table 1*).

Seropositivity for SARS-CoV-2 surrogate NABs

Additionally, we focused on SARS-CoV-2 surrogate NAB 12 weeks after second vaccination. Mean titers differed significantly between patients under treatment (61.38%; 95% CI: 53.29% to 69.48%; P<0.01) and patients in follow-up care (84.81%; 95% CI: 74.80% to 94.83%). Impairment was most obvious in patients with HCC (55.31%; 95% CI: 40.20% to 70.42%; P<0.01) and PBN (56.63%; 95% CI: 40.99% to 72.27%; P<0.01). Patients under immune checkpoint inhibition had non-significantly higher SARS-CoV-2 surrogate NAB titers (95.65%; 95% CI: 55.01% to 136.29%; P=0.61) (*Table 2* and *Figure 2*).

Again, the mean estimated titer of patients in follow-up care 12 weeks after second vaccination (≥85.0%) was defined as effective. Titers of SARS-CoV-2 surrogate NAB ≥85.0% were found in 32.7% (N=17/52) of patients under treatment versus 70.6% (N=24/34) in patients of the control group, marking a significant difference (P<0.01) (*Table 1*).

Impact of immunosuppression, age, sex, and overcome COVID-19 on SARS-CoV-2 immunogenicity after second vaccination

In a multivariate analysis, we focused on co-factors potentially influencing immunogenicity after vaccination for SARS-CoV-2.

In situations of additional immunosuppression, titers of SARS-CoV-2 anti-spike IgG (–0.62 $\log_{10}$ BAU/mL; 95% CI: –1.01 to –0.23; P<0.01) and of SARS-CoV-2 surrogate NAB (–35.49%; 95% CI: –52.59 to –18.38; P<0.01)

Table 2 Titers of SARS-CoV-2 anti-spike IgG and SARS-CoV-2 surrogate NAB following vaccination

	SARS-CoV-2 anti-spike IgG						SARS-CoV-2 surrogate NAB		
	4 weeks			12 weeks			12 weeks		
	Estimate (log_{10} BAU/mL)	95% CI	P	Estimate (log_{10} BAU/mL)	95% CI	P	Estimate (%)	95% CI	P
Treatment									
Off treatment >1 year (Ref.)	3.06	2.79–3.32		2.45	2.20–2.71	<0.01	84.81	74.80–94.83	
Under treatment	2.47	2.29–2.64	<0.01	2.11	1.92–2.29	0.08	61.38	53.29–69.48	<0.01
Tumor type									
Off treatment >1 year	3.06	2.79–3.32		2.45	2.20–2.71	<0.01	84.81	74.80–94.83	
PBN[a]	2.50	2.14–2.85	0.02	2.16	1.78–2.54	0.12	56.63	40.99–72.27	<0.01
CRC	2.18	1.78–2.57	<0.01	2.18	1.74–2.61	0.01	60.36	39.67–81.05	0.04
GEJC[b]	2.44	1.89–2.89	0.04	2.18	1.53–2.84	0.26	88.30	59.04–117.56	0.83
HCC	2.52	2.15–2.88	0.02	2.05	1.67–2.43	0.47	55.31	40.20–70.42	<0.01
NET	2.60	2.15–3.06	0.10	2.07	1.59–2.55	0.75	65.70	45.01–86.39	0.11
Other[c]	2.82	2.11–3.53	0.55	2.12	1.38–2.86	0.73	69.30	35.51–103.09	0.39
Disease status									
Local (Ref.)	2.80	2.59–3.01		2.30	2.09–2.51		74.81	65.89–83.74	
Metastatic	2.58	2.35–2.81	0.16	2.18	1.93–2.42	0.47	68.13	57.02–79.24	0.36
Tumor recurrence	1.85	1.30–2.39	<0.01	1.86	1.32–2.41	0.05	53.21	29.83–76.60	0.09
Type of treatment									
Off treatment >1 year (Ref.)	3.06	2.79–3.32		2.45	2.20–2.71	<0.01	84.81	74.80–94.83	
Chemotherapy	2.42	2.14–2.71	<0.01	2.02	1.71–2.33	0.25	60.22	47.03–73.40	<0.01
Immunotherapy	2.77	1.99–3.56	0.51	2.35	1.42–3.29	0.64	95.65	55.01–136.29	0.61
Targeted therapy	2.63	2.20–3.07	0.11	2.34	1.88–2.79	0.17	76.88	56.56–97.19	0.49
Local therapy	2.54	2.05–3.03	0.07	1.84	1.32–2.37	0.74	49.33	27.61–71.05	<0.01
Combined therapy[d]	2.37	2.03–2.70	<0.01	2.13	1.78–2.48	0.04	56.02	41.65–70.39	<0.01
Intention of treatment									
Off treatment >1 year (Ref.)	3.06	2.79–3.32		2.45	2.20–2.71	<0.01	84.81	74.80–94.83	
Neoadjuvant	2.00	1.55–2.45	<0.01	1.72	1.25–2.19	0.15	58.37	38.72–78.01	0.02
Adjuvant	2.41	1.95–2.88	0.02	2.38	1.87–2.89	0.03	54.60	28.24–80.96	0.04
Palliative	2.58	2.37–2.78	0.01	2.15	1.93–2.37	0.22	62.99	53.43–72.55	<0.01

Shown are the results of linear mixed model analysis for the SARS-CoV-2 anti-spike IgG and linear model analysis for the SARS-CoV-2 surrogate NAB. Results are reported by mean estimates, 95% confidence intervals and associated P values. [a], pancreas cancer, cholangiocellular cancer, gallbladder cancer; [b], gastric cancer, oesophageal cancer; [c], gastrointestinal stromal tumor, carcinoma of unknown primary; [d], chemotherapy + targeted therapy or chemotherapy + local therapy. IgG, immunoglobulin G; NAB, neutralization antibody; Ref., reference; PBN, pancreaticobiliary neoplasm; CRC, colorectal cancer; GEJC, gastro-oesophageal-junction cancer; HCC, hepatocellular cancer; NET, neuroendocrine tumor; BAU, binding antibody units.

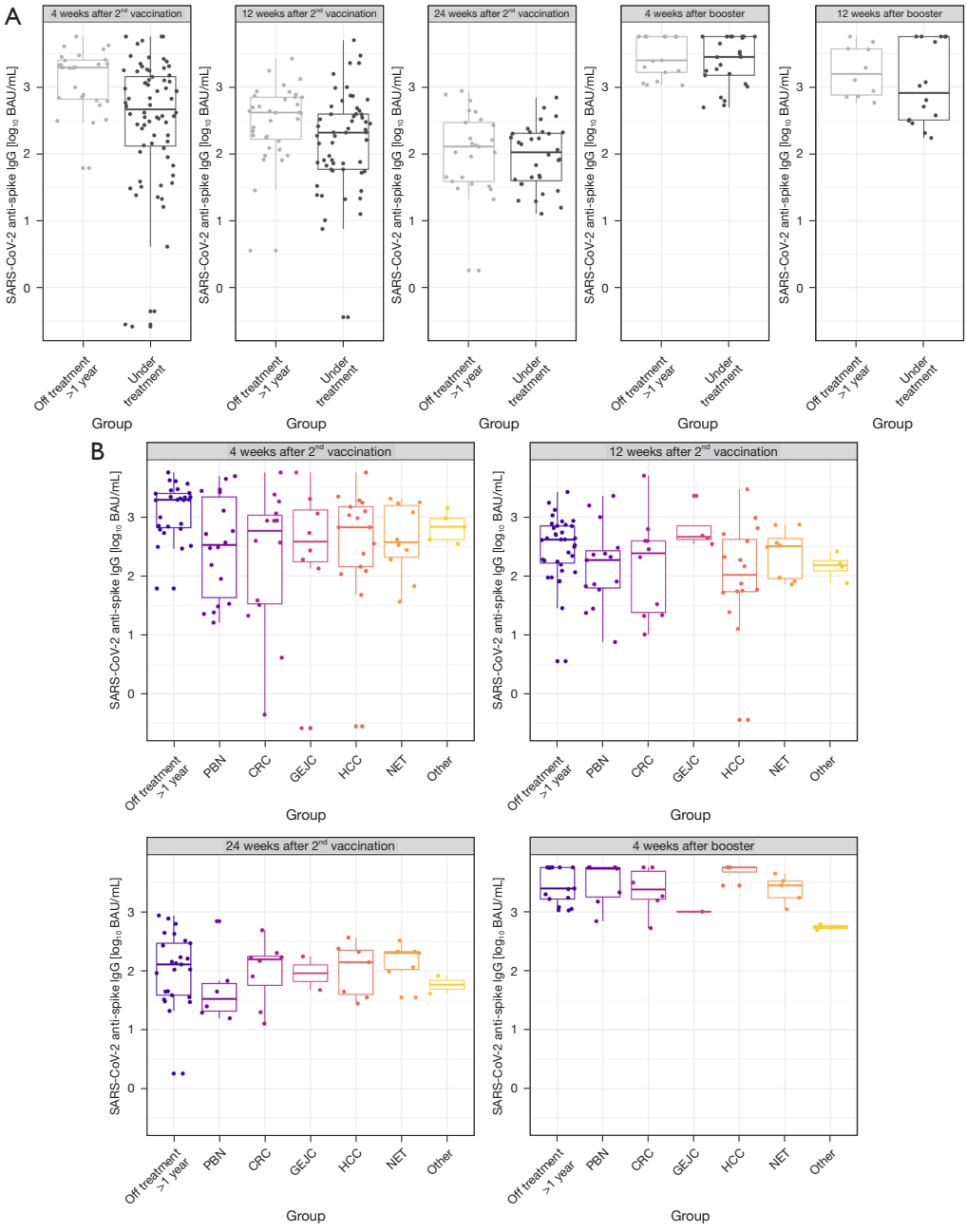

Figure 1 SARS-CoV-2 anti-spike IgG titers. Log_{10} SARS-CoV-2 anti-spike IgG titer of patients off treatment >1 year and patients under treatment at week 4, 12, 24 following second vaccination and at week 4 and week 12 after booster vaccination (A), impact of tumor types (B). Length of box represents the interquartile range, horizontal line shows the mean log_{10} SARS-CoV-2 anti-spike IgG titer, the whiskers denote the area which contains 95% of the data. PBN = pancreas cancer, cholangiocellular cancer, gallbladder cancer; GEJC = gastric cancer, oesophageal cancer; Other = gastrointestinal stromal tumor, carcinoma of unknown primary, combined therapy = chemotherapy + targeted therapy or chemotherapy + local therapy. IgG, immunoglobulin G; BAU, binding antibody units; PBN, pancreaticobiliary neoplasms; CRC, colorectal cancer; GEJC, gastro-oesophageal-junction cancer; HCC, hepatocellular cancer; NET, neuroendocrine tumor.

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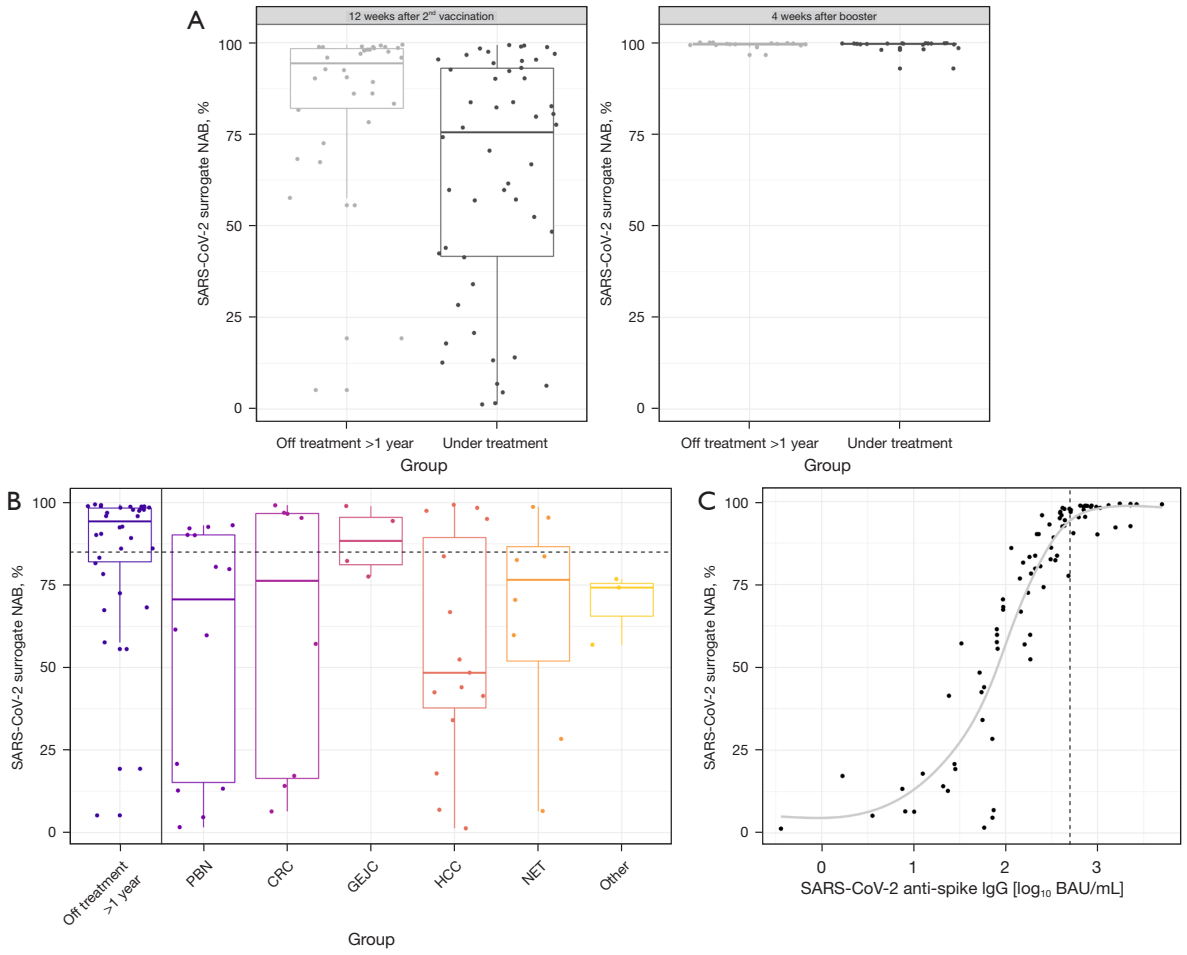

Figure 2 SARS-CoV-2 surrogate NAB titers and association between $\log_{10}$ SARS-CoV-2 anti-spike IgG titers and SARS-CoV-2 surrogate NAB titers. SARS-CoV-2 surrogate NAB titers of patients off treatment >1 year and patients under treatment at week 12 following second vaccination and week four after booster vaccination (A), impact of tumor types (B), association between $\log_{10}$ SARS-CoV-2 anti-spike IgG titer and SARS-CoV-2 surrogate NAB titer with coefficient of 0.93 (C). Length of box represents the interquartile range, horizontal line shows the mean SARS-CoV-2 surrogate NAB titer, the whiskers denote the area which contains 95% of the data; horizontal dashed line corresponds to a proportion of 85.0% SARS-CoV-2 surrogate NAB; vertical dashed line corresponds to the $\log_{10}$ equivalent of 482.0 BAU/mL (corresponding to 2.68 $\log_{10}$ BAU/mL) being linked to effective titers of SARS-CoV-2 surrogate NAB (≥85.0%). PBN = pancreas cancer, cholangiocellular cancer, gallbladder cancer; GEJC = gastric cancer, oesophageal cancer; Other = gastrointestinal stromal tumor, carcinoma of unknown primary, combined therapy = chemotherapy + targeted therapy or chemotherapy + local therapy. NAB, neutralization antibody; PBN, pancreaticobiliary neoplasms; CRC, colorectal cancer; GEJC, gastro-oesophageal-junction cancer; HCC, hepatocellular cancer; NET, neuroendocrine tumor; IgG, immunoglobulin G.

were significantly lower. Active tumor treatment was an independent factor for reduced immunogenicity and led to additionally significantly decreased SARS-CoV-2 anti-spike (–0.52 $\log_{10}$ BAU/mL; 95% CI: –0.84 to –0.20; P<0.01) and SARS-CoV-2 surrogate NAB titers (–17.88%; 95% CI: –30.59 to –5.16; P<0.01) (*Table 3* and *Figure 3*).

With increasing age and in male sex, there were minor, non-significant decreases in antibody titers. Infection with

Table 3 Suspected factors influencing immunogenicity for SARS-CoV-2

	SARS-CoV-2 anti-spike IgG			SARS-CoV-2 surrogate NAB		
	Effect (log_{10} BAU/mL)	95% CI	P	Effect (%)	95% CI	P
Intercept (Ref.)	3.36	2.53 to 4.20	–	95.31	56.14 to 134.47	–
Under treatment	−0.52	−0.84 to −0.20	<0.01	−17.88	−30.59 to −5.16	<0.01
Additional immunosuppression[a]	−0.62	−1.01 to −0.23	<0.01	−35.49	−52.59 to −18.38	<0.01
Age	−0.01	−0.02 to 0.01	0.51	−0.12	−0.70 to 0.47	0.69
Sex (male)	−0.03	−0.30 to 0.24	0.84	−1.80	−14.04 to 10.44	0.77
History of SARS-CoV-2 infection	0.51	−0.17 to 1.18	0.15	10.80	−29.30 to 50.91	0.59
Time effect 12 weeks	−0.60	−0.82 to −0.38	<0.01	–	–	–
Treatment + time effect 12 weeks	0.25	−0.02 to 0.52	0.07	–	–	–

Shown are the results of multivariable linear mixed model analysis for the SARS-CoV-2 anti-spike IgG and multivariable linear model analysis for SARS-CoV-2 surrogate NAB. Results are reported by point estimates, 95% confidence intervals and associated P values. [a], co-medication with corticosteroids, methotrexate or calcineurin inhibitor, or HIV-infection. Ref., reference; IgG, immunoglobulin G; BAU, binding antibody units; NAB, neutralization antibody; CI, confidence interval; HIV, human immunodeficiency virus.

SARS-CoV-2 prior to vaccination led to a slight increase in SARS-CoV-2 anti-spike IgG as well as SARS-CoV-2 surrogate NAB (*Table 3*).

Vaccination failure

Ineffective vaccination response rates were considered when levels of SARS-CoV-2 anti-spike IgG were <282.0 BAU/mL and/or of SARS-CoV-2 surrogate NAB <85.0% at week 12 after second vaccination.

Overall, 59.6% (N=31/52) of patients in the treatment group versus 29.4% (N=10/34) of patients in follow-up care failed to reach levels of SARS-CoV-2 anti-spike IgG ≥282.0 BAU/mL and of SARS-CoV-2 surrogate NAB ≥85.0% at week 12 after second vaccination (P=0.01). In our cohort, we discussed this constellation as total vaccination failure.

Ineffective levels of SARS-CoV-2 surrogate NAB (<85.0%) significantly differed between patients in the treatment group (67.3%; N=35/52) and patients in the control group (29.4%; N=10/34; P<0.01) 12 weeks after second vaccination. Concerning ineffective levels of SARS-CoV-2 anti-spike IgG (<282.0 BAU/mL), this difference was minor and non-significant at the same time-point: ineffective levels were found in 60.7% (N=34/56) in patients under active treatment versus in 40.0% (N=14/35) in patients in follow-up care (P=0.09). Isolated insufficient levels of SARS-CoV-2 surrogate NAB but sufficient levels of SARS-CoV-2 anti-spike IgG (N=4/86) or vice versa (N=4/86) were discussed as partial vaccination failure.

In view of the association between total and NABs with a coefficient of 0.93 (*Figure 2*), reaching titers of SARS-CoV-2 anti-spike IgG ≥482.0 BAU/mL (corresponding to 2.68 log_{10} BAU/mL), effective levels of SARS-CoV-2 surrogate NAB (≥85.0%) could be taken for granted probably indicating full protection from severe COVID-19 by vaccination in our cohort of patients (*Figure 2*). This could be found in 29.1% of patients in both groups (N=25/86).

Effect of booster vaccination on SARS-CoV-2 anti-spike IgG and surrogate NABs

Finally, we assessed the effect of booster vaccination. Prior to booster vaccination, titers of SARS-CoV-2 anti-spike IgG further decreased in all patients (24 weeks after second vaccination) (*Figure 1*). Four weeks after booster vaccination, titers of SARS-CoV-2 anti-spike IgG significantly increased, both, in patients under active treatment (3.33 log_{10} BAU/mL; 95% CI: 3.10 to 3.56; P<0.01) and in patients in follow-up care (3.50 log_{10} BAU/mL; 95% CI: 3.22 to 3.79; P<0.01) compared to patients under active treatment and to patients in follow-up-care 12 weeks after second vaccination, respectively (*Figure 1*). Correspondingly, titers of SARS-CoV-2 surrogate NAB also increased in all patients (*Figure 2*). There was no significant difference between mean titers of patients under active treatment, independent of tumor type, and patients in

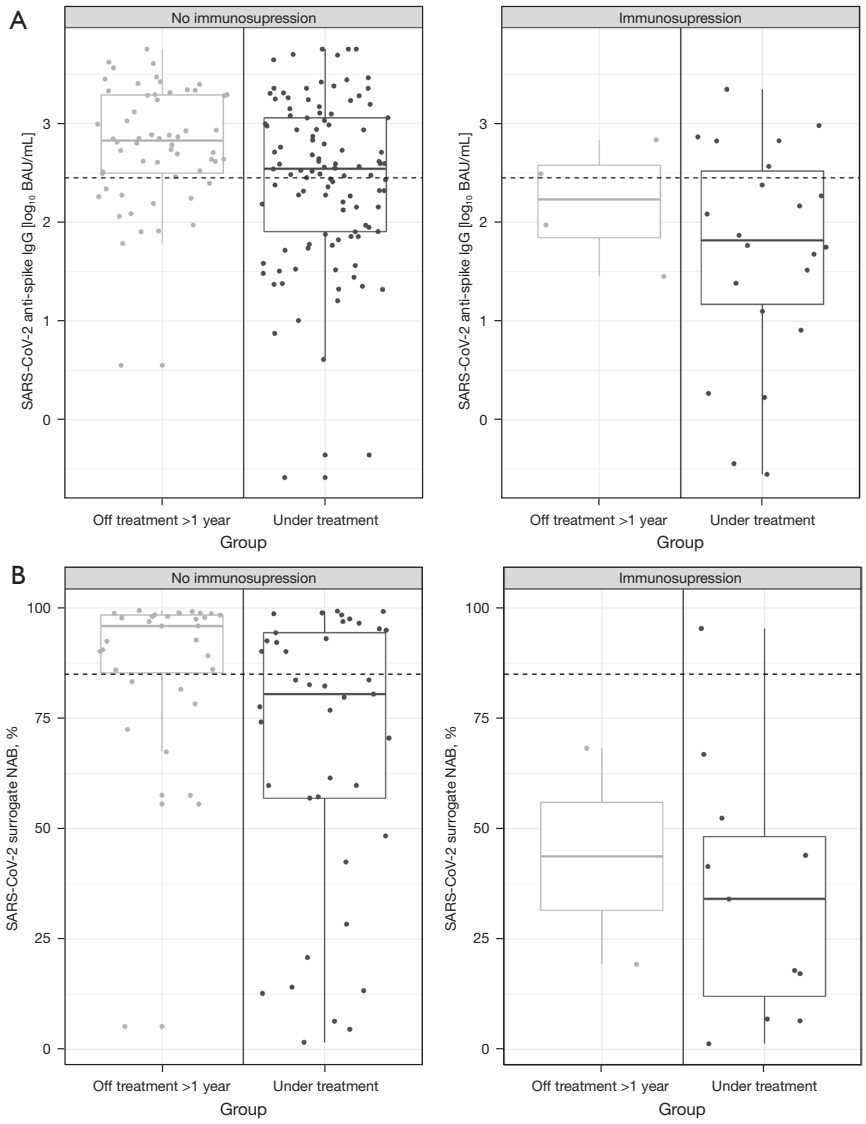

Figure 3 Effect of immunosuppression on antibody titers. Log_{10} SARS-CoV-2 anti-spike IgG titer (A) and SARS-CoV-2 surrogate NAB titer (B) of patients off treatment >1 year and patients under treatment according to the effect of immunosuppression (co-medication with corticosteroids, methotrexate or calcineurin inhibitor, HIV infection, or chronic hepatitis B/C-infection), data 12 weeks after vaccination. Length of box represents the interquartile range, horizontal line shows the mean log_{10} SARS-CoV-2 anti-spike IgG titer (B)/SARS-CoV-2 surrogate NAB titer (A), the whiskers denote the area which contains 95% of the data; dashed line correspond to the log_{10} equivalent of 282.0 BAU/mL (2.45 log_{10} BAU/mL) (A) or 85.0% (B). IgG, immunoglobulin G; BAU, binding antibody units; NAB, neutralization antibody; HIV, human immunodeficiency virus.

follow-up care after booster vaccination (*Figure 1*). Effective titers of SARS-CoV-2 anti-spike IgG (≥282.0 BAU/mL) and of SARS-CoV-2 surrogate NAB (≥85%) were found in all patients. Patients with additional immunosuppression still showed significantly reduced titers of SARS-CoV-2 anti-spike IgG (–0.39 log_{10} BAU/mL; 95% CI: 0.18 to

–2.22; P=0.03).

Titers of SARS-CoV-2 anti-spike IgG were again found to decrease 12 weeks after booster vaccination in patients under treatment (3.19 $\log_{10}$ BAU/mL; 95% CI: 2.91 to 3.46; P=0.55) as well as in patients in follow-up care (3.23 $\log_{10}$ BAU/mL; 95% CI: 2.88 to 3.58; P=0.63) compared to titers four weeks after booster vaccination.

Infections with SARS-CoV-2 (including B1.1.529.2) were found in three patients with active GI cancer and one patient in follow-up care.

Adverse side effects

In self-report, injection-side pain (17.0%, N=21/125), erythema (6.0%, N=7/125) and swelling (6.0%, N=7/125) were the most common local side effects in all patients of our cohort. Regarding systemic side effects, fatigue (17.0%, N=21/125) was stated most frequently.

A case of grade IV pneumonitis after therapy with immune checkpoint inhibitor atezolizumab (programmed death-ligand 1 antibody) in a patient with HCC was discussed as possibly increased toxicity of anticancer therapy following SARS-CoV-2 vaccination. Moreover, a cutaneous reaction under panitumumab possibly related to the vaccination was documented in a patient with CRC.

Discussion

In the present study, we showed that two vaccinations for SARS-CoV-2 in patients with active GI cancer under therapy was safe but induced significantly lower efficient response rates and higher rates of vaccination failure compared to patients in follow-up care being at least one year off any anticancer therapy. As impairment was particularly pronounced in patients with CRC, HCC and/or PBN, an association with the tumor type must be discussed. First booster vaccination improved antibody titers and balanced the differences between the groups. Since titers decreased again already 12 weeks after booster vaccination, earlier further booster vaccinations may be individually recommended in patients with GI cancer after antibody assessment.

No antibody titer associated with protection from severe COVID-19 was defined so far which would be decisive for recommendations concerning antiviral therapy, passive immunization and/or individual booster vaccinations. Titers of SARS-CoV-2 anti-spike IgG ≥264.0 BAU/mL were described as effective against B1.1.7 (alpha) previously (31). Since there was only one case of mild infection with SARS-CoV-2 in our cohort after second vaccination in patients in follow-up care, we could assume that mean titers of SARS-CoV-2 anti-spike IgG (≥282.0 BAU/mL) and of SARS-CoV-2 surrogate NAB (≥85.0%) were possibly effective to prevent severe COVID-19. This was also true after booster vaccination indicating even protection from B1.1.529.2 (i.e., omicron BA.2).

In our cohort of patients, we could demonstrate a stable association between titers of SARS-CoV-2 surrogate NAB and SARS-CoV-2 anti-spike IgG with a strong correlation coefficient of 0.93. Correlations in that range with values of up to 0.91 were described (32,33). By reaching a titer of SARS-CoV-2 anti-spike IgG ≥482.0 BAU/mL, protective levels of SARS-CoV-2 surrogate NAB (≥85.0%) could be assumed, indicating full protection by vaccination in our cohort.

Contrary to published studies so far, we analyzed a cohort of patients with active GI cancer and patients in follow-up care without current anticancer therapy. Unexpectedly, only 62.2% of patients with GI cancer under active anticancer treatment compared to 96.3% of patients in follow-up care (P<0.01) reached effective titers of SARS-CoV-2 anti-spike IgG (≥282.0 BAU/mL) four weeks after second vaccination in our cohort. Thus, we could identify active GI cancer and anticancer therapy as high-risk factors for reduced response to SARS-CoV-2 vaccination since patients in follow-up care achieved significantly higher titers for SARS-CoV-2 anti-spike IgG almost similar to that found in healthy people.

Consistent with several studies including patients with different kind of solid tumors, mean antibody titers of SARS-CoV-2 anti-spike IgG were also significantly lower in the treatment group. However, the proportion of patients with solid cancer with positive antibody responses was comparatively higher than in our cohort and ranged from 84.1% to 95.0% of cases versus 100% in participants of healthy control groups (10-12,14-16). Interestingly, our patients seemed to resemble more patients with hematological cancer who also showed low positive response rates of only 60.0% after two vaccinations than patients with solid tumors (13,15).

Similar to our findings, titers decreased overtime which we could be proved in both groups of patients highlighting the necessity of booster vaccination. However, we observed a more pronounced decreasing in the group of patients with active GI cancer under treatment. Other cohorts including all solid cancer still had positive response rates of 79.0%

compared to 84.0% in healthy participants (P=0.32) in a follow up of 6 months after second vaccination (34).

Regarding levels of surrogate NAB, we found a more pronounced impairment in patients with active GI cancer compared to patients in follow-up care. Twelve weeks after second vaccination, only 32.7% of patients under treatment versus 70.6% of patients in follow-up care reached protective levels of SARS-CoV-2 surrogate NAB (≥85.0%), empathizing again a clinically relevant impairment in patients with active GI cancer. Data of SARS-CoV-2 surrogate NAB in patients with cancer are rare and therefore difficult to compare. Rates of positive levels of SARS-CoV-2 surrogate NAB ranged from 81.3% to 69.0% in patients with solid cancer without distinguishing between tumor types and without a definition concerning protective levels (12,21). Interestingly, patients with hematological malignancies showed worse rates, down to 41.0% in patients with chronic lymphatic leukemia (CLL) or B-cell-non-Hodgkin-lymphoma (B-NHL), which was similar to our findings (17,19).

Finally, rates of total vaccination failure (SARS-CoV-2 anti-spike IgG <282.0 BAU/mL and SARS-CoV-2 surrogate NAB <85.0%) were significantly higher in patients with GI cancer under active treatment (59.6%) than in follow-up patients with a past medical history of GI cancer (29.4%). Studies focusing only on SARS-CoV-2 anti-spike IgG found positive levels between 81.0% and 95.5% in patients with solid tumors (11,13). Patients with CLL or B-NHL had even lower rates with only 52% and 49%, respectively (17-19). Comparing hematological and solid cancers, vaccination response rates differed significantly (13). Again, our cohort with only GI-cancer seemed to resemble patients with hematological malignancies, more than patients with other solid tumors.

Partial vaccination failure (isolated insufficient levels of SARS-CoV-2 surrogate NAB but sufficient levels of SARS-CoV-2 anti-spike IgG or vice versa) was rare in our cohort but must also be taken into consideration indicating a considerable discrepancy in real effectiveness of vaccination. Although the exact form of association is yet unclear, both, SARS-CoV-2 anti-spike IgG as well as SARS-CoV-2 surrogate NAB are crucial for protection from infection or severe COVID-19 (22,35-38).

In order to understand, why our cohort of patients with active GI cancer showed unexpectedly highly impaired immunogenicity to vaccination for SARS-CoV-2 compared to data in patients with solid tumors in general published to date, we further analyzed our patients regarding tumor entity, therapy and further risk factors. Interestingly, our cohort of patients included a comparatively high proportion of patients with primary pancreatico-hepato-biliary tumors: 23.5% of cases were HCC and 24.5% PBN, followed by 20% CRC. Studies proving impaired immunogenicity after vaccination for SARS-CoV-2 in patients with solid cancer only included up to 25% patients suffering from GI cancer (11,12). Moreover, for the time being, no differentiated analyses regarding different GI tumor types separately were performed in these studies so far. However, the differentiated analysis presented here evidently explained our results, since SARS-CoV-2 anti-spike IgG were lowest in CRC and SARS-CoV-2 surrogate NAB were lowest in patients with HCC and PBN. Especially, hepato-pancreato-biliary tumors as well as secondary hepatic metastases from GI cancers are well known to impair host immunity, somewhat explaining the results concerning impaired SARS-CoV-2 immunogenicity (24,25). Furthermore, underlying liver cirrhosis/ fibrosis especially in patients with HCC and in part with CCC is linked to immunodeficiency (26). In general, the capacity to develop adequate response rates to vaccinations in patients with liver dysfunction is reduced as shown for hepatitis B and/ or pneumococcal vaccines previously (27,28). Finally, an under-release of cytokines must be taken into account in patients with CRC as shown previously (39).

Regarding the effects of anticancer therapy, we found that patients under immune checkpoint inhibition seemed to form higher SARS-CoV-2 surrogate NAB titers compared to patients under cytostatic chemotherapy as shown previously (12). Compared to therapy regimes of other solid tumor types (for instance breast or lung cancer), use of immune checkpoint inhibitors is less common in patients with GI cancer also partly explaining the more pronounced impairment of SARS-CoV-2 immunogenicity in patients with GI cancer.

Considering the high proportion of patients with HCC, PBN and CRC under anticancer and the reduced use of immunotherapy, impaired COVID-19 immunogenicity in patients with active GI cancer seems obviously to be tumor-related. Of note, no impact of the disease status (local, metastatic, recurrent) on immune responses was observed. Regarding risk for impaired COVID-19 immunogenicity, GI tumor types could be classified as a solid borderline tumor group with similarities to hematological malignancies most likely explained by an underlying immunodeficiency. Further studies in other cohort of patients to confirm these findings and further particularities of these patients should

be studied next.

Furthermore, additional immunosuppression was found to worsen SARS-CoV-2 immunogenicity with significantly lower titers of SARS-CoV-2 anti-spike IgG and SARS-CoV-2 surrogate NAB. This reduction was more evident in patients under active tumor treatment and was in line with other studies in cancer patients (10,12,14,16). Contrary, infection with SARS-CoV-2 prior to vaccination appeared to increase immunogenicity after SARS-CoV-2 vaccination since these patients showed similar increase in total and in surrogate NAB compared to patients in follow-up care. Other studies also demonstrated a favorable effect of a past medical history of COVID-19 on antibody titers in healthy persons as well as in patients with cancer (12,40,41).

Regarding age and sex, no significant influence on antibody titers could be observed. Data on the impact of age and sex are inconsistent as some showed no evidence for an effect (10), while others demonstrated a better immune response in female patients and patients under the age of 65 (12,17).

According to our data, booster vaccination can overcome differences between patients under active treatment and in follow-up care, since all patients reached effective levels of SARS-CoV-2 anti-spike IgG (≥282.0 BAU/mL) and NAB (≥85.0%). This is in line with clinical observations that rates of confirmed COVID-19 or severe COVID-19 were significantly reduced after booster vaccination (42). Moreover, the immunogenic potential of booster vaccination could be confirmed, even for patients with cancer having been seronegative after first and second vaccination (43,44). In patients with solid cancer, NAB against omicron was detectable in 90% of patients after booster vaccination (45). Especially for patients with hepatobiliary carcinoma, booster vaccinations are thus already recommended (20). Of note, data on the effect of booster vaccination in patients with cancer are sparse for the time being. However, as we found that titers of SARS-CoV-2 anti-spike IgG again decreased 12 weeks after booster vaccination, earlier further booster vaccinations should be taken into consideration individually in patients with GI cancer after antibody assessment.

The present study has some limitations. Consequent follow-up of all patients was not possible as some patients died, and some patients had a reduced performance status while continuing their medical treatment in the department of palliative care. While presenting extensive data on humoral immunogenicity for SARS-CoV-2, data on cellular immune response are missing.

The strengths of our study is a more conservative analysis than previous studies since we not only regarded positive response rates to vaccination but defined titers being linked to protection. However, this makes it difficult to compare our data with other studies due to different cut-off values for antibody testing (sole test positivity versus reaching individually defined effective titers), different time points for antibody testing and heterogeneous distribution of tumor entities in other studies. This might have contributed to the overall worse response rates in patients with GI-cancer under active treatment compared to patients with other solid tumors in the same situation.

Conclusions

In summary, we showed that patients with GI cancer under anticancer therapy reached unexpectedly worse response rates to vaccination for SARS-CoV-2 than comparable patients in follow-up care. Impairment was particularly pronounced for patients with HCC, PBN and CRC. Thus, we claim that effects are tumor-related due to underlying immunodeficiency. However, we could define levels of SARS-CoV-2 anti-spike IgG (≥282.0 BAU/mL) as well as of SARS-CoV-2 surrogate NAB (≥85.0%) having been associated with protection from severe COVID-19 marking a landmark for clinicians as well as for future studies. Booster vaccination finally led to effective antibody titers in all tested patients. Considering waning immunity as well as antibody escape phenomena of variant of concern Omicron (46,47), follow-up analyses putting emphasis on long-term immunogenicity following vaccination with Omicron-adapted vaccines into account.

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Footnote

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Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1065/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1065/coif). MBM received consulting fees; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events; and support for attending meetings and/or travel from Gilead, Pfizer and Virology Education. CB received grants or contracts from any entity; consulting fees; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events; and support for attending meetings and/or travel; from DZIF, Hector Foundation, DFG, NEAT ID, AbbVie, Gilead JnJ, MSD and ViiV. He participated on a Data Safety Monitoring Board or Advisory Board; and was leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid (Mavmet Study, DAIG, EACS). GJR received travel expenses and honoraria from Gilead. SS received grants or contracts from any entity; and support for attending meetings and/or travel from German Center for Infection Research, DFG, Gilead, Abbvie and Johnson&Johnson. He holds stock or stock options (MIG9 Fonds containing BionTech). MAGC has contributed to advisory boards for Roche, Eisai. BMS, MSD and AZ. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the institutional review board of the Medical Faculty of the University of Bonn (No. 341/17 and 023/22). Written informed consent was obtained from all participating patients.

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2.2 Longitudinale SARS-CoV-2-Impfstudie bei PatientInnen mit gastrointestinalen Karzinomen – Effekt der ersten Auffrischungsimpfung

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Nachdem vor allem PatientInnen mit hepatobiliären Karzinomen besonders niedrige humorale Titer nach der Primärimpfserie gegen SARS-CoV-2 aufwiesen, standen diese PatientInnen im Fokus der Folgearbeit. Dabei wurden zwischen Januar 2021 und Juli 2022 der Effekt einer ersten wildtypbasierten Auffrischungsimpfung und damit die Basisimmunität analysiert. Neu wurden ergänzend die zelluläre Immunantwort und neben der Immunogenität insbesondere auch der klinische Verlauf etwaiger Durchbruchinfektionen betrachtet (vgl. Kapitel 1.8.1).

Von 101 in die Studie aufgenommenen PatientInnen hatten 59 PatientInnen ein hepatobiliäres Karzinom unter tumorspezifischer Therapie (Referenzgruppe). In der Kontrollgruppe hatten von 42 PatientInnen mit gastrointestinalen Karzinomen 12 PatientInnen ein hepatobiliäres Karzinom ohne Hinweis auf Tumoraktivität und ohne Indikation einer fortlaufenden Erhaltungstherapie.

Die signifikant niedrigeren humoralen Impftiter bei PatientInnen der Referenzgruppe im Vergleich zu PatientInnen der Kontrollgruppe nach einer Primärimpfserie gegen SARS-CoV-2 (vgl. Kapitel 2.1) ließen sich in dieser Kohorte bestätigen. In einer multivariablen Analyse waren eine chemotherapeutische Behandlung, eine systemische Immundefizienz und/oder ein Diabetes mellitus mit signifikant niedrigeren Titern assoziiert. Im zeitlichen Intervall fielen die Titer in beiden Gruppen weiter ab, wobei dies in der Referenzgruppe ausgeprägter war. Durch eine Auffrischungsimpfung, die zwei Drittel der PatientInnen zu diesem Zeitpunkt erhalten hatten, konnten die Titer stabilisiert werden. Dabei war die Höhe der Titer zwischen der Referenz- und der Kontrollgruppe ausgeglichen. Außerdem konnte bei allen PatientInnen, die nach zwei Impfungen noch keinen Titer aufgewiesen hatten, eine Serokonversion nachgewiesen werden. Auch in Bezug auf die zelluläre Immunantwort ergab sich nach der SARS-CoV-2-Auffrischungsimpfung kein Unterschied zwischen der Referenz- und der

Kontrollgruppe. Insgesamt kam es zu wenigen, mild verlaufenden Durchbruchinfektionen mit SARS-CoV-2 nach der Auffrischungsimpfung in unserer Kohorte (n=3).
Ein erneuter Abfall der Spiegel von SARS-CoV-2 anti-spike IgG bei allen in die Studie aufgenommenen PatientInnen im zeitlichen Intervall war deutlich geringer als nach der Primärimpfserie. Diese Entwicklung galt es im Weiteren aber zu reevaluieren (vgl. Kapitel 2.3).

PatientInnen mit hepatobiliären Karzinomen sollten entsprechend motiviert werden, die Impfempfehlungen, insbesondere auch die Auffrischungsimpfung, umzusetzen. Hierdurch ist eine effektive Basisimmunität gegen SARS-CoV-2 zu erzielen. Speziell PatientInnen, bei denen die Impfempfehlungen der STIKO und der Fachgesellschaften nicht umgesetzt worden sind, sollten weiterhin antivirale Therapien angeboten werden. Die insgesamt geringe Zahl und die milden Verläufe von Durchbruchinfektionen mit SARS-CoV-2 werteten wir als klinisches Korrelat einer effektiven Immunantwort. Offen blieb die Frage, ob die Basisimmunität ohne erneuten, variantenadaptierten SARS-CoV-2-Antigenkontakt aufrechterhalten werden kann und ob die Basisimmunität auch einen effektiven Schutz gegen neue, speziell die Omikron-Varianten von SARS-CoV-2 würde bieten können.

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Deficient Immune Response following SARS-CoV-2 Vaccination in Patients with Hepatobiliary Carcinoma: A Forgotten, Vulnerable Group of Patients

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Keywords
COVID-19 · Vaccination · SARS-CoV-2 neutralization · Hepatobiliary carcinoma · Chronic liver disease · Liver cirrhosis · Waning immunity

Abstract

Introduction: Data on immune response rates following vaccination for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in patients with hepatobiliary carcinoma (HBC) are rare. However, impaired immunogenicity must be expected due to the combination of chronic liver diseases (CLDs) with malignancy and anticancer treatment. ***Methods:*** In this prospective, longitudinal study, 101 patients were included, of whom 59 were patients with HBC under anticancer treatment. A cohort of patients with a past medical history of gastrointestinal cancer, of whom 28.6% had HBC without detectable active tumor disease having been off therapy for at least 12 months, served as control. Levels of SARS-CoV-2 anti-spike IgG, surrogate neutralization antibodies (sNABs), and cellular immune responses were compared. In uni- and multivariable subgroup analyses, risk factors for impaired immunogenicity were regarded. Data on rates and clinical courses of SARS-CoV-2 infections were documented. ***Results:*** In patients with HBC under active treatment, levels of SARS-CoV-2 anti-spike IgG were significantly lower (2.55 $\log_{10}$ BAU/mL; 95% CI: 2.33–2.76; $p < 0.01$) than in patients in follow-up care (3.02 $\log_{10}$ BAU/mL; 95% CI: 2.80–3.25) 4 weeks after two vaccinations. Antibody levels decreased over time, and differences between the groups diminished. However, titers of SARS-CoV-2 sNAB were for a longer time significantly lower in patients with HBC under treatment (64.19%; 95% CI: 55.90–72.48; $p < 0.01$) than in patients in follow-up care (84.13%; 95% CI:

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76.95–91.31). Underlying CLD and/or liver cirrhosis Child-Pugh A or B (less than 8 points) did not seem to further impair immunogenicity. Conversely, chemotherapy and additional immunosuppression were found to significantly reduce antibody levels. After a third booster vaccination for SARS-CoV-2, levels of total and neutralization antibodies were equalized between the groups. Moreover, cellular response rates were balanced. Clinically, infection rates with SARS-CoV-2 were low, and no severe courses were observed. ***Conclusion:*** Patients with active HBC showed significantly impaired immune response rates to basic vaccinations for SARS-CoV-2, especially under chemotherapy, independent of underlying cirrhotic or non-cirrhotic CLD. Although booster vaccinations balanced differences, waning immunity was observed over time and should be monitored for further recommendations. Our data help clinicians decide on individual additional booster vaccinations and/or passive immunization or antiviral treatment in patients with HBC getting infected with SARS-CoV-2.

Introduction

In October 2022, over 600 million cases of coronavirus disease-2019 (COVID-19) caused by the newly identified severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) were confirmed. By that time, more than 12.5 billion SARS-CoV-2 vaccine doses had been administered [1]. While for all persons – regardless of any comorbidities – three vaccinations were recommended [2], immunocompromised patients were encouraged to receive at least four vaccine doses. Most recently, immunization with vaccines adapted to the SARS-CoV-2 variant of concern (VOC), Omicron, has additionally been recommended for people aged 12 years or older [3].

Patients with chronic liver disease (CLD) are facing higher rates of SARS-CoV-2-associated morbidity and mortality [4, 5]. However, studies on response rates to well-known vaccines in patients with CLD have revealed impaired immune responses and faster antibody decline over time [6–9]. These effects were especially pronounced in cases of decompensated liver cirrhosis or in patients undergoing immunosuppressive therapy due to autoimmune hepatitis [10]. As expected, poor response rates to vaccinations for SARS-CoV-2 have also been found in almost a quarter of patients with CLD in published studies to date [11, 12]. Unfortunately, the group of patients with hepatobiliary carcinoma (HBC), i.e., hepatocellular carcinoma (HCC), cholangiocarcinoma (CCC), and gallbladder cancer (GBC), is underrepresented in these cohorts, leading to incomplete data concerning these patients [13, 14]. One would assume a pronounced impairment of immune responses. First, these patients are frequently confronted with underlying CLD, which is associated with immunodeficiency, resulting in reduced immune responses to vaccines as detailed above [13, 15]. Second, in patients with hematologic and in patients with different solid cancer types, immunogenicity after vaccination for SARS-CoV-2 was worse than in healthy people [16–18], especially under active anticancer treatment [19]. Taking these facts into consideration, patients with HBC were expected to be at high risk for severe COVID-19 due to impaired immune response rates to vaccination for SARS-CoV-2. The question remains whether malignancy per se, anticancer treatment, underlying liver pathologies, or a combination of these factors mainly contribute to this impairment. Here, we present prospective data on humoral and cellular response rates as well as on clinical efficacy and safety in a large cohort of patients with HBC under active anticancer treatment to further clarify the mentioned dubieties concerning immunogenicity following basic and booster vaccination for SARS-CoV-2.

Patients and Methods

Study Design

This prospective, longitudinal, observational study explores humoral and cellular immune response rates to SARS-CoV-2 vaccinations in a cohort of patients with HBC and gastrointestinal (GI) cancer treated at the Department of Internal Medicine I, Gastroenterology Oncology Section at the University Hospital of Bonn, Germany, between January 2021 and July 2022. Blood samples were drawn 4, 12, and 24 weeks after the second (basic vaccination) and third vaccination (first booster vaccination) for SARS-CoV-2. Seroprevalence of SARS-CoV-2 anti-spike antibodies (IgG) was analyzed at all time points, while SARS-CoV-2 surrogate neutralization antibodies (sNABs) were measured 12 weeks after the basic and 4 and 24 weeks after the first booster vaccination. To determine cellular immune response rates, a SARS-CoV-2 interferon gamma release assay (IGRA) was performed 4 and 24 weeks after the third vaccination. In addition, all patients who reported having received a second booster vaccination had their blood drawn again 4 weeks thereafter to re-measure humoral and cellular response rates.

The study was performed in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Medical Faculty of the University of Bonn (Nos. 341/17 and 023/22). Written informed consent was obtained from all participating patients.

Patient Characteristics and Eligibility Criteria

Patients with HBC (HCC, CCC, or GBC) under any active anticancer treatment or having finished their anticancer treatment

within the last 12 months without detectable cancer disease for the time being were eligible to be included. Those patients who were under best supportive care continuing their treatment in the department of palliative care were not included. Patients with a past medical history of GI cancer without detectable cancer disease for at least 12 months prior to inclusion were included as control group. Additionally, these patients did not receive oncologic treatment during the study period and in the last 12 months prior to inclusion to avoid any effects of active cancer disease and/or of anticancer therapy. Of note, at least 28.6% of the patients in this follow-up group had a past medical history of HBC. The control group features the following additional advantages and is therefore especially matchable. First, patients shared comparable risk factors, both for cancer pathogenesis and for severe COVID-19 (shown in Table 1). Second, all patients were treated in the same center according to standardized procedures. Basic vaccination with one of the SARS-CoV-2 vaccines authorized by the European Medicines Agency was obligatory for inclusion. Patients who refused to receive booster vaccinations were not excluded.

Relevant clinical data regarding risk factors for severe COVID-19, increased immunosuppression (suspected in patients with long-term immunosuppressive medication and chronic HIV infection with impaired immune status), as well as underlying cirrhotic and non-cirrhotic liver diseases (alcoholic liver disease, non-alcoholic fatty liver disease, chronic viral hepatitis, autoimmune hepatitis, primary sclerosing cholangitis, hemochromatosis, Budd-Chiari syndrome) were acquired from standardized medical records. Of note, only patients with liver cirrhosis Child-Pugh A or B (score <8) were included as patients with decompensated liver cirrhosis are not eligible for anticancer treatment. Finally, clinical data on side effects potentially associated with the vaccinations as well as on the course of infections with SARS-CoV-2 despite vaccinations were collected.

Assessment of Humoral and Cellular Response Rates

To quantify antibodies against SARS-CoV-2 spike receptor-binding domain (SARS-CoV-2 anti-spike IgG), SARS-CoV-2 IgG II Quant chemiluminescent microparticle immunoassay (Abbott) was used. SARS-CoV-2 sNABs in relation to all antibodies [%] were identified using a blocking ELISA detection tool (cPass^{TM} SARS-CoV-2 Neutralization Antibody Detection Kit; GenScript). This test detects functional virus neutralization strongly correlating with live-cell neutralization [20, 21]. Of note, its relevance concerning current SARS-CoV-2 (VOCs) is limited as neutralization of the SARS-CoV-2 wild type is identified by this test.

Cellular immune response rates, i.e., SARS-CoV-2 spike-protein-specific T-cell response, were determined by a standardized IGRA (EUROIMMUN Quan-T-Cell SARS-CoV-2 and EUROIMMUN Quan-T-Cell ELISA). For qualitative detection of SARS-CoV-2 anti-nucleocapsid IgG in patients having been infected with SARS-CoV-2, Elecsys Anti-SARS-CoV-2 electrochemiluminescence immunoassay (Roche) was used. All assays were performed according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was carried out using R version 4.1.1 (R Core Team 2021: R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria). Descriptive analyses included the calculation of medians and interquartile ranges for continuous variables and frequencies (absolute and relative) for categorical variables. The association between levels of SARS-CoV-2 anti-spike IgG and SARS-CoV-2 surrogate NAB was analyzed using Spearman's rank correlation coefficient.

Univariate linear mixed effects models were used to compare ($\log_{10}$ transformed) levels of SARS-CoV-2 anti-spike IgG (at all points of time) with respect to treatment status, tumor type (HCC or CCC), and type of treatment, with time treated as factor variable. Each model contains main effects, interaction terms between risk factor and time, as well as a patient-specific random intercept. To compare antibody levels between the different points of time in each group and between the two groups at each point of time, we performed an additional post hoc pairwise comparison based on these mixed effects models. Moreover, ($\log_{10}$ transformed) levels of SARS-CoV-2 anti-spike IgG were compared with respect to underlying CLD and a compensated liver cirrhosis in the group of patients with HBC under anticancer treatment using univariate linear mixed effects models. Analogously, SARS-CoV-2 surrogate NAB (measurements at 12 weeks after basic as well as 4 and 24 weeks after the first booster vaccination) was compared with respect to the discussed influencing factors. Also, applying univariate linear mixed effects regression, levels of SARS-CoV-2 IGRA (measurements four and 24 weeks after the third vaccination) were compared with respect to treatment status.

Furthermore, multivariable regression analyses (for SARS-CoV-2 anti-spike IgG and SARS-CoV-2 surrogate NAB) were performed to examine a possible effect of age, sex, history of SARS-CoV-2 infection, additional immunosuppression, and diabetes mellitus (DM), respectively. p values ≤ 0.05 were regarded as statistically significant. Bonferroni-Holm adjustments were applied as appropriate.

Results

Baseline Characteristics

In this study, 101 patients were included: 58.4% (n = 59) suffered from active HBC and were under anticancer treatment, while 41.6% (n = 42) had a past medical history of GI cancer being in follow-up care. There were 64.4% patients with HCC (n = 38) and 35.6% patients with CCC/GBC (n = 21) under anticancer treatment. In the follow-up care group, 16.7% of patients had HCC (n = 7), 11.9% had CCC/GBC (n = 5), and 71.4% had other GI cancer types (n = 30) ($p < 0.01$). The different distribution of tumor types as well as higher rates of immunosuppression and CLD in patients with active HBC was related to the different prognosis and pathogenesis of the tumor types. Overall, the groups were well matchable as summarized in Table 1.

Humoral Response Rates, Clinical Efficacy, and Safety after Second Vaccination

Four weeks after the second vaccination for SARS-CoV-2, titers of SARS-CoV-2 anti-spike IgG were significantly lower in patients with HBC under active

Table 1. Baseline characteristics

	Under treatment (n = 59)	Off treatment >1 year (n = 42)	p value
Age, years			
Median	67 (40–84)	65 (31–85)	0.59
IQR			
Sex			
Female	32.2% (19)	47.6% (20)	0.15
Male	67.8% (40)	52.4% (22)	
Tumor type			
Hepatobiliary cancers	100% (59)	28.6% (12)	<0.01
HCC	64.4% (38)	16.7% (7)	
CCC/GBC	35.6% (21)	11.9% (5)	
GI cancers*	–	71.4% (30)	
Type of treatment			
Local therapy	39% (23)	–	
Targeted therapy and/or immune checkpoint inhibition	28.8% (17)	–	
Chemotherapy	27.1% (16)	–	
Off treatment <1 year**	5.1% (3)	–	<0.01
Underlying liver disease	69.5% (41)	19% (8)	
Cirrhotic (Child-Pugh A or B, score <8)	63.4% (26)	62.5% (5)	
ALD	38.5% (10)	40% (2)	
NAFLD	38.5% (10)	60% (3)	
Hepatitis B/C	15.4% (4)	–	
Hemochromatosis	3.8% (1)	–	
Autoimmune	3.8% (1)	–	
Non-cirrhotic	36.6% (15)	37.5% (3)	
NAFLD	46.7% (7)	33.3% (1)	
Hepatitis B/C	20% (3)	33.3% (1)	
PSC	13.3% (2)	–	
Hemochromatosis	13.3% (2)	–	
Budd-Chiari syndrome	–	33.3% (1)	
ALD	6.7% (1)	–	
Additional immunosuppression	16.9% (10)	2.4% (1)	0.02
Co-medication with corticosteroids	50% (5)	100% (1)	
Calcineurin inhibitors	30% (3)	–	
Co-medication with azathioprine	10% (1)	–	
HIV infection (T helper cells <400/μL)	10% (1)	–	
Risk factors for severe COVID-19			
Age >65 years	67.8% (40)	73.8% (31)	0.65
BMI >30 kg/m^2	23.7% (14)	7.1% (3)	0.03
History of smoking	33.9% (20)	31% (13)	0.53
Hypertension	61% (36)	50% (21)	0.31
Chronic respiratory disease	13.6% (8)	19% (8)	0.58
Cardiovascular disease	20.3% (12)	26.2% (11)	0.63
Chronic kidney disease	13.6% (8)	2.4% (1)	0.07
CLD	69.5% (41)	19% (8)	<0.01
Neurological disorder	5.1% (3)	4.8% (2)	1
Autoimmune disease	11.9% (7)	2.4% (1)	0.13
DM	33.9% (20)	14.3% (6)	0.03
SARS-CoV-2 infection before initial vaccination ***	3.4% (2)	2.4% (1)	1
Clinical outcome after vaccination			
SARS-CoV-2 infection after second vaccination ***	5.1% (3)	2.4% (1)	0.64
SARS-CoV-2 infection after third vaccination ***	6.8% (4)	9.5% (4)	0.71

Table 1 (continued)

	Under treatment (n = 59)	Off treatment >1 year (n = 42)	p value
Vaccine			
Initial vaccine			0.79
BNT162b2 (Pfizer & BioNTech)	86.4% (51)	83.4% (35)	
AZD1222 (AstraZeneca)	11.9% (7)	9.5% (4)	
mRNA-1273 (Moderna)	1.7% (1)	7.1% (2)	
Third vaccine			0.21
BNT162b2 (Pfizer & BioNTech)	87.2% (34)	75% (21)	
mRNA-1273 (Moderna)	12.8% (5)	25% (7)	
Fourth vaccine			1
BNT162b2 (Pfizer & BioNTech)	91.7% (11)	100% (7)	
mRNA-1273 (Moderna)	8.3% (1)	–	
Vaccine side effects****			
Initial vaccination			
Local side effects	13.6% (8)	28.6% (12)	
Systemic side effects	16.9% (10)	33.3% (14)	
Third vaccination			
Local side effects	10.2% (4)	7.1% (2)	
Systemic side effects	17.9% (7)	17.9% (5)	
Fourth vaccination			
Local side effects	16.7% (2)	–	
Systemic side effects	25% (3)	33.3% (2)	

Baseline characteristics were compared between treatment and control group using Student's t test for age and Fisher's exact tests for the categorical variables. CCC, cholangiocellular cancer; GBC, gallbladder cancer; HCC, hepatocellular carcinoma; GI cancers, gastrointestinal cancers; CRC, colorectal carcinoma; CUP, cancer of unknown primary; GIST, gastrointestinal stromal tumor; NET, neuroendocrine tumor; NAFLD, non-alcoholic fatty liver disease; ALD, alcoholic liver disease; PSC, primary sclerosing cholangitis; IQR, interquartile range. * Gastrointestinal% (GI) cancers – pancreatic cancer, duodenal cancer, gastric and esophageal cancer, CRC, CUP, GIST, NET. ** Patients who received oncological treatment within the last 12 months currently under no anticancer treatment and without detectable tumor. *** SARS-CoV-2 anti-nucleocapsid IgG positive. **** Local side effects: erythema or swelling of injection side, local pain, lymph node swelling; systemic side effects: fever, nausea/vomiting, diarrhea, headaches, allergic reactions.

treatment (2.55 $\log_{10}$ BAU/mL; 95% CI: 2.33–2.76; $p < 0.01$) than in patients in follow-up care (3.02 $\log_{10}$ BAU/mL; 95% CI: 2.80–3.25). Over time, a decrease in mean antibody levels was observed, which was pronounced in patients under treatment (shown in Tables 2A, 3A; Fig. 1a). At week 12 after the second vaccination, differences between the groups were minor and not significant ($p = 0.20$, shown in Tables 2A, 3B; Fig. 1a). However, levels of SARS-CoV-2 sNAB determined at week 12 after the second vaccination were still significantly lower in patients with active HBC under treatment (64.19%; 95% CI: 55.90–72.48; $p < 0.01$) compared to patients in follow-up care (84.13%; 95% CI: 76.95–91.31) (shown in Tables 2C and 3B; Fig. 1b). Of note, 10.6% ($n = 6$) of patients with active HBC and 4.8% ($n = 2$) of patients in follow-up care failed to develop any SARS-CoV-2 antibody titer after the basic vaccination. Levels of total and neutralization antibodies were associated with a correlation coefficient of 0.93 at week 12 after the second vaccination (shown in Fig. 1c).

From a clinical point of view, infections with SARS-CoV-2 after the second vaccination (either self-reported or validated by PCR test) were found in 5.1% ($n = 3$) of patients with active HBC under treatment and 2.4% ($n = 1$) of patients in follow-up care, all with a mild course and most of them with SARS-CoV-2 VOC B1.617.2 (Delta). No severe adverse side effects related to the vaccinations were reported (shown in Table 1).

Humoral Response Rates, Clinical Efficacy, and Safety after Booster Vaccinations

A total of 66.1% ($n = 39$) of patients with active HBC under treatment and 66.7% ($n = 28$) of patients in follow-up care received a third vaccination 6 months after the second vaccination (shown in Table 1). Prior to that, mean levels of SARS-CoV-2 anti-spike IgG were re-measured (24 weeks after the second immunization), revealing a further decline of antibody levels in patients with active HBC (1.95 $\log_{10}$ BAU/mL; 95% CI: 1.71–2.19;

Table 2. Titers of SARS-CoV-2 antibodies

A SARS-CoV-2 anti-spike IgG following second vaccination

Time after vaccination	4 weeks			12 weeks			24 weeks		
	estimate [log_{10} BAU/mL]	95% CI	*p* value	estimate [log_{10} BAU/mL]	95% CI	*p* value	estimate [log_{10} BAU/mL]	95% CI	*p* value
Treatment									
Off treatment >1 year*	3.02	2.80–3.25		2.48	2.27–2.68	<0.001	2.07	1.85–2.29	<0.001
Under treatment	2.55	2.33–2.76	0.003	2.18	1.99–2.38	0.29	1.95	1.71–2.19	0.06
Tumor type									
Off treatment >1 year*	3.02	2.80–3.25		2.48	2.27–2.68	<0.001	2.07	1.85–2.29	<0.001
HCC	2.58	2.31–2.84	0.01	2.17	1.93–2.40	0.46	2.05	1.75–2.36	0.05
CCC/GBC	2.51	2.13–2.89	0.02	2.26	1.92–2.59	0.24	1.79	1.41–2.16	0.39

B SARS-CoV-2 anti-spike IgG following third vaccination

Time after vaccination	4 weeks			12 weeks			24 weeks		
	estimate [log_{10} BAU/ml]	95% CI	*p*-value	estimate [log_{10} BAU/ml]	95% CI	*p*-value	estimate [log_{10} BAU/ml]	95% CI	*p*-value
Treatment									
Off treatment > 1 year*	3.53	3.25–3.80		3.22	2.92–3.53	0.24	3.18	2.89–3.48	0.33
Under treatment	3.47	3.23–3.71	0.05	3.43	3.13–3.72	<0.01	3.42	3.09–3.75	<0.001
Tumor type									
Off treatment > 1 year*	3.53	3.25–3.80		3.22	2.92–3.53	0.24	3.18	2.89–3.48	0.32
HCC	3.56	3.27–3.85	0.04	3.67	3.27–4.06	<0.01	3.46	3.08–3.84	<0.001
CCC/GBC	3.28	2.86–3.70	0.37	3.09	2.64–3.54	0.24	3.23	2.47–3.99	0.22

C SARS-CoV-2 neutralization antibodies following second and third vaccinations

Time after vaccination	12 weeks after second vaccination			4 weeks after third vaccination			24 weeks after third vaccination		
	estimate [%]	95% CI	*p* value	estimate [%]	95% CI	*p*-value	estimate [%]	95% CI	*p* value
Treatment									
Off treatment > 1 year*	84.13	76.95–91.31		99.36	89.20–100.00	0.02	91.18	79.92–100.00	0.3
Under treatment	64.19	55.90–72.48	<0.001	98.54	89.22–100.00	0.03	94.91	81.37–100.00	0.03
Tumor type									
Off treatment > 1 year*	84.13	76.95–91.31		99.36	89.20–100.00	0.02	91.18	79.92–100.00	0.3
HCC	61.77	52.15–71.39	<0.001	99.39	88.07–100.00	0.03	98.49	83.06–100.00	0.01
CCC/GBC	71.45	54.79–88.11	0.17	96.68	80.02–100.00	0.46	82.40	53.54–100.00	0.83

CI, confidence interval; CCC, cholangiocellular carcinoma; HCC, hepatocellular carcinoma; GBC, gallbladder cancer. The results of linear mixed model analysis for the SARS-CoV-2 anti-spike IgG and neutralization antibodies are shown. Results are reported by mean estimates, 95% confidence intervals, and associated *p* values. *p* values refer to the comparison to patients off treatment >1 year 4 weeks after second vaccination. * Patients with a history of GI cancer in follow-up care being at least 1 year off therapy.

Table 3. Time and group effect on antibody levels

A Comparisons between different points of time for patients under treatment and patients off treatment >1 year		
Regarded points of time	SARS-CoV-2 anti-spike IgG	
	under treatment (*p* value)	off treatment >1 year (*p* value)
2–1	<0.01	0.02
3–1	<0.01	<0.01
4–1	<0.01	<0.01
5–1	1.0	<0.01
6–1	1.0	<0.01
3–2	<0.01	0.58
4–2	<0.01	<0.01
5–2	<0.01	<0.01
6–2	<0.01	<0.01
4–3	<0.01	<0.01
5–3	<0.01	<0.01
6–3	<0.01	<0.01
5–4	0.80	1.0
6–4	0.46	1.0
6–5	1.0	1.0

B Comparison between patients under treatment and patients off treatment >1 year at each point of time						
	Point of time					
	1	2	3	4	5	6
Comparison patients under treatment versus patients off treatment >1 year (*p* value) for SARS-CoV-2 anti-spike IgG	0.02	0.20	1.0	1.0	1.0	1.0
Comparison patients under treatment versus patients off treatment >1 year (*p* value) for SARS-CoV-2 neutralization antibodies	/	<0.01	/	1.0	/	1.0

The results of a post hoc pairwise comparison based on the mixed regression model reported in Table 2 A–C are shown. The numbers are referred to the different points of time as follows: 1–3 = 4, 12, and 24 weeks after second vaccination, respectively; 4–6 = 4, 12, and 24 weeks after third vaccination, respectively.

$p < 0.01$) and patients in follow-up care (2.07 $\log_{10}$ BAU/mL; 95% CI: 1.85–2.29; $p = 0.06$) compared to levels 4 weeks after the second vaccination (shown in Table 2A; Fig. 1a).

Four weeks after the first booster vaccination, predicted mean antibody levels of SARS-CoV-2 anti-spike IgG showed a significant increase in patients with HBC under treatment (3.47 $\log_{10}$ BAU/mL; 95% CI: 3.23–3.71; $p = 0.05$) and in patients in follow-up care (3.53 $\log_{10}$ BAU/mL; 95% CI: 3.25–3.80; $p < 0.01$) compared to patients in follow-up care at week four after the second vaccination. Titers between the groups were almost equal (shown in Table 2B; Fig. 1a). This effect could also be observed for SARS-CoV-2 sNAB in both groups (98.54%; 95% CI: 89.22–100.0; $p = 0.04$ and 99.36%; 95% CI: 89.20–100.0; $p = 0.02$) (shown in Table 2C; Fig. 1b). Importantly, all of the patients who did not develop SARS-CoV-2 antibody titers following the basic vaccination ($n = 8$) showed a positive immune response after the first booster vaccination.

However, levels of total as well as neutralization antibodies decreased again in patients with HBC under treatment and in patients in follow-up care over time (shown in Tables 2B, 2C; Fig. 1a, b). In comparison to the decrease of antibody levels after the first two vaccinations, the decrease after the third vaccination was less pronounced (shown in Tables 2B, C, 3A, B; Fig. 1a, b). Despite official recommendations and our reinforcement, only 20.4% ($n = 12$) of patients under treatment and 16.7% ($n = 7$) of patients in follow-up care opted to receive a fourth vaccination (shown in Table 1). In patients with active HBC under treatment as well as in patients in follow-up care, this second booster vaccination stabilized and, again, improved antibody levels of total (3.75 $\log_{10}$ BAU/mL; 95% CI:

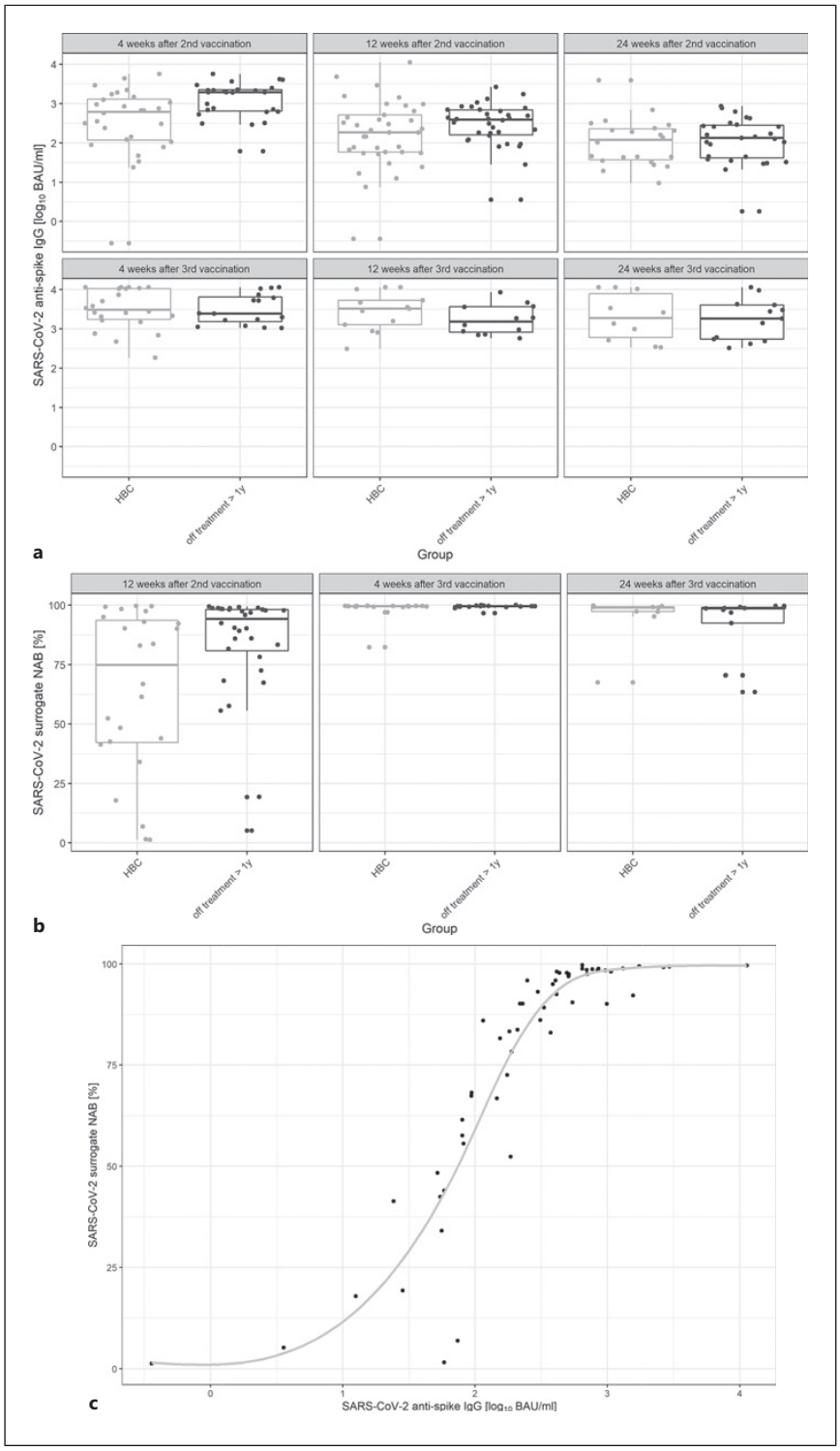

Fig. 1. Comparison of SARS-CoV-2 antibody titer between patients with HBC undergoing active anticancer treatment and patients being off treatment >1 year. **a** log10 SARS-CoV-2 anti-spike IgG titer at weeks 4, 12, and 24 after the second and third vaccinations, respectively. **b** SARS-CoV-2 surrogate neutralization antibody titer at week 12 after the second and at weeks 4 and 24 after the third vaccination, respectively. **c** Association between log10 SARS-CoV-2 anti-spike IgG and SARS-CoV-2 surrogate NAB titer with a correlation coefficient of 0.93. Lower and upper ends correspond to the 25% and 75% quartiles, respectively; length of boxes represents interquartile range; horizontal line shows median log10 SARS-CoV-2 anti-spike and SARS-CoV-2 surrogate neutralization antibody titer. BAU, binding antibody units; NABs, neutralization antibodies; HBC, hepatobiliary cancer.

2.86–4.17 and 3.60 $\log_{10}$ BAU/mL; 95% CI: 2.93–4.10) as well as of neutralization antibodies (99.14%; 95% CI: 97.00–99.70 and 98.88%; 95% CI: 98.40–99.60).

Up until May 2022, infections with SARS-CoV-2 were found in 8 patients of our cohort – all with a mild course. Infections with VOCs BA.1 and BA.2 (Omicron) were documented in 1.7% ($n = 1$) of patients with active HBC under treatment and 4.8% ($n = 2$) of patients in follow-up care despite booster vaccination. With VOCs BA.4 and BA.5 (Omicron), 2 patients in follow-up care (4.8%) and 3 patients under anticancer treatment (5.1%) were infected (shown in Table 1). The third and fourth vaccinations in our cohort of patients were also well tolerated with only mild side effects (shown in Table 1).

Cellular Response Rates after Booster Vaccination

In addition, we examined cellular response rates in both groups after the third vaccination for SARS-CoV-2. Levels of SARS-CoV-2-stimulated interferon gamma release were balanced with similar titers in patients with HBC under treatment (1,372.39 mIU/L; 95% CI: 940.69–1,804.08) and in patients in follow-up care (1,248.34 mIU/L; 95% CI: 843.20–1,653.49; $p = 0.68$) 4 weeks after the third vaccination. At week 24 after the third vaccination, levels of patients in follow-up care were stable (1,341.70 mIU/L; 95% CI: 852.87–1,830.53), while those of patients with HBC under anticancer treatment decreased distinctly (921.98 mIU/L; 95% CI: 349.14–1,494.83) (shown in Table 4).

Subgroups Analyses and Factors Influencing Humoral Immune Response Rates

In the group of patients with active HBC, we analyzed factors potentially impairing immune response rates. Neither underlying CLD nor liver cirrhosis was found to be associated with significantly reduced antibody levels (shown in Fig. 2). Concerning tumor types, no significant differences between patients with HCC and patients with CCC/GBC could be determined at any point of time (shown in Table 2; Fig. 3 A, B). Of note, in the control group, time-averaged mean predicted total (2.74 $\log_{10}$ BAU/mL; 95% CI: 2.41–3.07 vs. 2.77 $\log_{10}$ BAU/mL; 95% CI: 2.57–2.97; $p = 0.89$) and neutralization SARS-CoV-2 antibody levels (84.91%; 95% CI: 72.87–88.53 vs. 91.17%; 95% CI: 84.29–98.04; $p = 0.38$) did not differ between patients with a past medical history of HBC and patients with a past medical history of other GI cancer types. In patients under treatment, chemotherapy significantly reduced levels of total (−0.50 $\log_{10}$ BAU/mL; 95% CI: −0.85 to −0.15; $p < 0.01$) as well as of neutralization antibodies (−15.23%; 95% CI: −28.50 to −1.96; $p = 0.03$). By contrast, the potential impact of local, targeted, or immune checkpoint therapy was insignificant and minor (shown in Fig. 3c, d).

In a multivariable analysis, we again regarded factors potentially influencing humoral response rates to vaccinations for SARS-CoV-2. Additional immunosuppression was associated with significantly reduced levels of total 0.70 $\log_{10}$ BAU/mL; 95% CI: −1.06 to −0.34; $p < 0.01$) and neutralization antibodies (26.99%; 95% CI: −42.51 to −11.45; $p < 0.01$). Of note, immunosuppression was only suspected in patients under co-medication with corticosteroids, calcineurin inhibitors, or azathioprine and/or in patients with an underlying HIV infection and T helper cells <400/μL (shown in Table 1). Thus, in the present model, immunosuppression was not linked to chemotherapy, identifying immunosuppression as an independent risk factor for impaired response rates. Conversely, chemotherapy itself significantly impaired levels of SARS-CoV-2 anti-spike IgG (−0.79 $\log_{10}$ BAU/mL; 95% CI: −1.28 to −0.29; $p < 0.01$), while its impact on neutralization antibodies was less pronounced (−16.94%; 95% CI: −35.97 to +2.09; $p = 0.08$). Vice versa, DM as comorbidity reduced levels of neutralization antibodies significantly (−16.75%; 95% CI: −29.06 to −4.45; $p < 0.01$) with an inferior impact on levels of total antibodies (−0.29 $\log_{10}$ BAU/mL; 95% CI: −0.55 to −0.02; $p = 0.04$). Being off treatment <1 year without detectable tumor activity and/or a past medical history of COVID-19 prior to vaccinations were linked to distinctly higher levels of total and neutralization antibodies by trend. Other therapy regimes (immune checkpoint therapy, targeted therapy, local therapy), age, sex, CLD, and/or compensated liver cirrhosis (i.e., Child-Pugh A or B) showed no substantial impact on antibody levels (shown in Fig. 4a, b).

Regarding levels of total and neutralization antibodies after the first booster vaccination, negative effects of additional immunosuppression, chemotherapy, and/or DM were minor and insignificant. Moreover, the positive impacts of infections with SARS-CoV-2 on antibody levels and/or of finishing anticancer treatment within 1 year diminished (shown in Fig. 4c, d).

Discussion

In a large cohort of patients with GI cancer, we showed that patients with active HBC under anticancer treatment are facing significantly impaired immune responses to basic vaccination for SARS-CoV-2 without safety concerns. Although response rates could eventually be

Table 4. Cellular response rates after SARS-CoV-2 vaccination

	Estimate, mIU/mL	95% CI	*p* value
Four weeks after third vaccination			
Treatment			
Off treatment >1 year *	1,248.34	843.20–1,653.49	<0.001
Under treatment	1,372.39	940.69–1,804.08	0.68
24 weeks after third vaccination			
Treatment			
Off treatment >1 year *	1,341.70	852.87–1,830.53	0.7
Under treatment	921.98	349.14–1,494.83	0.18

The results of linear mixed model analysis for the SARS-CoV-2 IGRA are shown. Results are reported by mean estimates, 95% confidence intervals, and associated *p* values. *p* values refer to the comparison to patients off treatment >1 year 4 weeks after second vaccination. IGRA, interferon gamma release assay; mIU/mL, milli-international units per milliliter; CI, confidence interval. *Patients with a history of GI cancer in follow-up care being at least 1 year off therapy.

improved by booster vaccinations in our cohort of patients to levels similar to those in patients without active cancer disease in follow-up care, waning immunity over time was again observed and should be taken into consideration for further recommendations.

Patients with CLD and with solid cancer have been shown to develop worse immune responses to basic vaccination for SARS-CoV-2 than healthy people [13, 16]. Unfortunately, differentiated data on patients with active HBC are missing to date. These patients have been suspected to be especially vulnerable during the ongoing COVID-19 pandemic due to the underlying combination of CLD and malignant disease. Thus, in this study, we focused on immune response rates to vaccination for SARS-COV-2 in a relatively large cohort of patients with active HBC receiving anticancer treatment (being or having been under local therapy, chemotherapy, targeted therapy, or immune checkpoint inhibition within the last 12 months). Patients with a past medical history of GI cancer having been at least 1 year without detectable tumor disease and without anticancer treatment were included as control group. Within the control group, there was a proportion of 28.6% of patients with a past medical history of HBC. All patients were well matched as detailed above, particularly as they shared comparable risk factors for cancer development and for severe COVID-19 which could not be found in a cohort of healthy people.

As expected, the present data reveal that patients with active HBC are especially challenged by significantly reduced levels of total and neutralization SARS-CoV-2 antibodies when compared to patients with a past medical history of GI cancer in follow-up care. Levels of total and neutralization antibodies were strongly associated (correlation coefficient of 0.93) as described before [22]. However, while differences in total antibodies diminished over time, significant differences in neutralization antibodies persisted marking a discrepancy in real effectiveness. Previous studies on response rates to vaccinations for seasonal influenza, hepatitis A or B, and *Streptococcus pneumoniae* in patients with CLD revealed impaired immunogenicity compared to healthy controls [6–9]. Recently, these effects could be shown in patients with CLD after having received SARS-CoV-2 vaccines [11, 12].

HBC per se as well as underlying CLD and liver fibrosis/cirrhosis in most patients are associated with augmented immunodeficiency [23, 24], resulting in worse immune responses to vaccines. In order to further dissect the influence of additional underlying hepatological conditions, subgroup analyses were performed. Interestingly, neither underlying CLD nor liver cirrhosis was associated with significantly reduced levels of total and neutralizing antibodies, in our cohort of patients with active HBC. These results were also confirmed in a multivariable analysis. Concerning liver cirrhosis, only patients with compensated liver cirrhosis (Child-Pugh A or B) were included in our cohort, explaining in part the missing effect of cirrhosis on levels of SARS-CoV-2 anti-spike IgG. In general, patients with CLD had positive SARS-CoV-2 anti-spike IgG levels after basic vaccination in >85.0% of cases [25]. The positive titers found in 89.4% of cases after basic vaccination in our cohort of patients are in line with these data. Previously, it has been shown that compensated liver cirrhosis did not impair immune response rates to vaccinations for SARS-CoV-2 in patients with CLD other than HBC [12] which is confirmed here for patients with

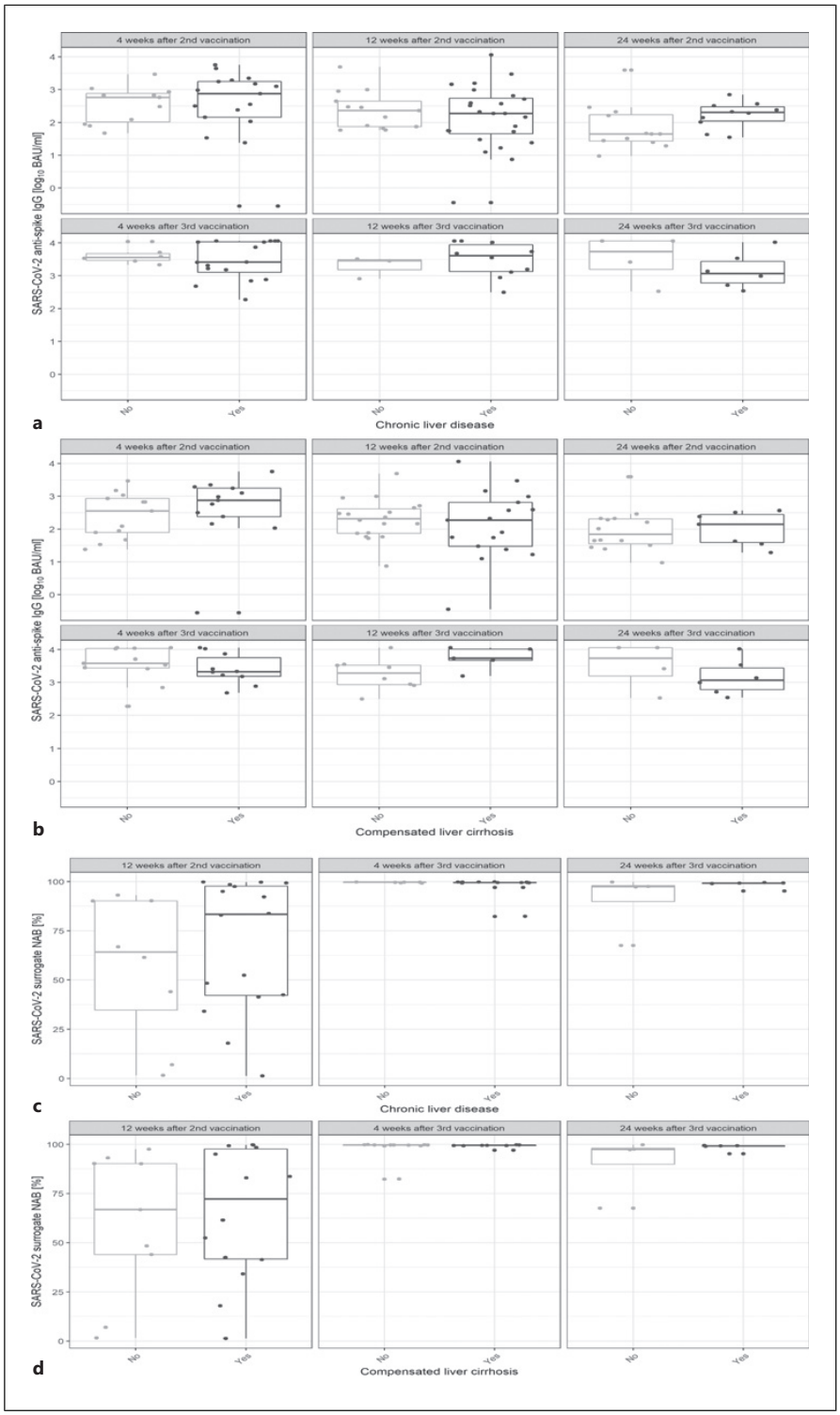

Fig. 2. Comparison of SARS-CoV-2 antibody titer of patients with HBC with and without underlying liver diseases and compensated liver cirrhosis, respectively. **a** log10 SARS-CoV-2 anti-spike IgG titer of patients undergoing active anticancer treatment with and without an underlying liver disease. **b** log10 SARS-CoV-2 anti-spike IgG titer of patients undergoing active anticancer treatment with and without underlying compensated liver cirrhosis. **c** SARS-CoV-2 surrogate neutralization antibody titer of patients undergoing active anticancer treatment with and without an underlying liver disease. **d** SARS-CoV-2 surrogate neutralization antibody titer of patients undergoing active anticancer treatment with and without an underlying compensated liver cirrhosis. Lower and upper ends correspond to the 25% and 75% quartiles, respectively; length of boxes represents interquartile range; horizontal line shows median log10 SARS-CoV-2 anti-spike and SARS-CoV-2 surrogate neutralization antibody titer. Compensated liver cirrhosis was defined as liver cirrhosis Child Pugh A or B with a score less than 8. BAU, binding antibody units; NABs, neutralization antibodies; HBC, hepatobiliary cancer.

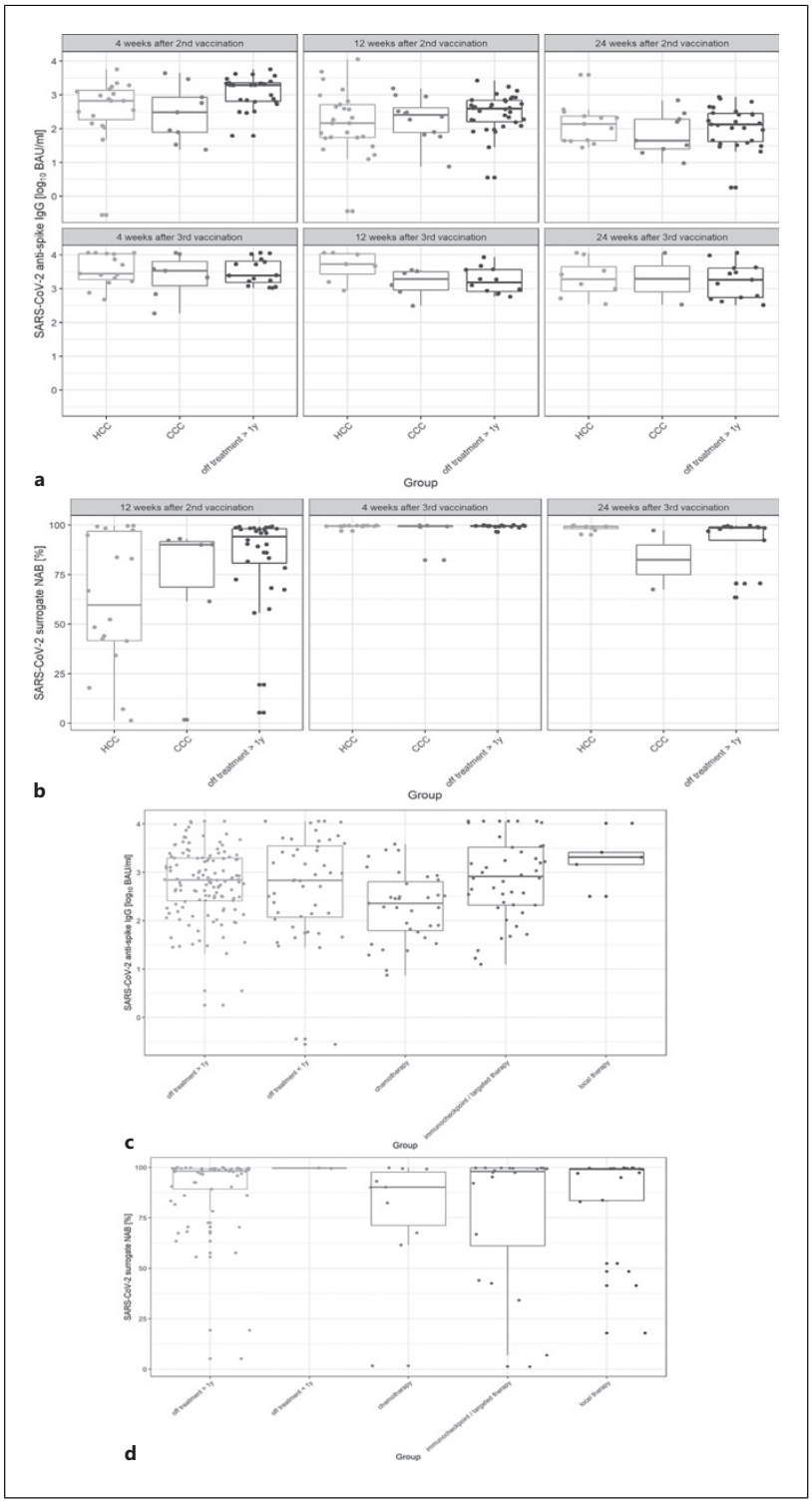

Fig. 3. Comparison of SARS-CoV-2 antibody titer between patients with HCC and CCC undergoing different types of anticancer treatment and patients being off treatment >1 year. **a** log10 SARS-CoV-2 anti-spike IgG titer at weeks 4, 12, and 24 after second and third vaccinations, respectively. **b** SARS-CoV-2 neutralization antibody titer at week 12 after second vaccination and at weeks 4 and 24 after third vaccination, respectively. **c** Comparison of log10 SARS-CoV-2 anti-spike IgG titer of patients with HBC undergoing different types of anticancer treatment. **d** Comparison of SARS-CoV-2 neutralization antibody titer of patients with HBC undergoing different types of anticancer treatment. Lower and upper ends correspond to the 25% and 75% quartiles, respectively; length of boxes represents interquartile range; horizontal line shows median log10 SARS-CoV-2 anti-spike and SARS-CoV-2 surrogate neutralization antibody titer. BAU, binding antibody units; NABs, neutralization antibodies; HCC, hepatocellular cancer; CCC, cholangiocellular cancer; HBC, hepatobiliary cancer.

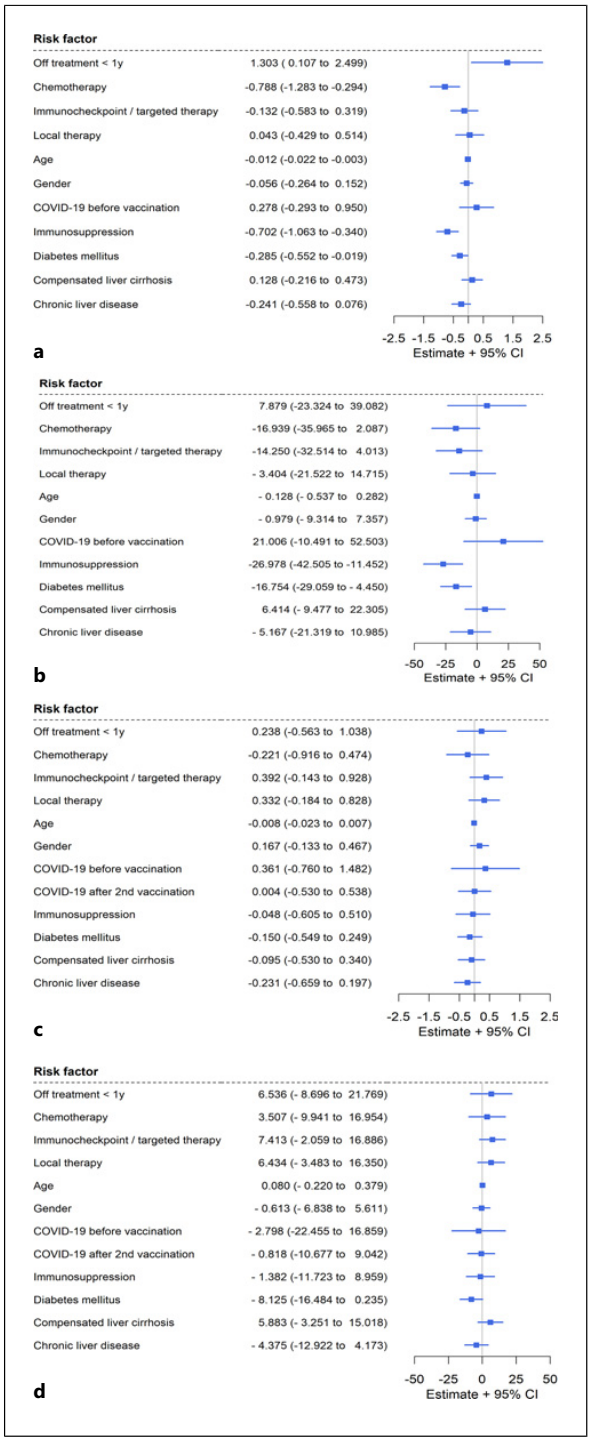

Fig. 4. Effects of suspected factors influencing immunogenicity for SARS-CoV-2 antibody titer. **a** Effects on log10 SARS-CoV-2 anti-spike IgG titer after second vaccination. **b** Effects on SARS-CoV-2 surrogate neutralization antibody titer after the second vaccination. **c** Effects on log10 SARS-CoV-2 anti-spike IgG titer after the third vaccination. **d** Effects on SARS-CoV-2 surrogate neutralization antibody titer after third vaccination. The results of multivariable linear mixed effects analysis for the log10 SARS-CoV-2 anti-spike IgG and SARS-CoV-2 neutralization antibodies are shown. Forest plots are showing point estimates and 95% CIs. Compensated liver cirrhosis was defined as liver cirrhosis Child-Pugh A or B with a score less than 8. CI, confidence interval.

HBC. However, immunogenicity after vaccination for hepatitis A and B was especially impaired in cases of decompensated liver cirrhosis correlating with clearly reduced liver synthesis [10]. One would thus also assume worse levels of SARS-CoV-2 antibodies in patients with HBC in situations of decompensated liver conditions, which were not included as these patients are mostly not eligible for anticancer treatment.

Although no significant differences between solid tumor types could be demonstrated to date [26], differentiated data for several tumor types are still missing. In our own preliminary analysis on efficacy of SARS-CoV-2 vaccinations in patients with GI cancer, we could identify patients with active HBC (n = 39) as especially facing impaired immunogenicity compared to patients with other types of active GI cancer and to patients in follow-up care [27]. Separating HCC from CCC, there were no differences in antibody levels between the 2 groups of patients with HBC in our cohort. Moreover, patients with a past medical history of HBC did not show worse immune responses than patients with a past medical history of other GI cancers. While, as far as studies are comparable, the finding of positive antibody titers in 89.4% of patients with active HBC after basic vaccination in our cohort of patients is worse than in other solid cancer patients (95.0%), it is better than in patients with hematological malignancy (60.0%) [28]. By contrast, 96.2% of patients in follow-up care showed better positive antibody titers, resembling response rates of healthy people. We therefore conclude for our cohort of patients that those with undetectable cancer and without any treatment for at least 12 months are well protected from severe COVID-19.

Moreover, anticancer treatment in general was identified as an outstanding risk factor for significantly reduced levels of SARS-CoV-2 antibodies as shown before by Lee LY et al. [19]. In detail, patients, especially those under chemotherapy, were facing reduced antibody levels, while antibody levels of patients under local or targeted/immunological therapy were not substantially impaired in our cohort of patients. Chemotherapy has previously been identified as an outstanding risk factor for lower antibody levels [29].

According to our multivariable analysis, additional immunosuppression could be identified as a main risk factor for impaired immunogenicity following SARS-CoV-2 vaccination. This is in line with data in patients on immunosuppressive medication following liver transplantation [11, 30]. Of note, this immunosuppression was not linked to anticancer treatment, especially not to chemotherapy. Interestingly, chemotherapy was confirmed as another risk factor which mainly impaired levels of total antibodies with a lesser impact on neutralization antibodies. Moreover, DM as comorbidity showed similar negative effects on SARS-CoV-2 immunogenicity, especially on levels of sNAB. The effects of chemotherapy as well as of DM can be at least partly explained by the underlying immunodeficiency associated with both conditions [31].

A past medical history of COVID-19 was linked to higher levels of total and neutralization antibodies by trend following basic vaccination for SARS-CoV-2. This has previously been revealed for patients with cancer [26] and could here be confirmed for patients with HBC. Natural infections have been shown to possess higher immunogenic potential than vaccinations [32]. Moreover, hybrid immunity, i.e., vaccination after having undergone COVID-19, showed best immune responses compared to natural as well as immunity by vaccination [33]. Higher SARS-CoV-2 antibody levels were also observed in patients who finished their anticancer treatment within 1 year. The formally positive effect of finishing anticancer treatment within 1 year and of a past medical history of COVID-19 on antibody levels in our cohort of patients are though limited in its meaning as only very few patients showed these features.

Differences between antibody levels have eventually been overcome by a third vaccination. Correspondingly, only rare infections with BA.1 and BA.2 were observed in both groups of patients thereafter, highlighting the clinical importance of booster vaccinations, especially for patients at high risk of impaired SARS-CoV-2 immunogenicity. Moreover, cellular response rates were balanced between the groups after the first booster vaccination. It has been shown that the course of COVID-19 was less severe after booster vaccination [34]. In patients with solid as well as blood cancer having been seronegative after the first and the second vaccination for SARS-CoV-2, seropositivity could be traced after the first booster vaccination [35]. This was also true for patients under immunosuppressive treatment after liver transplantation [36] and could also be observed in 8 patients of our cohort. Contrary to our reinforced recommendations, only about two-thirds of patients in our cohort received a third vaccination. Of note, this is in line with the general German booster vaccination rate of 61.95% [1], prompting more intense awareness campaigns, especially for vulnerable groups, such as patients with active HBC.

Despite vaccination, infections with new VOCs (BA.4 and BA.5) were found to increase in our cohort of patients up to about 5.0%, in line with observations of antibody escape of these VOCs from vaccines [37,

38]. Fortunately, no severe courses of COVID-19 were observed. This indicates that the decrease of antibody levels over time was not critical and that the maintained neutralizing capacity still was sufficient to prevent severe COVID-19 from a clinical point of view. Waning immunity over time was confirmed by data from the UK [39], which we also observed in our cohort of patients, making them a vulnerable group concerning infections with SARS-CoV-2. Of note, levels of cellular response were especially diminished in patients with active HBC and must be further monitored. Fortunately, decrease of antibody levels was less pronounced after the third vaccination compared to the same time points after the second vaccination stressing the need for booster vaccinations. However, it is difficult to define a titer effectively preventing infections with SARS-COV-2 and/or a severe course of COVID-19. Titers ≥264.0 BAU/mL were described as being most likely linked to protection from infections with VOC B1.1.7 (alpha) before [40]. Taking the mentioned escape phenomena of new VOCs with increasing infection rates into consideration, for the authors of this study, it is thus speculative to name titers being probably linked to protection for the time being.

The mentioned negative effects of additional immunosuppression, anticancer treatment, particularly of chemotherapy, and DM on immune responses were overcome by the first booster vaccination. Improved immune responses following the third vaccination for SARS-CoV-2 could already be shown for immunocompromised patients, particularly for patients with past medical history of liver transplantation [41, 42].

The overall low rates of infections in our patient cohort reflect that chronically ill patients do stick to hygienic and social distancing rules. As clinical benefits of a fourth vaccine dose have recently been documented [43, 44], which was therefore recommended for immunocompromised patients in the meantime [3], we encouraged our patients to obtain a fourth vaccine for SARS-CoV-2. However, only less than a quarter of the patients in our cohort received a second booster vaccination, resulting in improved and stabilized antibody levels. At least, this vaccination rate is markedly higher than that in the general German population with a vaccination rate of 8.7% [45].

To the best of our knowledge, this is the first report focusing on immune responses to vaccination for SARS-CoV-2 in a large cohort of patients with HBC and with emphasis on underlying hepatological conditions as well as oncological therapy regimes. Due to the limited life expectancy of most of the patients with active HBC, follow-up of all patients was difficult as some patients died and others experienced reduced performance status while continuing their medical treatment in the department of palliative care. Patients under best supportive care were not included. Data on cellular immune response were only evaluated after the first booster vaccination, while data after the second vaccination and thus a longitudinal analysis of this parameter are missing.

In conclusion, patients with HBC are facing significantly impaired immune responses to basic vaccinations for SARS-CoV-2. This seemed to be more related to the malignant disease in general, to therapy regimes (especially chemotherapy), and to any additional immunocompromising circumstances (therapy related or comorbidities) than to underlying cirrhotic or non-cirrhotic CLD or compensated cirrhosis. The currently recommended booster vaccinations effectively overcame discrepancies in effectiveness of vaccination with low infection rates and/or mostly mild courses of COVID-19 thereafter. Patients should be encouraged to receive at least three, better still four, vaccinations according to our data due to waning immunity. Continued monitoring including antibody assessment of the vulnerable group of patients with HBC may help decide on individual extra booster vaccinations, passive immunization, and/or antiviral treatment for patients with active HBC. In future studies, the effects of booster vaccinations on long-term SARS-CoV-2 immunogenicity should be analyzed putting emphasis on new SARS-CoV-2 VOCs, VOC-adapted vaccines, and potential escape phenomena of VOCs from vaccines.

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Statement of Ethics

The study was performed in accordance with the Declaration of Helsinki and approved by the institutional review board of the Medical Faculty of the University of Bonn (Nos. 341/17 and 023/22). Written informed consent was obtained from all participating patients.

Conflict of Interest Statement

M.A.G.C. has contributed to advisory boards for Roche, Eisai, BMS, MSD, and AZ. C.B. received honoraria for lectures and/or consultancies from AbbVie, Gilead, Janssen, MSD, and ViiV as

well as funding from Dt. Leberstiftung, DZIF, Hector Stiftung, and NEAT ID. M.B.M. received travel expenses and honoraria from Gilead, Pfizer, and Virology Education. JKR has received honoraria for lectures and/or consultancies from Abivax, Galapagos, Gilead, Merck, Janssen, Theratechnologies, and ViiV. However, these activities have no potential conflicts of interest with the manuscript. None of the other authors have any potential conflicts (financial, professional, or personal) that are relevant to the manuscript.

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Author Contributions

Malte B. Monin: study concept and design; analysis and interpretation of data; drafting of the manuscript; statistical analysis; administrative, technical, or material support; and study supervision. Leona I. Baier: acquisition of data; analysis and interpretation of data; drafting of the manuscript; and statistical analysis. Jens G. Gorny: acquisition of data; analysis and interpretation of data; and critical revision of the manuscript for important intellectual content. Moritz Berger: analysis and interpretation of data; critical revision of the manuscript for important intellectual content; and statistical analysis. Taotao Zhou, Robert Mahn, Christian Möhring, and Anna-Maria Eis-Hübinger: acquisition of data; critical revision of the manuscript for important intellectual content; and administrative, technical, or material support. Farsaneh Sadeghlar: study concept and design; acquisition of data; critical revision of the manuscript for important intellectual content; administrative, technical, or material support; and study supervision. Christoph Boesecke, Kathrin van Bremen, Jürgen K. Rockstroh, and Christian P. Strassburg: critical revision of the manuscript for important intellectual content and administrative, technical, or material support Matthias Schmid: critical revision of the manuscript for important intellectual content and statistical analysis. Maria A. Gonzalez-Carmona: study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; statistical analysis; administrative, technical, or material support; and study supervision.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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2.3 Longitudinale SARS-CoV-2-Impfstudie bei PatientInnen mit gastrointestinalen Karzinomen – Effekt eines ersten Antigenkontaktes mit Omikron-Varianten von SARS-CoV-2

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In der finalen Arbeit der longitudinalen SARS-CoV-2-Impfstudie wurde zunächst die Entwicklung der Basisimmunantwort gegen SARS-CoV-2 im zeitlichen Verlauf ohne weiteren stattgehabten Antigenkontakt bei PatientInnen mit verschiedenen gastrointestinalen Karzinomen betrachtet. Dabei erfolgte speziell eine Subanalyse zur kreuzreaktiven Neutralisation der Omikron-Varianten durch prä-Omikron-Antigenkontakte. Abschließend wurde der Effekt eines ersten Omikron-Antigenkontaktes auf die Gesamtimmunantwort untersucht (vgl. Kapitel 1.8.1).

Von 168 in die Studie aufgenommenen PatientInnen hatten 109 ein aktives gastrointestinales Karzinom unter Therapie. Weitere 59 PatientInnen hatten ein gastrointestinales Karzinom in der Vorgeschichte, wobei es seit mindestens 12 Monaten keinen Hinweis auf Tumoraktivität gab und auch keine fortlaufende Erhaltungstherapie notwendig war.

Im September 2022 zeigten sich die Spiegel von SARS-CoV-2 anti-spike IgG nach ausschließlich prä-Omikron-Antigenkontakten noch ausgeglichen zwischen den Patientengruppen. Dabei war die Kapazität zur kreuzreaktiven Neutralisation der Omikron-Varianten mit 71,48% signifikant niedriger als die Kapazität zur Neutralisation des Wildtyps von SARS-CoV-2 (93,24%; $p<0,01$). Gleichzeitig wiesen bereits 83,3% aller PatientInnen eine effektive zelluläre SARS-CoV-2-Immunantwort auf. Bei Ausbleiben eines erneuten SARS-CoV-2-Antigenkontaktes fiel insgesamt jedoch ein Abfall der humoralen Immunantwort im Vergleich zu den in Kapitel 2.2 dargestellten Daten auf.

Ein erster Omikron-Antigenkontakt erfolgte bei 70,5% der PatientInnen über Infektionen mit einer der Omikron-Varianten von SARS-CoV-2. Dabei waren die Krankheitsverläufe überwiegend mild. Bei nur 29,5% der PatientInnen wurde die empfohlene Impfung mit einem Omikron-adaptierten Impfstoff durchgeführt. Im September 2023 zeigten sich nach einem ersten stattgehabten Antigenkontakt mit einer Omikron-Variante von SARS-CoV-2 signifikant angestiegene Spiegel von SARS-CoV-2 anti-spike IgG. Auch war insbesondere die Kapazität zur Neutralisation der Omikron-Varianten von SARS-CoV-2 signifikant verbessert (93,86%; $p<0,01$). Im Vergleich war ein erster Omikron-Antigenkontakt bei PatientInnen mit aktiven gastrointestinalen Karzinomen unter Therapie, insbesondere mit pankreatikobiliären Karzinomen, mit systemischer Immundefizienz und/oder unter Chemotherapie signifikant weniger immunogen.

Gemäß diesen Daten konnte eine erste Auffrischungsimpfung die humorale Immunantwort bei PatientInnen mit gastrointestinalen Karzinomen wie in Kapitel 2.2. schon vermutet nur temporär stabilisieren. Aus klinischer Sicht ergab sich bei milden Verläufen von Infektionen mit neuen SARS-CoV-2-Varianten, wie hier exemplarisch für die Omikron-Varianten gezeigt, dennoch das Bild einer effizienten Basisimmunität. Zu berücksichtigen ist aber, dass ein Erstkontakt mit einer der neuen Omikron-Varianten des Virus in Subgruppen von PatientInnen weniger immunogen war. Somit stützen unsere Daten die aktuellen Empfehlungen der STIKO zu jährlichen Auffrischungsimpfungen bei PatientInnen mit soliden Karzinomen[41], der wir uns für PatientInnen mit gastrointestinalen Karzinomen explizit anschlossen.

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Longitudinal Study of SARS-CoV-2 Vaccinations and Infections in Patients with Gastrointestinal Cancer: Stabilizing Immune Responses and Neutralizing Emerging Variants with Variant-Adapted Antigen Exposures †

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Abstract: This longitudinal study examined how active gastrointestinal (GI) cancer types affect immune responses to SARS-CoV-2, focusing on the ability to neutralize the Omicron variants. Patients with GI cancer ($n = 168$) were categorized into those with hepatocellular carcinoma, hepatic metastatic GI cancer, non-hepatic metastatic GI cancer, and two control groups of patients with and without underlying liver diseases. Humoral and cellular immune responses were evaluated before and after Omicron antigen exposures. In the pre-Omicron era, humoral SARS-CoV-2 immunity decreased after three antigen contacts without further antigen exposure. While Omicron neutralization was significantly lower than wildtype neutralization ($p < 0.01$), Omicron infections were yet mild to moderate. Additional Omicron exposures improved IgG levels ($p < 0.01$) and Omicron neutralization ($p < 0.01$). However, this effect was significantly less intense in patients with active GI cancer, particularly in patients with pancreaticobiliary neoplasms (PBN; $p = 0.04$), with underlying immunodeficiency ($p = 0.05$), and/or under conventional chemotherapy ($p = 0.05$). Pre-Omicron SARS-CoV-2 immunity prevented severe clinical courses of infections with Omicron variants in patients with GI cancer. However, in patients with PBN, with underlying immunodeficiency, and/or under conventional chemotherapy initial contacts with Omicron antigens triggered only reduced immune responses. Thus, subgroups could be identified for whom booster vaccinations are of special clinical significance.

Keywords: SARS-CoV-2; immune responses; gastrointestinal cancer; metastases; hepatocellular cancer; waning immunity; booster antigen contacts; omicron neutralization

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1. Introduction

Coronavirus disease 2019 (COVID-19) is currently classified as less dangerous than at the beginning of the pandemic [1]. Newer variants of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), in particular the currently predominant Omicron variants, are more transmissible than the wildtype [2]. However, the course of the infections is usually milder, as the average immunity has increased significantly due to vaccinations and/or infections [3–5]. Additionally, antiviral drugs improved the clinical outcome of high-risk patients [6–9]. Nevertheless, the Omicron variants are more resistant to neutralization by vaccines targeting the wildtype [10,11], and thus, remain associated with significant morbidity and mortality worldwide [12]. Patients with immunodeficiency, especially those with hematological neoplasms, and presumably impaired immune responses to SARS-CoV-2 antigen exposures, are still at particular risk of severe courses of COVID-19 in the event of infection with an Omicron variant [13,14].

Differentiated data on immune responses in subgroups of patients with solid neoplasms are scarce, and recommendations are often based on a transfer of data from patients with hematological neoplasms [15–18]. However, immune responses appeared to be more effective in patients with solid neoplasms than in patients with hematological neoplasms [19]. In a longitudinal study, we previously demonstrated that immune responses in the subgroup of patients with active gastrointestinal (GI) cancer, especially those with hepatocellular carcinoma (HCC), were less effective than in patients in follow-up care with a past medical history of GI cancer after basic SARS-CoV-2 immunization [20]. Impaired immune responses were closely related to the malignant disease itself, chemotherapeutic treatment regimens, and/or additional immunodeficiency rather than to any underlying liver dysfunction [20]. Although the differences were largely mitigated by a first pre-Omicron booster antigen exposure, a decline in titers was observed over time [21,22], which was confirmed by another study in patients with GI cancer [23].

The focus of our study so far has been on the ability to neutralize wildtype SARS-CoV-2, without considering emerging variants, i.e., currently the Omicron variants. Recent studies have shown that there was a weaker cross-reactive B- and T-cell response against Omicron variants following vaccination against wildtype SARS-CoV-2 in both healthy controls and oncological patients [24–30]. However, there is still insufficient knowledge about the ability to neutralize the Omicron variants in patients with GI cancer, and recommendations of national and international medical societies for these patients are largely based on pre-Omicron data [15–18].

As part of our longitudinal cohort study, we present new data on long-term immune responses in patients with HCC and other GI cancers. We additionally analyzed the ability to neutralize the Omicron variants before their emergence and after a first Omicron antigen exposure by variant-matched vaccination and/or infections. Thereby, we studied the impact of the cancer type, of the type of oncological treatment, in particular chemotherapeutic treatment, of any underlying liver disease, and of underlying immunodeficiency.

2. Results

2.1. Patients' Characteristics

A total of 168 patients were enrolled in this cohort study (Table 2). In total, 39 (23.2%) patients had HCC (group 1), 26 (15.5%) patients had hepatic metastatic GI cancer (group 2), and 44 (26.2%) patients had non-hepatic metastatic GI cancer (group 3). CRC and PBN were the most represented non-hepatocellular GI cancer types. The control group consisted of 59 (35.1%) patients in follow-up care for GI cancer without active cancerous disease and not under maintenance therapy. Of these control patients, 37 (62.7%) did not have underlying liver diseases (control 1), while 22 (37.3%) did (control 2). The most common liver diseases among them were metabolic dysfunction associated steatotic liver disease and alcoholic liver disease.

2.2. Pre-Omicron Immune Responses

By September 2022, all patients had comparable relative levels of SARS-CoV-2 anti-spike IgG after a mean of 3.45 (2–5) SARS-CoV-2 antigen exposures (vaccinations against the wildtype and/or infections with pre-Omicron variants), with no significant differences compared to patients in follow-up care without any underlying liver disease (control 1: 3.24 $\log_{10}$ BAU/mL; 95% CI: 3.02–3.46; Figure 1a; Table 1a). Additionally, the capacities to neutralize wildtype SARS-CoV-2 (control 1: 93.24%; 95% CI: 87.86–98.63; Figure 1b; Table 1a) and/or the Omicron variants (control 1: 71.48%; 95% CI: 62.78–80.18; Figure 1c; Table 1a) were balanced among all groups of patients. Yet, the capacity to neutralize the Omicron variant was significantly lower than that of the wildtype in all patients of our cohort regardless of cancer activity, cancer type, and/or any underlying liver disease ($p < 0.01$; Figure 1d). The assumed effective titer of SARS-CoV-2 anti-spike IgG of 847.0 BAU/mL was achieved by 68.35% (n = 108/158) of all patients (Table 3). A specific subgroup of patients who did not achieve this titer could not been identified. In 83.3% (n = 50/60) of cases, a qualitative cellular immune response was detectable with no significant differences between the subgroups (Table 3, Interferon-Gamma Releasing Assay).

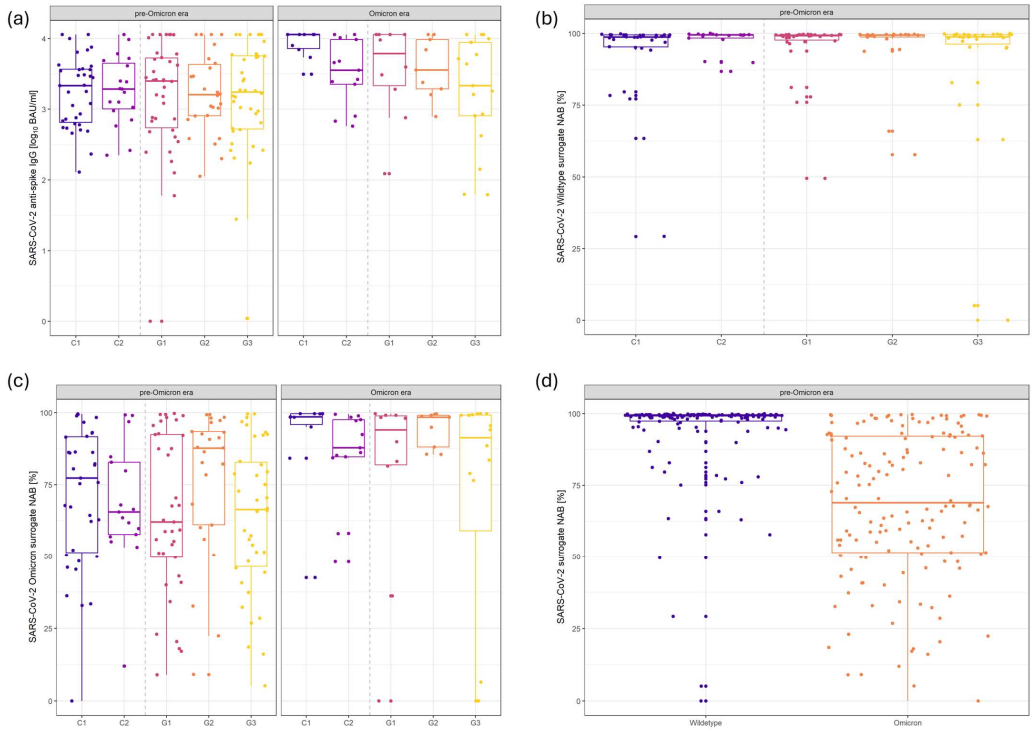

Figure 1. Humoral immune responses to pre-Omicron and Omicron SARS-CoV-2 antigen exposures. Balanced levels of SARS-CoV-2 anti-spike IgG (**a**) in all patients in the pre-Omicron era. Moreover, the capacities to neutralize the wildtype (**b**) as well as the Omicron variants (**c**) of SARS-CoV-2 were comparable in all patients. However, neutralization of the wildtype was significantly higher than that of the Omicron variants (**d**). Following Omicron antigen exposures, both levels of SARS-CoV-2 anti-spike IgG (**a**) as well as the capacity to neutralize the Omicron variants (**c**) increased significantly. Abbreviations: C1: control 1 (patients in follow-up care without underlying liver disease); C2: control 2 (patients in follow-up care with underlying liver disease); G1: group 1 (patients with hepatocellular carcinoma); G2: group 2 (patients with hepatic metastic gastrointestinal cancer); G3: group 3 (patients with non-hepatic metastic gastrointestinal cancer); IgG: immunoglobulin G; sNABs: surrogate neutralization anti-bodies.

Table 1. Titers of SARS-CoV-2 anti-spike IgG and wildtype- and Omicron-neutralization.

	SARS-CoV-2 Anti-Spike IgG [log_{10} BAU/mL]						Wildtype sNAB [%]			Omicron sNAB [%]					
Timepoint	**Pre-Omicron (1)**			**Omicron (2)**			**Pre-Omicron (1)**			**Pre-Omicron (1)**			**Omicron (2)**		
	Estimate	**95% CI**	***p***	**Estimate**	**95% CI**	***p***	**Estimate**	**95% CI**	***p***	**Estimate**	**95% CI**	***p***	**Estimate**	**95% CI**	***p***
(a) Group comparison (intercept: liver-healthy control group)															
Control 1 (Ref.)	3.24	3.02–3.46		3.96	3.58–4.34	<0.01	93.24	87.86–98.63		71.48	62.78–80.18		93.86	78.21–100.0	<0.01
Control 2	3.26	2.97–3.55	0.91	3.52	3.18–3.86	0.09	97.52	90.14–100.0	0.36	68.00	55.60–80.41	0.65	87.41	73.39–100.0	0.80
Group 1	3.17	2.96–3.38	0.64	3.50	3.13–3.88	0.17	95.57	90.35–100.0	0.54	64.78	56.31–73.25	0.28	79.10	63.51–94.68	0.50
Group 2	3.22	2.97–3.48	0.93	3.49	3.10–3.89	0.12	95.47	88.98–100.0	0.60	76.75	66.45–87.05	0.44	90.15	73.63–100.0	0.47
Group 3	3.16	2.96–3.36	0.61	3.26	2.97–3.55	0.01	90.08	84.61–95.55	0.42	63.75	55.39–72.11	0.21	70.70	58.27–83.12	0.16
(b) Group comparison (intercept: control group with underlying liver disease)															
Control 2 (Ref.)	3.26	2.95–3.57		3.52	3.16–3.88	0.18	97.52	89.98–100.0		67.84	55.12–80.55		87.93	73.72–100.0	0.01
Group 1	3.17	2.94–3.39	0.64	3.50	3.10–3.89	0.81	95.57	90.24–100.0	0.68	64.81	56.08–73.54	0.70	79.26	63.82–94.70	0.61
Group 2	3.22	2.95–3.49	0.87	3.49	3.07–3.90	1.00	95.47	88.84–100.0	0.69	76.83	66.22–87.43	0.29	88.63	72.18–100.0	0.48
Group 3	3.16	2.94–3.38	0.62	3.26	2.95–3.57	0.51	90.08	84.49–95.67	0.12	63.76	55.14–72.38	0.60	70.92	58.52–83.33	0.20
(c) Cancer type															
No cancerous activity >1 year (Ref.)	3.24	3.07–3.42		3.71	3.46–3.96	<0.01	94.73	90.47–98.99		70.29	63.13–77.45		90.96	80.69–100.0	<0.01
HCC	3.17	2.96–3.37	0.57	3.50	3.13–3.87	0.57	95.57	90.46–100.0	0.80	64.80	56.27–73.34	0.33	79.21	63.93–94.50	0.52
GEJC	2.90	2.41–3.38	0.19	3.43	2.82–4.03	0.85	74.30	59.40–89.20	0.01	66.98	45.74–88.23	0.77	100.0	74.15–100.0	0.35
PBN	3.52	3.21–3.83	0.12	3.34	2.89–3.79	0.02	98.93	90.67–100.0	0.37	71.89	59.00–84.78	0.83	64.68	46.03–83.33	0.02

Table 1. *Cont.*

	SARS-CoV-2 Anti-Spike IgG [log_{10} BAU/mL]						Wildtype sNAB [%]			Omicron sNAB [%]					
Timepoint	**Pre-Omicron (1)**			**Omicron (2)**			**Pre-Omicron (1)**			**Pre-Omicron (1)**			**Omicron (2)**		
	Estimate	**95% CI**	***p***	**Estimate**	**95% CI**	***p***	**Estimate**	**95% CI**	***p***	**Estimate**	**95% CI**	***p***	**Estimate**	**95% CI**	***p***
CRC	3.05	2.78–3.33	0.25	3.46	3.02–3.90	0.81	93.47	86.45–100.0	0.76	68.32	57.47–79.17	0.76	81.40	63.14–99.67	0.49
NET	3.29	2.96–3.62	0.82	3.35	2.86–3.83	0.15	93.23	84.97–100.0	0.75	73.55	59.64–87.46	0.68	85.38	65.37–100.0	0.46
Other *	2.71	2.14–3.28	0.08	2.64	1.80–3.48	0.24	82.99	69.66–96.31	0.10	50.83	24.81–76.84	0.16	45.62	10.70–80.55	0.18
(d) Type of treatment															
Off treatment > 1 year (Ref.)	3.24	3.07–3.42		3.71	3.45–3.97	<0.01	94.73	90.22–99.24		70.34	63.09–77.59		90.31	79.69–100.0	<0.01
Chemotherapy	3.25	2.95–3.55	0.99	3.10	2.56–3.64	0.05	94.93	86.78–100.0	0.97	67.22	55.21–79.24	0.66	60.52	37.90–83.14	0.05
Immune Checkpoint inhibitors	3.09	2.62–3.55	0.53	3.87	3.01–4.72	0.49	98.34	84.22–100.0	0.63	63.74	45.17–82.30	0.51	93.64	58.11–100.0	0.62
Targeted therapy	3.19	2.77–3.60	0.80	3.45	2.75–4.16	0.61	96.91	86.93–100.0	0.69	64.18	46.67–81.68	0.52	73.57	44.41–100.0	0.53
Local therapy	3.16	2.88–3.43	0.60	3.65	3.22–4.10	0.91	90.56	83.50–97.62	0.33	64.54	52.79–76.28	0.41	91.96	74.03–100.0	0.51
Combined therapy	3.07	2.84–3.31	0.25	3.13	2.76–3.50	0.09	91.97	85.78–98.16	0.48	65.41	56.00–74.83	0.41	67.19	51.20–83.19	0.09

The results of linear mixed model analysis for the SARS-CoV-2 anti-spike IgG and neutralization antibodies for wildtype SARS-CoV-2 and the Omicron variants are shown. Results are reported by mean estimates, 95% confidence intervals, and associated *p*-values. Here, *p*-values refer to the comparison to the shown reference/intercept. Control 1 (patients in follow-up care without underlying liver disease), Control 2 (patients in follow-up care with underlying liver disease), Group 1 (patients with hepatocellular carcinoma), Group 2 (patients with hepatic metastatic gastrointestinal cancer), and Group 3 (patients with non-hepatic metastatic cancer). * duodenal cancer, gastrointestinal stromal tumor, cancer of unknown primary. Abbreviations: CI: confidence interval; HCC: hepatocellular carcinoma; GI: gastrointestinal cancer; GEJN: gastroesophageal junction cancer; IgG: immunoglobulin G; PBN: pancreaticobiliary neoplasms; CRC: colorectal cancer; NET: neuroendocrine tumor; ref: reference; sNAB: surrogate neutralizing antibody.

2.3. Impact of an Omicron Antigen Exposure

Omicron antigen contacts occurred mainly through infections (n = 67; 70.5%) but also through variant-adapted vaccines (n = 28; 29.5%). The course of infections was predominantly mild, with 97.0% (n = 65/67) of cases remained in the outpatient setting. Only two patients required hospitalization, and none needed intensive care (score 4–5 on the WHO Clinical Progression Scale; Table 3). Following any additional Omicron antigen contact, total SARS-CoV-2 anti-spike IgG levels (control 1: 3.96 $\log_{10}$ BAU/mL; 95% CI: 3.58–4.34; $p < 0.01$; Figure 1a; Table 1a) and the capacity to neutralize Omicron (control 1: 93.86%; CI: 78.21–100.0; $p < 0.01$; Figure 1c; Table 1a) increased significantly until September 2023. There was neither a qualitative nor a quantitative difference in the humoral immune responses between patients who had undergone an infection and those who were vaccinated. Only patients with non-hepatic metastatic GI cancer had a significantly weaker booster response for SARS-CoV-2 anti-spike IgG levels (group 3: 3.26 $\log_{10}$ BAU/mL; 95% CI: 2.97–3.55; p = 0.01; Figure 1a; Table 1a) compared to patients in follow-up care without an underlying liver disease. They also achieved the assumed effective titer of SARS-CoV-2 anti-spike IgG of 847.0 BAU/mL less frequently (70.59%; n = 12/17) than all other patients (81.36%; n = 48/59; p = 0.08) in the Omicron era.

A significant booster effect of Omicron antigen contacts on the cellular immune response was not observed in our cohort of patients.

2.4. Impact of Cancer Types and Underlying Liver Diseases on Immune Responses

Patients with GEJC showed a significantly lower capacity to neutralize wildtype SARS-CoV-2 (74.30%; 95% CI 59.40–89.20; p = 0.01; Figure 2b; Table 1c) compared to liver-healthy patients without cancer activity (94.73%; 95% CI: 90.47–98.99) in the pre-Omicron era. Following an additional Omicron antigen contact, immune responses significantly improved in most patients with cancer activity including those with GEJC (Figure 2a,c; Table 1c). Only patients with PBN showed significantly reduced levels of SARS-CoV-2 anti-spike IgG (3.34 $\log_{10}$ BAU/mL; 95% CI: 2.89–3.79; p = 0.02; Figure 2a; Table 1c) as well as a significantly lower capacity to neutralize the Omicron variants (64.68%; 95% CI: 46.03–83.33; p = 0.02; Figure 2c; Table 1c) compared to liver-healthy patients without cancer activity (SARS-CoV-2 anti-spike IgG: 3.71 $\log_{10}$ BAU/mL; 95% CI: 3.46–3.96; Omicron sNAB: 90.96%; CI: 80.69–100.0).

Regarding different types of oncological treatment (chemotherapy, targeted therapy, immune-checkpoint inhibitors, local therapies, and combined therapies), no regimen was associated with a significantly impaired immune response in the pre-Omicron era (Table 1d). However, following an Omicron antigen contact, total levels of SARS-CoV-2 anti-spike IgG (p = 0.05) and the capacity to neutralize the Omicron variants (p = 0.05) were significantly lower in patients receiving conventional chemotherapy. This was not true for patients receiving targeted therapy, immune checkpoint inhibitors, local therapies, or combination therapies (Table 1d).

Compared to patients with underlying liver diseases (control 2), no differences concerning levels of SARS-CoV-2 anti-spike IgG (3.26 $\log_{10}$ BAU/mL; 95% CI: 2.95–3.57; Table 1b) or the capacities to neutralize the wildtype (97.52%; 95% CI: 89.98–100.0; Table 1b) and/or the Omicron variants of SARS-CoV-2 (67.84%; 95% CI: 55.12–80.55; Table 1b) were observed between the groups in the pre-Omicron era. Following an additional Omicron antigen exposure, a significantly increased capacity to neutralize the Omicron variants was revealed (control 2: 87.93%; 95% CI: 73.72–100.0; p = 0.01; Table 1b). In contrast, there was no booster effect on the levels of SARS-CoV-2 anti-spike IgG (control 2: 3.52 log10 BAU/mL; 95% CI: 3.16–3.88; p = 0.18; Table 1b).

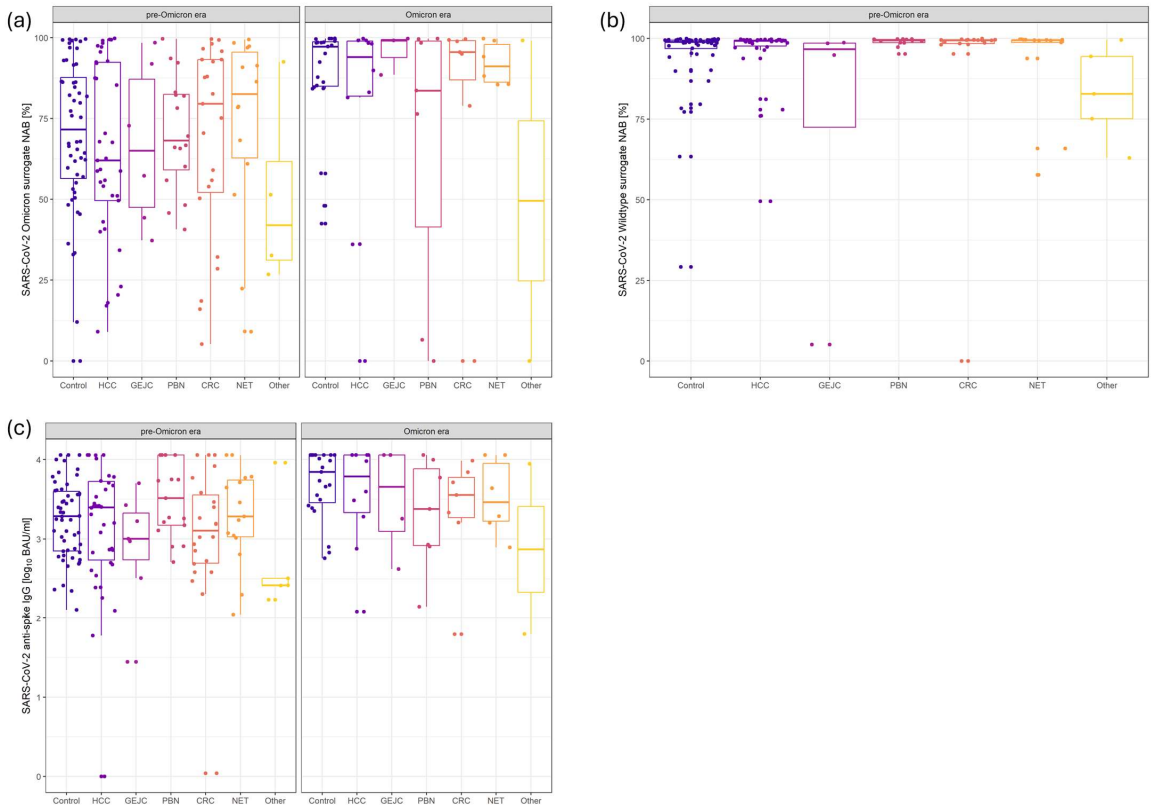

Figure 2. Impact of the type of cancer on humoral immune responses to pre-Omicron and Omicron SARS-CoV-2 antigen exposures. Balanced levels of SARS-CoV-2 anti-spike IgG (**a**) in all patients in the pre-Omicron era. Moreover, the capacity to neutralize the wildtype and/or the Omicron variants were comparable (**b**,**c**). Following Omicron antigen exposures, both levels of SARS-CoV-2 anti-spike IgG (**a**) as well as the capacity to neutralize the Omicron variants (**c**) increased significantly in all patients except for those with pancreaticobiliary cancer. "Other" included duodenal carcinoma, gastrointestinal stroma tumors, and cancer of unknown primary with most likely GI origin. Abbreviations: CRC: colorectal cancer; GEJC: gastroesophageal junction cancer HCC: hepatocellular carcinoma; IgG immunoglobulin; NET: neuroendocrine tumors; PBN: pancreaticobiliary neoplasms; sNABs: surrogate neutralization antibodies.

2.5. Multivariable Analysis of Factors Influencing Immune Responses

After Omicron antigen contacts, any active cancerous disease under oncological treatment was associated with significantly lower SARS-CoV-2 anti-spike IgG levels ($p = 0.04$) and a lower capacity to neutralize Omicron variants ($p = 0.03$) in a multivariable analysis. In contrast, any underlying liver disease or any type of oncological treatment showed no significant effects on the immune response (Figure 3a,c). The ability to neutralize Omicron variants was additionally impaired in patients with underlying relevant immunodeficiency ($p = 0.05$; Figure 3c), which was also true for the ability to neutralize wildtype SARS-CoV-2 in the pre-Omicron era ($p = 0.01$; Figure 3b). Considering different cancer types, it was confirmed that the capacity to neutralize Omicron variants was significantly impaired in patients with PBN ($p = 0.04$).

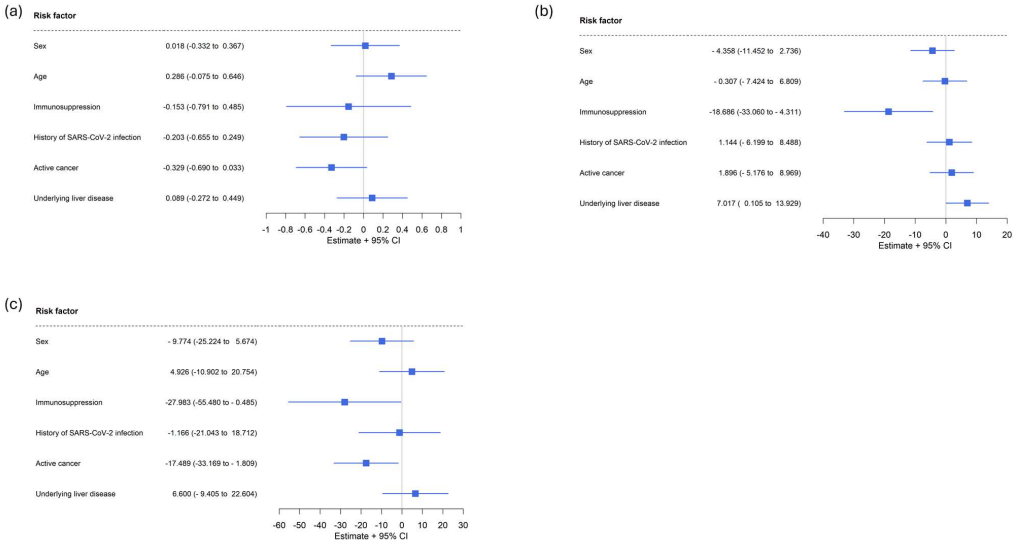

Figure 3. Multivariable analyses on factors potentially influencing immune responses to SARS-CoV-2 antigen exposures. (**a**) Effects on levels of SARS-CoV-2 anti-spike IgG. (**b**) Effects on the capacity to neutralize the wildtype of SARS-CoV-2. (**c**) Effects on the capacity to neutralize the Omicron variants of SARS-CoV-2. Any active cancerous disease under oncological treatment was associated with significantly lower SARS-CoV-2 anti-spike IgG levels ($p = 0.04$; (**a**)) and a lower capacity to neutralize the Omicron variants ($p = 0.03$; (**c**)). The ability to neutralize either the wildtype or the Omicron variants of SARS-CoV-2 was significantly impaired in patients with underlying relevant immunodeficiency ($p = 0.01$; (**b**) and $p = 0.05$; (**c**)). Considering different tumor types, it was confirmed that the capacity to neutralize the Omicron variant was significantly impaired in patients with PBN ($p = 0.04$).

3. Discussion

Patients with active GI cancer showed impaired immune responses after initial antigen contact with an Omicron variant of SARS-CoV-2 and especially a reduced ability to neutralize the Omicron variants. This was particularly pronounced in patients with systemic immunodeficiency and in patients with PBN (univariable and multivariable analysis) and to a lesser extent also in patients undergoing conventional chemotherapy (univariable analysis only).

Investigating longer-term immune responses and especially capacities to cross-reactively neutralize new variants of SARS-CoV-2 (i.e., Omicron in this case) in high-risk patients during the pre-Omicron era is crucial to understand their ability to maintain protective immunity and to evaluate their vulnerability to emerging variants. We found that humoral immune responses were lower than 24 weeks after the first pre-Omicron booster [22], but still balanced in September 2022 in all patients after a mean of 3.45 pre-Omicron antigen exposures. However, 93.24% neutralization of the wildtype was still ensured. Without any Omicron antigen contact having taken place before, a significantly lower cross-reactive neutralization of Omicron variants of 71.48% was observed for all patients of our cohort. Several studies have shown that Omicron neutralization improved after at least three pre-Omicron antigen contacts [31–34]. Nevertheless, cross-reactivity with Omicron variants after antigen contacts with pre-Omicron variants of SARS-CoV-2 were reported to be up to 8-fold lower [30]. Of note, in a cohort of cancer patients, of whom 58% suffered from solid cancer, a much better Omicron neutralization of 90% after three pre-Omicron antigen contacts was described [29], classifying patients with GI cancer as particularly vulnerable.

In a minority of only 16.7% of all study participants ($n = 28/168$), Omicron antigen exposures occurred through variant-adapted vaccination, as recommended by national

and international guidelines for this patient group [15–18]. In line with the higher contagiousness compared to other variants of SARS-CoV-2 [2], most patients of our cohort were exposed to the Omicron variants through infections (39.9%; n = 67/168). In contrast, infections with pre-Omicron variants were significantly lower, at 5.1% in patients with active GI cancer and 2.4% in patients in follow-up care [22]. Consistent data from the pre-Omicron era indicated that overall clinical efficacy, with an 80–90% prevention rate of symptomatic courses of COVID-19 cases, can be assumed even in patients with hematological neoplasms [35]. Importantly, infections with the Omicron variants were generally mild in our cohort, with most patients being managed as outpatients, suggesting that exposures to pre-Omicron SARS-CoV-2 antigens provide substantial protection against infections with the Omicron variants from a clinical perspective. An efficient cellular immune response following an average of three exposures to pre-Omicron SARS-CoV-2 antigens was observed in over 83.3% of our patients, which likely contributed to the mild clinical outcomes. T-cell recognition appears to be relatively well-preserved against most SARS-CoV-2 variants, including Omicron, which is crucial for preventing severe COVID-19 [23–27].

After a continued steady decline in total levels of SARS-CoV-2 anti-spike IgG compared to our previous analyses [21,22], these levels increased significantly and were, thus, stabilized after an additional Omicron antigen exposure. Compared to the group of patients in follow up care with underlying liver disease (control 2), the titers were not stabilized by an Omicron antigen contact. This discrepancy may be attributed to an impaired liver function, since antibody titers in patients with cirrhosis have been shown to decrease more rapidly over time compared to healthy controls [36–38], suggesting a lower booster effect in this population.

Even more importantly, the capacity to specifically neutralize the Omicron variants increased significantly in all patients except those with non-hepatic metastatic GI cancer, particularly in patients with PBN. Interestingly, patients with GEJC showed significantly reduced neutralization of the wildtype prior to an Omicron antigen contact, which was not revealed in our previous studies [21,22]. Additionally, patients with underlying immunodeficiency were consistently less able to neutralize SARS-CoV-2 variants throughout the entire survey period as described for comparable patients with myelodysplastic syndromes and/or acute myeloid leukemia [39]. Following the basic vaccination against SARS-CoV-2, neutralization of the wildtype was also significantly reduced, especially in patients with PBN, as well as those with HCC and/or CRC [20]. Compensation by a third antigen exposure with pre-Omicron variants [21,22] was evidently not sustainable in the long term. Regarding various types of treatment, systemic conventional chemotherapy with cytostatic drugs was especially associated with a significantly poorer neutralization of the Omicron variants. Chemotherapy regimens are particularly used in patients with PBN and/or GEJC, suggesting an association. In patients with active GI cancer, those who received conventional chemotherapy appeared to respond less favorably to the vaccination and must, therefore, be considered particularly at risk. Among patients with solid tumors, especially older individuals and those undergoing chemotherapy showed a faster decline in humoral immune responses compared to cellular immune responses after vaccination against various pathogens, including SARS-CoV-2 [40–42]. This decline was particularly pronounced in patients with hematological neoplasms and those receiving immunosuppressive therapies [43,44], but also in patients with GI cancer [23].

The strength of our study is that, to the best of our knowledge, it is the only longitudinal, albeit monocentric, prospective study of SARS-CoV-2 vaccination in the subgroup of patients with GI cancer, covering the period from January 2021 to September 2023. Thereby, we provide short-term and long-term data on immune responses to different variants of SARS-CoV-2. While the data are robust, we experienced a relatively high loss to follow-up due to the high COVID-19-independent, cancer-associated mortality in the cohort. Additionally, patients who were under best supportive care and/or had decompensated liver cirrhosis were excluded a priori, which relativizes some observations, especially in patients with impaired liver function. Subgroup analyses of the different GI cancerous entities were

not possible due to the small group sizes. Furthermore, we did not assess Omicron-specific cellular immune responses, as we assumed T-cell responses would be relatively stable over time regardless of the variant.

4. Patients and Methods

4.1. Study Design

This is the final report of our prospective, longitudinal cohort study in patients with GI cancer treated at the Department of Internal Medicine I, Gastroenterology Oncology Section at the University Hospital of Bonn, Germany. The timeline since the start of the observation period in January 2021 and design of the study are shown in Figure 4.

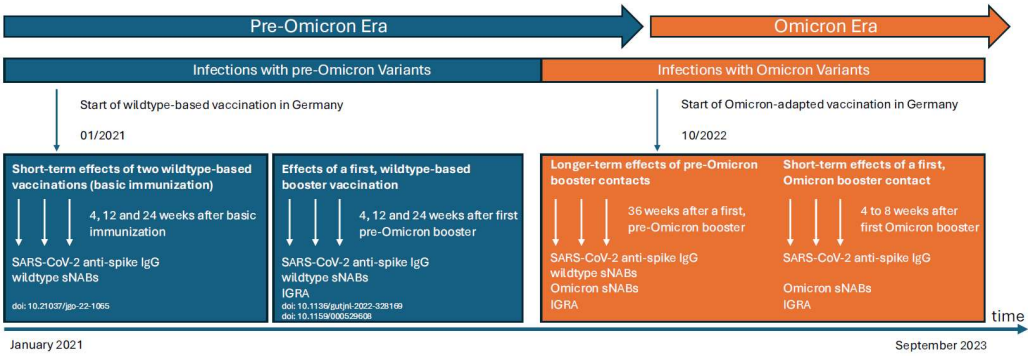

Figure 4. Study design. From the beginning of the second calendar week in 2022, Omicron was the predominant variant of SARS-CoV-2 in Germany, though the infection rate was initially very low in our cohort. Omicron-adapted vaccines became available in October 2022, and the number of Omicron infections significantly increased in our cohort at the same time. Based on this knowledge, we defined a pre-Omicron and an Omicron era. In three previous studies, we evaluated short-term effects of the basic immunization (4, 12, and 24 weeks after two wildtype-based vaccinations against SARS-CoV-2; first blue box), followed by analyses on the effects of a first wildtype-based booster vaccination (4, 12, and 24 weeks after a third wildtype-based booster vaccination; second blue box), as described elsewhere [19–21]. In the present analysis, we focused on the ability to neutralize the Omicron variants of SARS-CoV-2 (orange box). Thereby, we firstly investigated longer-term effects of pre-Omicron antigen booster contacts considering both wildtype-based vaccinations and/or infections with pre-Omicron variants. The patients of our cohort had a mean of 3.45 (2–5) pre-Omicron antigen contacts at this point of time. A second analysis focused on short-term effects of a first Omicron antigen booster contact by Omicron-adapted vaccination or infection with an Omicron variant (four to eight weeks after this Omicron contact). Abbreviations: IgG: immunglobulin G; IGRA: Interferon-Gamma Releasing Assay; sNABs: surrogate neutralizing antibodies.

During the initial phase, short-term immune responses to basic SARS-CoV-2 immunization, defined as two mRNA-based vaccinations against the wildtype of SARS-CoV-2, were evaluated until April 2022 [20]. In subsequent follow-up studies, effects of a first wildtype-based booster vaccination were investigated up to July 2022 [21,22].

For the current analysis, we focused on the effect of a first Omicron antigen contact. Therefore, we initially evaluated longer-term effects of pre-Omicron booster antigen contacts (36 weeks after first pre-Omicron booster) until September 2023 (pre-Omicron era). At this point, the patients had a mean of 3.45 (2–5) pre-Omicron antigen contacts. Vaccinations with mRNA-based vaccines against the wildtype and/or infections with pre-Omicron variants were categorized as contacts with a SARS-CoV-2 antigen. For the latter, both PCR- and antigen-confirmed infections as well as silent infections with detection of SARS-CoV-2 anti-nucleocapsid IgG were considered (Table 2). Variant determinations were completed for patients with PCR-confirmed infections to exclude patients with early Omicron infections.

In cases with antigen-confirmed infections, the currently locally predominant variant was assumed. The analysis included levels of SARS-CoV-2 anti-spike immunoglobulins (IgG), the capacities to neutralize wildtype and cross-reactively Omicron-variants of SARS-CoV-2, and the cellular immune response.

Table 2. SARS-CoV-2-associated immunological and clinical characteristics.

	Control 1 (*n* = 37)		**Control 2 (*n* = 22)**		**Group 1 (*n* = 39)**		**Group 2 (*n* = 26)**		**Group 3 (*n* = 44)**	
Number of vaccinations										
2	5.4%	(2)	4.5%	(1)	5.1%	(2)	3.8%	(1)	6.8%	(3)
3	43.2%	(16)	40.9%	(9)	61.5%	(24)	26.9%	(7)	43.2%	(19)
≥4	51.4%	(19)	54.6%	(12)	33.4%	(13)	69.2%	(18)	50.0%	(22)
SARS-CoV-2 anti-spike IgG ≥ 847.0 BAU/mL (pre-Omicron)										
No	37.8%	(14)	31.8%	(7)	38.5%	(15)	30.8%	(8)	36.4%	(16)
Yes	62.2%	(23)	68.2%	(15)	61.5%	(24)	69.2%	(18)	63.6%	(28)
IGRA count (pre-Omicron)										
Positive	5.4%	(2)	40.9%	(9)	33.3%	(13)	34.6%	(9)	25.0%	(11)
Negative	21.6%	(8)	4.5%	(1)	7.7%	(3)	3.8%	(1)	6.8%	(3)
Not analyzed	73.0%	(27)	54.5%	(12)	59.0%	(23)	61.5%	(16)	68.2%	(30)
Infected with a pre-Omicron variant										
No	78.4%	(29)	90.9%	(20)	89.7%	(35)	88.5%	(23)	79.5%	(35)
Yes	21.6%	(8)	9.1%	(2)	10.3%	(4)	11.5%	(3)	20.5%	(9)
Infected with an Omicron-variant										
No	70.3%	(26)	40.9%	(9)	69.2%	(27)	57.7%	(15)	54.5%	(24)
Yes	29.7%	(11)	59.1%	(13)	30.8%	(12)	42.3%	(11)	45.5%	(20)
Omicron-adapted vaccination										
No	81.1%	(30)	68.2%	(15)	100%	(39)	73.1%	(19)	84.1%	(37)
Yes	18.9%	(7)	31.8%	(7)	-		26.9%	(7)	15.9%	(7)
Clinical course of Omicron infections (WHO clinical progression Scale)										
Scale 1–3 (non-hospitalized)	100%	(11)	100%	(13)	100%	(12)	90.9%	(10)	95.0%	(19)
Scale ≥ 4 (hospitalized)	-		-		-		9.1%	(1)	5.0%	(1)

Comparison between Control 1 (patients in follow-up care without underlying liver disease), Control 2 (patients in follow-up care with underlying liver disease), Group 1 (patients with hepatocellular carcinoma), Group 2 (patients with hepatic metastatic gastrointestinal cancer), and Group 3 (patients with non-hepatic metastatic cancer). Abbreviations: IgG: immunoglobulin G; IGRA: Interferon-Gamma Releasing Assay; SARS-CoV-2: severe acute respiratory syndrome coronavirus type 2; WHO: World Health Organization.

From the beginning of the second calendar week in 2022, Omicron became the predominant variant of SARS-CoV-2 in Germany [45], which it still is today. Since October 2022, infection rates increased significantly in our cohort, for which we, therefore, assumed infections with the Omicron variant (Table 3). As most patients remained in the outpatient setting, variant determination was no longer performed. In addition, we assumed silent infections with an Omicron variant in patients who developed seroconversion for SARS-CoV-2 anti-nucleocapsid IgG for the first time from October 2022. Omicron-adapted vaccines also became available at that time. From October 2022 to September 2023, SARS-CoV-2 immune responses were re-evaluated 4 to 12 weeks after a first Omicron antigen booster via variant-adapted vaccination and/or infection (Omicron era). The period for titer measurement was extended because SARS-CoV-2 was less present in the public eye and patients were less motivated to report for additional study visits. SARS-CoV-2 anti-spike immunoglobulins (IgG), the capacities to neutralize Omicron-variants of SARS-CoV-2, and the cellular im-

mune response were remeasured, thereby presenting short-term data on the effect of an Omicron-based booster on the long-term immune response.

Table 3. Baseline characteristics.

	Control 1 (*n* = 37)		**Control 2 (*n* = 22)**		**Group 1 (*n* = 39)**		**Group 2 (*n* = 26)**		**Group 3 (*n* = 44)**	
Age [years]										
Mean (range)	65	(33–85)	69	(44–84)	69	(49–85)	67	(48–87)	67	(45–89)
Sex										
Male	45.9%	(17)	45.5%	(10)	74.4%	(29)	61.5%	(16)	70.5%	(31)
Female	54.1%	(20)	54.5%	(12)	25.6%	(10)	38.5%	(10)	29.5%	(13)
Cancer types										
HCC	10.8%	(4)	22.7%	(5)	100%	(39)	-		-	
GEJC	18.9%	(7)	9.1%	(2)	-		-		18.2%	(8)
Esophageal cancer	2.7%	(1)	9.1%	(2)	-		-		11.4%	(5)
Gastric cancer	16.2%	(6)	-		-		-		6.8%	(3)
PBN	13.5%	(5)	13.6%	(3)	-		15.4%	(4)	34.1%	(15)
Pancreatic cancer	10.8%	(4)	-		-		7.7%	(2)	18.2%	(8)
BTC	2.7%	(1)	13.6%	(3)	-		7.7%	(2)	15.9%	(7)
CRC	27.0%	(10)	40.9%	(9)	-		38.5%	(10)	29.5%	(13)
NET	18.9%	(7)	4.5%	(1)	-		42.3%	(11)	9.1%	(4)
Other	10.8%	(4)	9.1%	(2)	-		3.8%	(1)	9.1%	(4)
Duodenal cancer	2.7%	(1)	-		-		-		6.8%	(3)
GIST	5.4%	(2)	4.5%	(1)	-		3.8%	(1)	2.3%	(1)
CUP	2.7%	(1)	4.5%	(1)	-		-		-	
Type of treatment										
Chemotherapy *	-		-		-		23.1%	(6)	31.8%	(14)
Immune checkpoint inhibitors **	-		-		7.7%	(3)	7.7%	(2)	6.8%	(3)
Targeted therapy ***	-		-		10.3%	(4)	3.8%	(1)	11.4%	(5)
Local therapy ****	-		-		41.0%	(16)	3.8%	(1)	15.9%	(7)
Combined therapy *****	-		-		38.5%	(15)	26.9%	(7)	25.0%	(11)
Other ******	-		-		2.6%	(1)	34.6%	(9)	9.1%	(4)
Immunodeficiency #										
No	91.9%	(34)	95.5%	(21)	87.2%	(34)	88.5%	(23)	97.7%	(43)
Yes	8.1%	(3)	4.5%	(1)	12.8%	(5)	11.5%	(3)	2.3%	(1)
Underlying Chronic liver disease										
No	100%	(37)	-		15.4%	(6)	80.8%	(21)	77.3%	(34)
Yes	-		100%	(22)	84.6%	(33)	19.2%	(5)	22.7%	(10)
Cirrhotic	-		13.6%	(3)	78.8%	(26)	40.0%	(2)	-	
Non-cirrhotic	-		86.4%	(19)	21.2%	(7)	60.0%	(3)	100%	(10)
Etiology of liver diseases										
ASH	-		13.6%	(3)	36.4%	(12)	60.0%	(3)	30.0%	(3)
Autoimmune hepatitis	-		-		3.0%	(1)	-		-	
Budd-Chiari-Syndrome	-		4.5%	(1)	-		-		-	
Chemotherapy-induced hepatopathy	-		4.5%	(1)	-		-		-	
Post-resection liver dysfunction	-		4.5%	(1)	-		-		10.0%	(1)
Hemochromatosis	-		-		6.1%	(2)	-		-	
Hepatitis A	-		4.5%	(1)			-		-	
Hepatitis B	-		9.1%	(2)	9.1%	(3)	-		10.0%	(1)
Hepatitis C	-		9.1%	(2)	9.1%	(3)	-		10.0%	(1)
Idiopathic	-		4.5%	(1)	6.1%	(2)	-		-	
MASLD	-		40.9%	(9)	30.3%	(10)	40.0%	(2)	40.0%	(4)
PSC	-		4.5%	(1)	-		-		-	

Table 3. *Cont.*

	Control 1 (*n* = 37)		Control 2 (*n* = 22)		Group 1 (*n* = 39)		Group 2 (*n* = 26)		Group 3 (*n* = 44)	
Blood Transfusion received										
No	100%	(37)	95.5%	(21)	76.9%	(30)	92.3%	(24)	86.4%	(38)
Yes	-		4.5%	(1)	23.1%	(9)	7.7%	(2)	13.6%	(6)
Lethal outcome due to underlying cancer										
No	100%	(37)	100%	(22)	76.9%	(30)	88.5%	(23)	79.5%	(35)
Yes	-		-		23.1%	(9)	11.5%	(3)	20.5%	(9)

Comparison between Control 1 (patients in follow-up care without underlying liver disease), Control 2 (patients in follow-up care with underlying liver disease), Group 1 (patients with hepatocellular carcinoma), Group 2 (patients with hepatic metastatic gastrointestinal cancer), and Group 3 (patients with non-hepatic metastatic cancer). * Capecitabine, Cisplatin, Docetaxel, 5-Fluorouracil, Gemcitabine, Irinotecan, Oxaliplatin, Paclitaxel, Tipiracil, Trifluridine. ** Atezolizumab, Nivolumab, Pembrolizumab. *** Bevacizumab, Cabozantinib, Imatinib, Lenvatinib, Panitumumab, Sorafenib. **** Microwave Ablation, Radiotherapy, Selective Internal Radiation Therapy, surgical resection, Transarterial Chemoembolization. ***** immune checkpoint inhibitors + targeted therapy, immune checkpoint inhibitors + local therapy, immune checkpoint inhibitors + chemotherapy, chemotherapy + local therapy, chemotherapy + targeted therapy. ****** somatostatin analogues, therapies in the context of clinical studies. # Immunosuppression was assumed in patients with HIV infection and $CD4^+$-cell count < 200 cells/µL and/or under therapy with Calcineurin-Inhibitors or glucocorticoids > 10 mg/day. Abbreviations: ASH: alcoholic steatohepatitis; BTC: biliary tract cancer, i.e., cholangiocellular cancer and gallbladder cancer; CRC: colorectal cancer; CUP: cancer of unknown primary; GEJC: gastroesophageal junction cancer; GIST: gastrointestinal stromal tumor; HCC: hepatocellular carcinoma; IGRA: Interferon-Gamma Release Assay; MASLD: metabolic dysfunction-associated steatotic liver disease; NET: neuroendocrine tumor; PBN: pancreaticobiliary neoplasms; PSC: primary sclerosing cholangitis.

The study was conducted in accordance with the Declaration of Helsinki, approved by the Institutional Review Board of the Medical Faculty of the University of Bonn (Nos. 341/17 and 023/22). All patients signed an informed consent form, were informed about clinically relevant results of their immunological test results and received individual advice on further vaccinations and/or strategies in the event of infection with SARS-CoV-2.

4.2. Patients' Characteristics and Eligibility Criteria

All patients with active cancer underwent oncologic treatment at the time of study evaluation. Patients with HCC had previously shown particularly poor immune responses to SARS-CoV-2 antigen contacts [20–22,46]. In addition, liver metastases and/or underlying liver disease were considered to investigate the influence of morphological and/or functional changes in the liver on immune responses. Therefore, the following groups were defined: patients with HCC (group 1), patients with hepatic metastatic GI cancer (group 2), and patients with non-hepatic metastatic GI cancer (group 3). The latter included cases without any metastases under neoadjuvant or adjuvant therapy and cases with metastases at sites other than the liver. GI cancer other than HCC included gastroesophageal junction cancer (GEJC: gastric cancer, esophageal cancer), pancreaticobiliary neoplasms (PBN: pancreatic cancer and biliary tract cancer), colorectal cancer (CRC), neuroendocrine tumors, and other cancers (duodenal cancer, gastrointestinal stroma tumors, and cancer of unknown primary with most likely GI origin). Patients in follow-up care with a past medical history of GI cancer without active cancerous disease and not under maintenance therapy for at least 12 months prior to inclusion were involved as control groups. In these patients, a distinction was made as to whether underlying liver diseases were absent (control 1) or present (control 2).

Standardized medical records were used from the Hospital Information System Orbis (version 08044202.01000.DACHL, DH Healthcare GmbH, Bonn, Germany) to document other possible factors influencing the immune response to exposure to the SARS-CoV-2 antigen (Tables 1 and 2). Those included the location of the cancer, types of oncological treatment (systemic therapies including chemotherapy, targeted therapy, immune-checkpoint inhibitors, as well as combined local and systemic therapies), underlying immunodeficiency (suspected in patients with long-term immunosuppressive medication and chronic HIV

infection with $CD4^+$-cell count < 200/µL), underlying liver diseases, history of SARS-CoV-2 infection, age, and sex. Data on the course of infections with an Omicron variant were considered using the World Health Organization (WHO) Clinical Progression Scale [47–49], by which patients were categorized into a non-hospitalized (score 1–3) and a hospitalized cohort (score $\geq$ 4) for the present analysis.

Patients under best supportive care and/or with decompensated liver function (Child-Pugh B/C score $\geq$ 8) who were not eligible for cancer treatment were excluded from the study. Moreover, patients who had completed their treatment within the 12 months prior to recruitment were not considered. For the analysis during the Omicron era, those patients without any additional Omicron antigen exposure were excluded.

4.3. Assessment of Humoral and Cellular Response Rates

Quantification of SARS-CoV-2 anti-spike IgG levels, as well as of the capacity to neutralize the wildtype virus and analysis of the cellular immune response, was performed as previously described [20–22]. The capacity to neutralize the Omicron variants of SARS-CoV-2 was determined using cPassTM SARS-CoV-2 Neutralization Antibody Detection Kit Omicron Variant (GenScript). This enzyme-linked immunosorbent assay measures surrogate neutralizing antibodies (sNABs), and thus, detects functional effects of antibodies, which are strongly associated with live-virus neutralization of the Omicron variants [50,51].

For the present study, we assumed protection against severe courses of COVID-19 at a titer of 847.0 BAU/mL or higher. The value of 847.0 BAU/mL was above the 20th percentile in immunocompetent patients in a seroprevalence study, while values below the 20th percentile were associated with a higher mortality rate for breakthrough infections [52,53].

4.4. Statistical Analysis

Statistical analysis was carried out using R version 4.3.3 (R Core Team 2024: R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria). Descriptive analyses included the calculation of medians and interquartile ranges for continuous variables and frequencies (absolute and relative) for categorical variables.

Univariate linear mixed effects models were used to compare ($\log_{10}$ transformed) levels of SARS-CoV-2 anti-spike IgG as well as neutralization of the wildtype and the Omicron variants in the pre-Omicron era and in the Omicron era across the five groups and with respect to cancer type and type of treatment, respectively. Each model contains main effects, interaction terms with time, as well as a patient-specific random intercept. Additionally, a univariate logistic mixed effects model was used for modeling the probabilities of achieving an effective titer (SARS-CoV-2 anti-spike IgG of 847.0 BAU/mL) for the five groups at the two points in time. Neutralization of the wildtype and the Omicron variants were compared in the pre-Omicron era applying a simple linear model. Furthermore, multivariable regression analyses (for SARS-CoV-2 anti-spike IgG and the capacities to neutralize the wildtype and the Omicron variants) were performed to examine a possible effect of age, sex, history of SARS-CoV-2 infection, and additional immunosuppression. In this study, p-values $\leq$ 0.05 were regarded as statistically significant.

5. Conclusions

Clinical courses of a first infection with an Omicron variant of SARS-CoV-2 were predominantly mild to moderate in our cohort of patients with active GI cancer. This suggests that pre-Omicron immunological protection after at least three SARS-CoV-2 antigen contacts is also effective against these emerging variants of SARS-CoV-2. From a clinical point of view, pre-Omicron immunological protection after at least three SARS-CoV-2 antigen contacts was also effective in the case of a first infection with an Omicron variant in our cohort of patients with active GI cancer. However, we demonstrated that booster antigen contacts can only temporarily stabilize waning humoral immune responses and that initial contacts with SARS-CoV-2 antigens of emerging variants continues to trigger only reduced immune responses. Vaccination gaps should, thus, be closed urgently. Our study

underscores the importance of continued monitoring and tailored vaccination strategies for GI cancer patients to ensure sustained protection against evolving SARS-CoV-2 variants. Based on the presented data, among patients with GI carcinoma, especially patients with PBN, patients undergoing conventional chemotherapy and patients with systemic immunodeficiency show poorer responses to SARS-CoV-2 antigen contacts. For the time being, booster vaccinations should be recommended in line with the national and international guidelines to maintain long-term protection against SARS-CoV-2.

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

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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2.4 Klinische Bewertung antiviraler Monotherapien anhand der WHO-Ordinalskala

Hübner, Y.R., Spuck, N., Berger, M., Schlabe, S., Rieke, G.J., Breitschwerdt, S., van Bremen, K., Strassburg, C.P., Gonzalez-Carmona, M.A., Wasmuth, J.-C., Rockstroh, J.K., Boesecke, C., **Monin, M.B.** (2023). Antiviral treatment of COVID-19: which role can clinical parameters play in therapy evaluation? Infection *51*.

Ziel dieser Studie war es, den klinischen anstatt des virologischen Verlaufs von COVID-19 für Therapieentscheidungen und -bewertungen antiviraler Monotherapien zu exponieren (vgl. Kapitel 1.8.2). Die Analyse wurde schlussendlich korrespondierend zu den LEOSS-Daten (*Lean European Open Survey on SARS-CoV-2 infected patients*), die einen Einsatz von RDV im fortgeschrittenen Stadium von COVID-19 unterstützen[67], publiziert.

Insgesamt konnten 330 ältere, multimorbide PatientInnen mit COVID-19, einem hohen Risiko für einen schweren klinischen Verlauf und somit einer Indikation für eine antivirale Monotherapie in diese retrospektive Studie aufgenommen werden. Der Anteil von PatientInnen mit systemischer Immundefizienz und einem zusätzlich hohen Risiko für einen prologierten Virusnachweis war gering (n=9). Zur klinischen Beurteilung haben wir die WHO-Ordinalskala genutzt (vgl. Kapitel 1.3), wobei jeweils der schlechteste Score im Laufe der Infektion gewertet wurde. Die Effektivität des Einsatzes der mAB-Kombination CVIV in der Frühen Phase der Infektion wurde bei geimpften und ungeimpften PatientInnen daran gemessen, ob eine unterstützende Sauerstofftherapie notwendig wurde (Score ≥4). Der Einsatz von RDV ab einem Score von 4 auf der WHO-Ordinalskala, also in der Pulmonalen Phase der Infektion, wurde bei ungeimpften PatientInnen anhand des Fortschreitens der Erkrankung im Weiteren beurteilt (Score 4-8). PatientInnen in zwei gematchten Kontrollgruppen erhielten bei zum Zeitpunkt der Studie begrenzten Ressourcen weder eine Therapie mit CVIV in der Frühen Phase noch mit RDV in der Pulmonalen Phase der SARS-CoV-2-Infektion.

Bei frühem Einsatz von CVIV bestand die Notwendigkeit einer Sauerstofftherapie (Score ≥4) im Verlauf der Erkrankung im Vergleich zu unbehandelten PatientInnen signifikant seltener. PatientInnen, die in der späten Phase von COVID-19 (Score ≥4) mit RDV therapiert wurden, entwickelten im Weiteren signifikant niedrigere Scores auf der WHO-Ordinalskala. Die Gabe von RDV führte zu einer signifikant verkürzten Hospitalisierungszeit. Die Dauer des SARS-CoV-2-Nachweises mittels PCR konnte durch eine Monotherapie mit RDV in der Pulmonalen Phase von COVID-19 in dieser Kohorte jedoch nicht beeinflusst werden. Ein

Patientenalter über 65 Jahre wurde als starker Prädiktor für einen schwereren klinischen Verlauf trotz Monotherapie mit CVIV oder RDV anhand der WHO-Ordinalskala herausgearbeitet, was durch einen deutlichen Anstieg der notwendigen Sauerstofftherapien und Todesfälle in dieser Subgruppe belegt wurde.

Basierend auf diesen Daten formulierten wir die Empfehlung, klinische Gesichtspunkte bei der Therapieentscheidung und -bewertung stärker zu gewichten und Virusnachweise mittels PCR nur sekundär heranzuziehen.

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CORRESPONDENCE

Antiviral treatment of COVID-19: which role can clinical parameters play in therapy evaluation?

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Dear Editor,

With great interest, we read the article "Use and Effectiveness of Remdesivir for the Treatment of Patients with COVID-19 Using Data from the Lean European Open Survey on SARS-CoV-2 infected patients (LEOSS): A multicentre cohort study", published in Infection by Pilgram et al. [1]. According to the data, initiation of treatment with remdesivir in the advanced course of COVID-19 was effective.

Our own data basically confirm the results and add further insights. In summary, we do propose to use a simple score based on clinical data, both, for therapy decision-making and for therapy evaluation.

The COVID-19 pandemic has created a global public health crisis with the need for treatment options to alleviate symptoms and prevent spreading of the virus. Direct acting antivirals (DAA) and monoclonal antibodies (mABs) neutralizing SARS-CoV-2 are recommended for treatment by the World Health Organization (WHO) based on pivotal studies and real-world data mainly focusing on virological parameters [2]. In contrast, more robust clinical data as presented by Pilgram et al. are still limited [1]. Of note, for the time being, mABs are only effective to a very limited extent due to viral evolution and their use is therefore reserved for individual cases. The focus was therefore placed on the data on the use of RDV.

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We here mapped clinical outcomes using the WHO ordinal clinical severity scale (hereafter: WHO ordinal scale) to assess the effectiveness of remdesivir (RDV) and the mAB combination casirivimab/imdevimab (CVIV).

This retrospective, single-centre cohort study aimed to assess the effect of treatment of COVID-19 on the clinical course of patients admitted to the infectious disease department of the University Hospital Bonn, Germany, between March 2020 and November 2021.

The diagnosis of COVID-19 had to be PCR-confirmed. Patients were assigned to two groups based on the treatment they received during their hospital stay: the RDV group and the CVIV group.

RDV therapy was initiated immediately in patients requiring oxygen (the latest with an oxygen saturation of 90.0%). Median time from diagnosis to initiation of RDV therapy was 7 days (IQR 4–10 days). Vaccinated patients and patients receiving a combination of RDV and CVIV and/or dexamethasone were excluded. Therapeutic effect was assessed comparing unvaccinated patients having received RDV to unvaccinated, untreated control patients requiring oxygen support.

The CVIV group included patients who received this mAB within the first five days after onset of the infection. The control group comprised patients hospitalized within the first five days after onset of the infection not treated with CVIV. For those groups, vaccination and combination of CVIV and RDV and/or dexamethasone in the further course were no exclusion criteria.

The clinical course was assessed using the WHO ordinal scale. Each patient was scored with zero to eight points: 0: no clinical or virological evidence of infection; 1: ambulatory, no activity limitation; 2: ambulatory, activity limitation; 3: hospitalized, no oxygen therapy; 4: hospitalized, oxygen mask or nasal prongs; 5: hospitalized, noninvasive mechanical ventilation or high-flow nasal cannula; 6:

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hospitalized, intubation and invasive mechanical ventilation (IMV); 7: hospitalized, IMV + additional support such as pressors or extracardiac membranous oxygenation; 8: death [3]. The primary endpoint in the RDV group was the WHO ordinal scale score of patients requiring oxygen support (WHO ordinal scale score > 3) treated with RDV compared to untreated patients with WHO ordinal scale score > 3. To assess the efficacy of CVIV, it was analysed whether oxygen therapy was necessary under CVIV (WHO ordinal scale score > 3) compared to a control group not treated with CVIV. In both groups, the worst WHO ordinal scale score was determined for each patient for the comparative analyses. The effects of RDV on length of hospitalization and duration of positive PCR results were secondary endpoints.

Raw data were collected and organized in an electronic case report form. All data were de-identified and kept confidential to maintain patients' privacy. Statistical analysis was performed using R software (version 4.0.3). Inverse probability of treatment weights were determined using propensity scores in order to account for possibly unequal distributions of important risk factors between the treatment groups. That is, observations in the treatment group that are similar to the expected observation in the control group are weighted more strongly, and vice versa. Propensity scores were calculated based on the most frequently documented risk factors, i.e., age over 65 years, hypertension, obesity, diabetes, chronic kidney disease, heart disease, chronic obstructive pulmonary disease, and neurological disease, as defined by the Center for Disease Control and Prevention (CDC) [4], and the total number of present risk factors. Vaccination status was used as an additional factor in the CVIV analysis. We applied inverse probability weighting as it allows the use of the complete sample in the analysis and facilitates the estimation of the average treatment effect, whereas, when propensity score matching is used, patients for whom no suitable match is available may be excluded and only the average effect on the treated is estimated [6]. An ordered logistic regression model was applied to the weighted data to analyse the effect of RDV on the WHO ordinal scale. Cumulative incidence functions were calculated to assess the effects on the length of hospitalization and duration of positive PCR results. Confidence intervals (CI) were constructed based on 500 bootstrap samples. The effect of CVIV on need for oxygen support (WHO ordinal scale score > 3) was investigated using a binary logistic model. The study was conducted in accordance with the Declaration of Helsinki.

A total of 394 patients who were diagnosed with PCR-confirmed COVID-19 were included in this study, with a mean age of 60.3 ± 18 years. As shown in Table 1, age greater than 65 years was identified as the most frequently observed risk factor in the data. Of all patients, 10.9% (n = 43/392) received oxygen therapy and were treated with remdesivir (RDV) alone, while 19.3% (n = 76/392) were hospitalized within five days after infection and received CVIV treatment. 6.3% (n = 25/394) were treated with CVIV and, in the further course, additionally with RDV. The control groups included 12.4% (n = 49/394) and 41.2% (n = 162/394) of the patients, respectively. 16.2% (n = 64/394) were excluded due to the criteria mentioned above. The detailed distribution of risk factors for severe COVID-19 in the study population is shown in Table 1.

The results of the descriptive statistical analysis revealed a notable association between the number of risk factors and a worse WHO ordinal scale score, particularly with respect to the escalation of treatment to oxygen therapy and mortality rates (Fig. 1). Of note, age > 65 years was found to be an outstanding predictor of worse WHO ordinal scale outcome, as demonstrated by the substantial increases observed in rates of oxygen therapy and death (Fig. 2).

Our findings indicate that RDV treatment in patients with advanced COVID-19 (median time of 7 days between diagnosis and treatment initiation; IQR 4–10 days) and need for oxygen support was associated with a significantly lower probability of worse WHO ordinal scale outcomes compared to untreated controls (OR 0.12, 95% CI 0.05–0.29, $p < 0.001$) (Fig. 3). We additionally calculated cumulative incidence functions: a significantly reduced time until hospital discharge for patients treated with RDV was demonstrated based on the 95% CI (Fig. 4). An association of treatment with RDV and a shortened time until testing negative for COVID-19 could not be shown (Fig. 5).

Finally, early treatment initiation with CVIV within the first five days after diagnosis of COVID-19 in patients at high risk for a severe course was found to prevent the development of a WHO ordinal scale > 3 (OR 0.25, 95% CI 0.12–0.55, $p < 0.001$), i.e., requiring oxygen (Fig. 6).

Our study adds clinical data to the growing evidence supporting the use of RDV in patients with advanced COVID-19—especially in unvaccinated patients. Using the WHO ordinal scale, we found that clinical course was better in patients in need for oxygen support who received RDV thereby supporting the data of Pilgram et al. [1].

RDV was used in the late phase of infection in patients requiring oxygen—and thereby in part outside the current indication. High-risk patients who have missed early therapy with DAAs and/or mABs can therefore benefit from the use of RDV in the event of clinical deterioration.

However, the decisive use of mABs such as CVIV in the early phase of infection can effectively prevent such events. mABs have strongly been recommended due to their efficacy and lack of severe side effects [2]. Yet, the emergence of new viral variants complicates the use of mABs, as efficacy dropped dramatically with mutations at the SARS-CoV-2 spike protein. Currently, all available mABs have rapidly experienced a loss of efficacy due to viral evolution. Therefore, the determination of the underlying SARS-CoV-2

Table 1 Baseline characteristics of this studies population

	RDV analysis data		CVIV analysis data		Overall (N = 394)
	No RDV (N = 49)	RDV (N = 43)	No CVIV (N = 162)	CVIV (N = 76)	
Gender (male)	20 (40.8%)	25 (58.1%)	84 (51.9%)	42 (55.3%)	212 (53.8%)
Age in years, mean ± SD	66.5 ± 19.2	63.3 ± 14.6	62.8 ± 18.2	58.1 ± 18.6	60.3 ± 18
SARS-CoV-2 vaccination	–	–	21 (13%)	30 (39.5%)	76 (19.3%)
Risk factors for severe COVID-19					
Age > 65 years	29 (59.2%)	21 (48.8%)	84 (51.9%)	30 (39.5%)	186 (47.2%)
BMI > 25 kg/m^2	8 (16.3%)	12 (27.9%)	38 (23.5%)	17 (22.4%)	92 (23.4%)
Hypertension	26 (53.1%)	18 (41.9%)	68 (42%)	30 (39.5%)	164 (41.6%)
Diabetes mellitus	13 (26.5%)	5 (11.6%)	29 (17.9%)	19 (25%)	80 (20.3%)
CKD	9 (18.4%)	4 (9.3%)	18 (11.1%)	11 (14.5%)	40 (10.2%)
Heart disease	19 (38.8%)	11 (25.6%)	44 (27.2%)	19 (25%)	98 (24.9%)
COPD	5 (10.2%)	1 (2.3%)	13 (8%)	6 (7.9%)	24 (6.1%)
Neurological disease	7 (14.3%)	6 (14%)	19 (11.7%)	8 (10.5%)	38 (9.6%)
Number of risk factors					
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	47 (11.9%)
1	2 (4.1%)	6 (14%)	18 (11.1%)	0 (0%)	95 (24.1%)
2	9 (18.4%)	8 (18.6%)	33 (20.4%)	28 (36.8%)	80 (20.3%)
3	9 (18.4%)	6 (14%)	29 (17.9%)	16 (21.1%)	73 (18.5%)
4	15 (30.6%)	12 (27.9%)	41 (25.3%)	8 (10.5%)	42 (10.7%)
5	7 (14.3%)	6 (14%)	17 (10.5%)	9 (11.8%)	35 (8.9%)
6	5 (10.2%)	3 (7%)	15 (9.3%)	11 (14.5%)	16 (4.1%)
7	2 (4.1%)	2 (4.7%)	8 (4.9%)	1 (1.3%)	6 (1.5%)
WHO ordinal scale					
1	–	–	6 (3.7%)	4 (5.3%)	11 (2.8%)
2	–	–	2 (1.2%)	5 (6.6%)	10 (2.5%)
3	–	–	41 (25.3%)	44 (57.9%)	131 (33.2%)
4	18 (36.7%)	37 (86%)	57 (35.2%)	20 (26.3%)	134 (34%)
5	11 (22.4%)	2 (4.7%)	19 (11.7%)	0 (0%)	42 (10.7%)
6	2 (4.1%)	1 (2.3%)	3 (1.9%)	0 (0%)	9 (2.3%)
7	2 (4.1%)	0 (0%)	2 (1.2%)	0 (0%)	3 (0.8%)
8	16 (32.7%)	3 (7%)	32 (19.8%)	3 (3.9%)	54 (13.7%)

SD standard deviation, *BMI* body mass index, *CKD* chronic kidney disease, *COPD* chronic obstructive pulmonary disease, *IQR* interquartile range, *ICU* intensive care unit

variant plays a special role for individualised therapy decisions. The future use of mABs depends on newly developed mABs targeting evolving virus strains.

In comparison, RDV as a nucleotide-like antiviral drug against the SARS-CoV-2 RNA polymerase does not seem to have been affected by mutations in the past. RDV should therefore been used in the early and/or in the late phase of infection outside the current indication, especially in immunological naïve patients with regard to SARS-CoV-2 who are at high risk for severe COVID-19.

The WHO ordinal scale is a simple and easy-to-use tool that measures the disease course in clinical status based on a 9-point ordinal scale. The scale ranges from no clinical or virological evidence of infection (0) to death (8) and includes several intermediate categories that reflect different degrees of clinical deterioration. Significant steps in the progression of the disease include the transition from mild (WHO ordinal scale 1–3) to moderate disease, requiring supportive oxygen therapy (WHO ordinal scale 4). Additionally, the progression to severe disease involves the use of mechanical ventilation, such as non-invasive and/or invasive ventilation, and falls within the WHO ordinal scale 5–7 range [3].

The use of WHO ordinal scale has several benefits, including standardization of outcome measurement, ease of implementation, and applicability across different treatment modalities. Thus, the WHO ordinal scale can simplify the handling of COVID-19 for specialists from other clinical disciplines than infectious disease. Consequently, we think that the use of the WHO ordinal scale remains useful

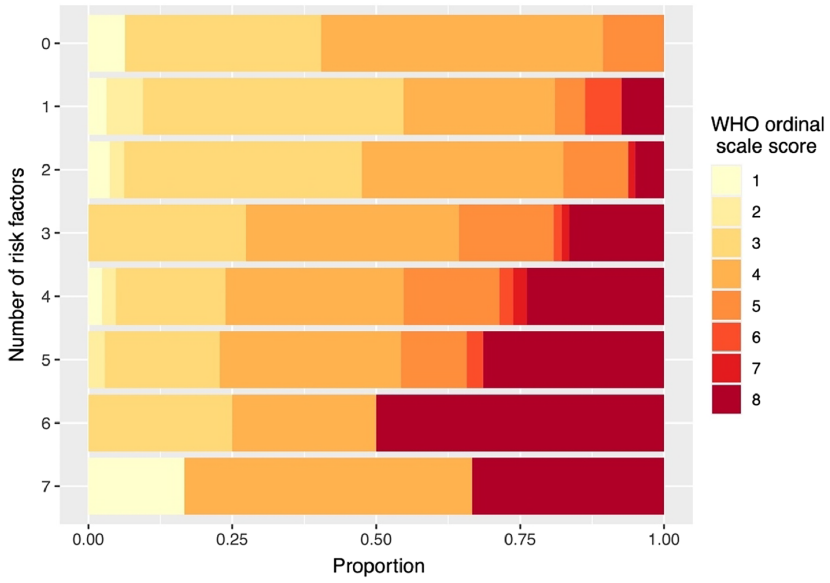

Fig. 1 Stacked bar chart depicting the relationship between the number of risk factors and the proportion of WHO ordinal scale outcomes, with darker shades indicating worse outcomes

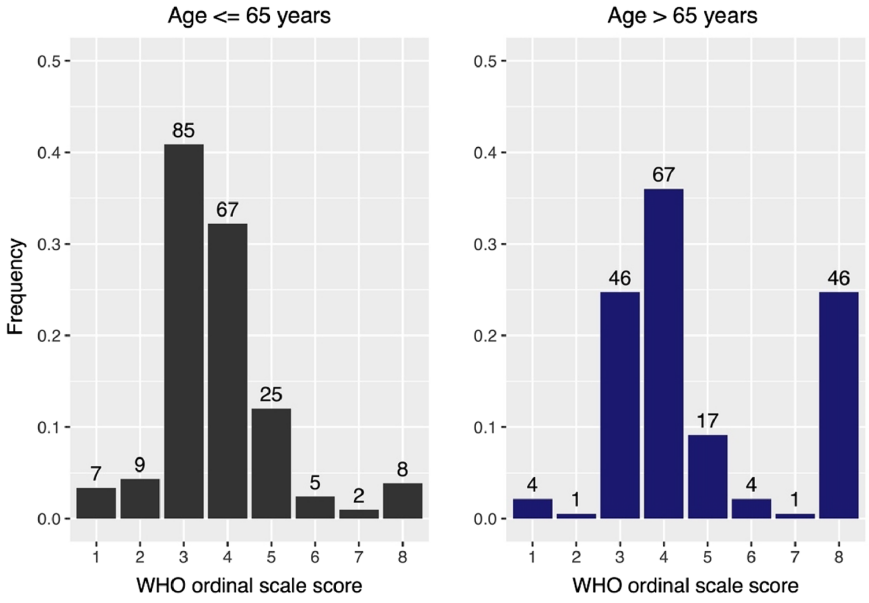

Fig. 2 Distribution of WHO ordinal scale scores in patients under and over the age of 65 years

both as a tool for clinical decision-making and for therapy evaluation.

It should be emphasized that an even more reliable classification into risk groups could be made with the implementation of inflammation markers resulting in a combined ordinal scale. In this case, each of the three previously mentioned groups would be divided into low risk and high risk [3]. Nevertheless, we see clear advantages using the simple WHO ordinal scale based only on clinical data, especially for general practitioners and specialists from other clinical disciplines than infectious disease—especially for reasons of time in a fast-paced everyday life.

While there are many studies supporting the use of PCR results as outcome measures, SARS-CoV-2 viral load has limited utility in evaluating treatment outcomes in COVID-19 patients especially due to prolonged viral shedding in high-risk patients. It should only be used as a secondary readout. Hospital discharge should not be reliant on negative PCR results since SARS-CoV-2 viral shedding kinetics make those results unreliable [5].

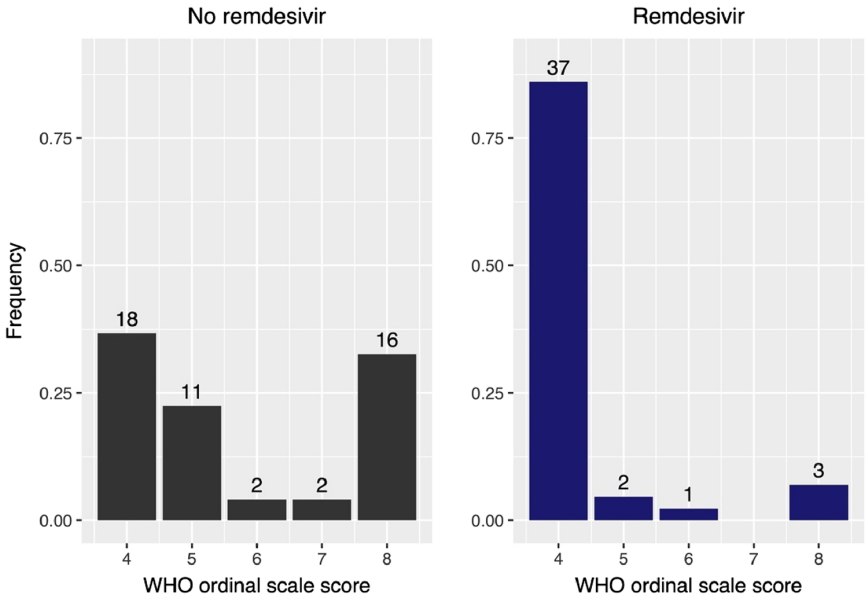

Fig. 3 Distribution of WHO ordinal scale scores for patients treated with or without remdesivir (RDV)

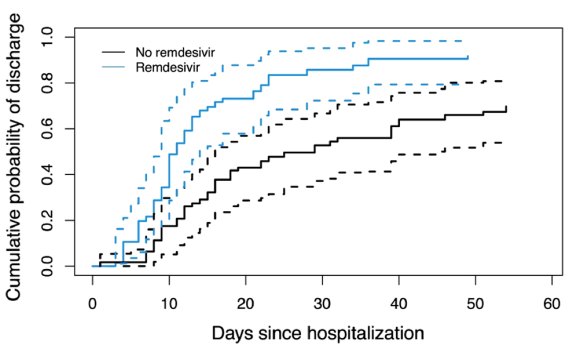

Fig. 4 Cumulative incidence function estimates based on the inverse probability weighted data for duration of hospitalization in days displaying the cumulative probability of discharge for patients hospitalized with COVID-19 (RDV vs. no RDV). Both curves are accompanied by dotted lines indicating the 95% confidence interval

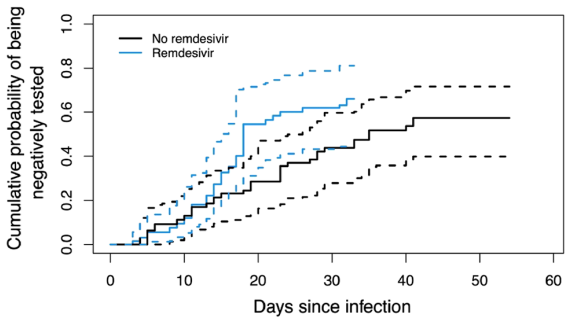

Fig. 5 Cumulative incidence function estimate based on the weighted data showing the probability of testing negative for COVID-19 over time since a positive test result (RDV vs. no RDV). Both curves are accompanied by dotted lines indicating the 95% confidence interval

Patients with underlying oncological/haematological diseases and/or B-cell-depleting therapies are particularly at risk of severe courses of COVID-19. Only a few of these patients (n = 9) were included in our analysis on RDV, which limits our data. Nevertheless, especially SARS-CoV-2 immunologically naïve patients are still at risk for severe courses of COVID-19. If they have risk factors for COVID-19, such as age over 65 years, hypertension, obesity, diabetes, chronic kidney disease, heart disease, chronic obstructive pulmonary disease, and/or neurological disease (which were considered for our propensity scores), they should also be treated.

In conclusion, our data support the findings of the LEOSS registry regarding the efficacy of RDV in improving clinical outcomes in patients with advanced COVID-19 [1]. Our study provides important insights into the use of RDV in advanced COVID-19, clinical decision-making and the evaluation of COVID-19 treatments.

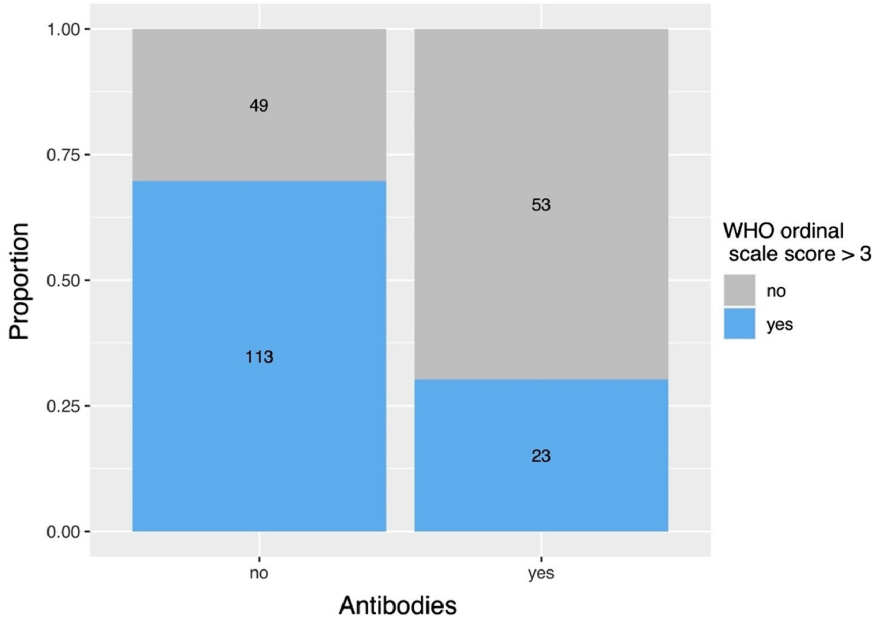

Fig. 6 Bar chart depicting the distribution of COVID-19 patients who received antibody treatment (no/yes) in relation to their WHO ordinal scale outcome

Author contributions YRH: acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis. NS: analysis and interpretation of data; statistical analysis; administrative, technical, or material support; critical revision of the manuscript for important intellectual content. MB: acquisition of data; study concept and design; analysis and interpretation of data; drafting of the manuscript; statistical analysis; administrative, technical, or material support; study supervision; acquisition of data; critical revision of the manuscript for important intellectual content. SS: acquisition of data; critical revision of the manuscript for important intellectual content. GJR: acquisition of data; critical revision of the manuscript for important intellectual content. SB: acquisition of data; critical revision of the manuscript for important intellectual content. KvB: acquisition of data; critical revision of the manuscript for important intellectual content. CPS: administrative, technical, or material support; critical revision of the manuscript for important intellectual content. MAG-C: administrative, technical, or material support; critical revision of the manuscript for important intellectual content. J-CW: administrative, technical, or material support; critical revision of the manuscript for important intellectual content. JKR: administrative, technical, or material support; critical revision of the manuscript for important intellectual content. CB: administrative, technical, or material support; critical revision of the manuscript for important intellectual content. MBM: acquisition of data; study concept and design; analysis and interpretation of data; drafting of the manuscript; statistical analysis; administrative, technical, or material support; study supervision.

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Availability of data and materials The data are available on request.

Declarations

Conflict of interest Yannis R. Hübner declares on conflicts of interest. Nikolai Spuck declares on conflicts of interest. Moritz Berger declares on conflicts of interest. Stefan Schlabe had a stipend from the German Centre for Infectious Diseases Research (DZIF); he received congress fees and travel expenses from Gilead, Abbvie and Johnson&Johnson and funding from the German Research Society (DFG) for initiation of an African-German research project; he holds Fonds-based shares from BionTech.. Gereon J. Rieke declares on conflicts of interest. Sven Breitschwerdt declares on conflicts of interest. Kathrin van Bremen declares on conflicts of interest. Christian P. Strassburg declares on conflicts of interest. Maria A. Gonzalez-Carmona has contributed to advisory boards for Roche, Eisai. BMS, MSD and AZ. M.B.M. received travel expenses and honoraria from Gilead, Pfizer and Virology Education. Jan-Christian Wasmuth declares on conflicts of interest. Jürgen K. Rockstroh has received honoraria for lectures and/or consultancies from Abivax, Galapagos, Gilead, Merck, Janssen, Theratechnologies and ViiV. Christoph Boesecke received honoraria for lectures and/or consultancies from AbbVie, Gilead, Janssen, MSD, ViiV as well as funding from Dt. Leberstiftung, DZIF, Hector Stiftung, NEAT ID. Malte B. Monin received received honoraria for lectures, travel expenses and/or consultancies from Pfizer, Gilead Sciences, AstraZeneca, NovaVax and Virology Education. However, these activities have no potential conflicts of interest with the manuscript.

Ethical approval The study was performed in accordance with the Declaration of Helsinki. Due to the retrospective study design, ethical approval and/or patient information was not required according to the North Rhine-Westphalian legislation.

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2.5 Antivirale Kombinationstherapien als Erstlinienregime bei PatientInnen mit systemischer Immundefizienz

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Prolongierte Virusnachweise sind vor allem bei PatientInnen mit systemischer Immundefizienz und entsprechend eingeschränkter Immunantwort auf SARS-CoV-2-Infektionen und/oder Impfungen klinisch und epidemiologisch relevant (vgl. Kapitel 1.6). Ziel der vorliegenden Studie war es daher, antivirale Kombinationstherapien in der Erstlinie bei diesen PatientInnen hin-sichtlich des klinischen Krankheitsverlaufs und der Dauer des Virusnachweises zu evaluieren (vgl. Kapitel 1.8.2).

144 vornehmlich geimpfte, immundefiziente PatientInnen, die sich mehrheitlich mit einer der Omikron-Varianten von SARS-CoV-2 infiziert hatten, wurden in die Studie aufgenommen. Kombinationstherapien aus RDV, NIR/r, MOL (zum Zeitpunkt der Gabe noch durch die EMA empfohlen) und/oder einem mAB/einer mAB-Kombination (CVIV, Tixagevimab/ Cilgavimab, Sotrovimab) wurden dabei als Erstlinientherapie eingesetzt, um schwere klinische Verläufe und prolongierte Virusnachweise zu verhindern. Dabei war zum Zeitpunkt der Gabe jeweils von einer effektiven Wirksamkeit der eingesetzten mABs gegen die SARS-CoV-2-Variante, die zur Infektion geführt hatte, auszugehen.

Unter den Kombinationstherapien waren die klinischen Verläufe, beurteilt anhand der WHO-Ordinalskala, leicht bis mittelschwer (medianer Score von 3). Nur zwei PatientInnen wurden intensivmedizinisch mit einer *high-flow*-Sauerstofftherapie (Score von 5) versorgt. Todesfälle wurden nicht dokumentiert. Hinweise auf eine erhöhte Toxizität unter den Kombinations-therapieregimen ergaben sich nicht. Prolongierte Virusnachweise (≥10^6 SARS-CoV-2-Kopien/ml an Tag 21 nach dem erstem Virusnachweis mittels PCR) konnten in 85.4 % der Fälle verhindert werden. Das Virus wurde mit einem Median von acht Tagen (Interquartilsabstand von 6,0-15,3) mittels PCR nachgewiesen. In einer multivariablen Analyse konnte gezeigt werden, dass das Virus bei PatientInnen mit malignen hämatologischen Grunderkrankungen

und/oder in Fällen mit einem späten Beginn der antiviralen Therapie (≥5 Tage nach erstem Virusnachweis mittels PCR) trotz primärer Kombinationstherapie signifikant länger nachgewiesen wurde. Bei PatientInnen, bei denen es trotz antiviraler Kombinationstherapie zu einem prolongierten Virusnachweis gekommen war, konnte kein Vorteil einer reaktiven zweiten antiviralen Mono- oder Kombinationstherapie belegt werden. Aufgrund von Infektionen mit SARS-CoV-2 wurden Therapiefortführungen und -einleitungen bei 12 % der PatientInnen mit malignen hämatologischen Erkrankungen verzögert. In der Studie wurde bei nicht klar definiertem Nachbeobachtungszeitraum in nur einem Fall ein Post COVID-19-Syndrom dokumentiert.

Vor allem PatientInnen mit malignen hämatologischen Erkrankungen profitierten zusammenfassend klinisch und bezüglich des virologischen Verlaufs von frühen antiviralen Kombinationstherapien bei Infektionen mit SARS-CoV-2 in der Erstlinie. Wir empfahlen, den Einsatz auch bei PatientInnen mit systemischer Immundefizienz anderer Genese in Erwägung zu ziehen.

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RESEARCH

Early combination therapy of COVID-19 in high-risk patients

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Abstract

Purpose Prolonged shedding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been observed in immunocompromised hosts. Early monotherapy with direct-acting antivirals or monoclonal antibodies, as recommended by the international guidelines, does not prevent this with certainty. Dual therapies may therefore have a synergistic effect.

Methods This retrospective, multicentre study compared treatment strategies for corona virus disease-19 (COVID-19) with combinations of nirmatrelvir/ritonavir, remdesivir, molnupiravir, and/ or mABs during the Omicron surge. Co-primary endpoints were prolonged viral shedding ($\geq 10^6$ copies/ml at day 21 after treatment initiation) and days with SARS-CoV-2 viral load $\geq 10^6$ copies/ml. Therapeutic strategies and risk groups were compared using odds ratios and Fisher's tests or Kaplan−Meier analysis and long-rank tests. Multivariable regression analysis was performed.

Results 144 patients were included with a median duration of SARS-CoV-2 viral load $\geq 10^6$ copies/ml of 8.0 days (IQR 6.0–15.3). Underlying haematological malignancies (HM) ($p = 0.03$) and treatment initiation later than five days after diagnosis ($p < 0.01$) were significantly associated with longer viral shedding. Prolonged viral shedding was observed in 14.6% ($n = 21/144$), particularly in patients with underlying HM (OR 3.5; 95% CI 1.2–9.9; $p = 0.02$). Clinical courses of COVID-19 were mild to moderate with only few adverse effects potentially related to combination treatment.

Conclusion Early combination treatment of COVID-19 effectively prevented prolonged viral shedding in 85.6% of cases. Considering the rapid viral clearance rates and low toxicity, individualized dual therapy approaches may be beneficial in high-risk patients.

Keywords COVID-19 · Immunocompromised host · Dual anti-SARS-CoV-2 therapies · Prolonged viral shedding · Individualized therapeutic approaches

Introduction

Based on the interventional studies and real-world data, the World Health Organization (WHO) recommends early monotherapy for coronavirus disease 2019 (COVID-19) in patients at risk of severe courses [1–4]. Several direct-acting antivirals (DAAs) and monoclonal antibodies (mABs) are therefore available. Older age, immunodeficiency, the extent of immune response after vaccination and/or infection and the number of risk factors are associated with a more severe clinical course [5]. Moreover, despite early initiation of monotherapy in immunocompromised hosts, shedding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was prolonged [6]. This favours the development of escape mutations against mAB therapies [7]. We form a network of four large university hospitals in North Rhine-Westphalia (Aachen, Bonn, Cologne and Düsseldorf) with over 5,000 beds and numerous highly specialised outpatient clinics. In this way, we provide COVID-19 care mainly for

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patients with haematological malignancies (HM) and for patients on immunosuppressive medication after solid-organ transplantation (SOT). These patients face prolonged infectiousness that limits their participation in daily life, including access to the medical system, which can interfere with appropriate management of underlying diseases [8].

Combination treatment to avoid the development of drug resistance or to improve therapeutic efficacy is well established, for example in human immunodeficiency virus infection or tuberculosis. The availability of three DAAs (nirmatrelvir/ritonavir, remdesivir, molnupiravir) and various mABs enables combination treatment against COVID-19. Molnupiravir-nirmatrelvir/ritonavir was more effective in vitro and in animal models, and improved survival in mice [9–11]. Molnupiravir−remdesivir combination was synergistic in hamsters [12]. In a case series of immunocompromised patients, the addition of a mAB to remdesivir was shown to increase the chance of sustained viral clearance as compared to remdesivir monotherapy [13]. Moreover, combination of nirmatrelvir/ritonavir with an mAB was effective in a small case series of immunocompromised patients with sustained viral shedding [14]. In humans, current experiences with combined DAAs are based on the few case reports and small case series [15].

Despite these encouraging reports, optimal combinations are unknown. In our large cohort of immunocompromised patients, we analysed first-line COVID-19 combination regimens for virological and clinical outcomes and safety.

Patients and methods

This is a retrospective, multicentre, observational cohort study conducted during the Omicron surge at four German university hospitals forming the Centre for Integrated Oncology Aachen Bonn Cologne Düsseldorf (CIO ABCD). Cases of mainly immunocompromised patients who received first-line combination treatment with DAAs ± mABs against COVID-19 between March 2022 and April 2023 were analysed. Therapeutic decisions were at the discretion of the treating physicians. The study was performed in accordance with the Declaration of Helsinki. Owing to the retrospective study design, ethical approval and/or patient information was not required according to the North Rhine-Westphalian legislation. According to internal standards, however, we submitted lead ethics proposals, which were approved by the Ethics Committees of the Medical Faculty of the Heinrich-Heine University Düsseldorf, Germany (study no. 2022-2240) and of the Medical Faculty of the University Hospital Bonn, Germany (file number 469/22).

For inclusion, patients were at risk of severe courses of COVID-19 and received first-line combination therapies for COVID-19 containing at least two of nirmatrelvir/ritonavir (administered five days orally), remdesivir (administered three, five or ten days intravenously), molnupiravir (administered five days orally), and/ or one mAB (administered intravenously or intramuscularly as single shot) at standard doses. The effectiveness of the mABs was tested in each individual case [16]. The dose of the mAB was doubled in cases of restricted neutralization capacity and/or in cases, in which sequencing of the viral genome was not performed, ensuring effectiveness. Combination therapy was assumed if the individual substances were administered overlapping for at least one day. Diagnosis of SARS-CoV-2 infection was confirmed by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) with or without viral sequencing. If viral sequencing was not available, the regionally predominant variant was assumed. The date of diagnosis was considered the date of first positive RT-PCR. Treatment initiation later than five days after diagnosis was defined as late treatment initiation.

Co-primary endpoints were days with SARS-CoV-2 viral load $\geq 10^6$ RNA copies/ml and occurrence of prolonged viral shedding, both overall, and compared among subgroups of antiviral therapy strategies ($2 \times$ DAAs $\pm 1 \times$ mAB versus $1 \times$ DAA plus $1 \times$ mAB). Prolonged viral shedding was defined as SARS-CoV-2 viral load $\geq 10^6$ RNA copies/ml 21 days after treatment initiation. The disease severity according to the WHO ordinal clinical scale [17], any complications of COVID-19 and any drug-related adverse effects among groups were chosen as secondary endpoints.

Potential predictors of increased and/ or prolonged viral shedding were analysed: sex (male vs. female), age groups (<65 years vs. ≥ 65 years), number of risk factors for severe COVID-19 according to the Centers for Disease Control and Prevention (<5 vs. ≥ 5 risk factors) [18], immunodeficiency (yes vs. no), HM (yes vs. no), history of allogenic stem cell transplantation (yes vs. no), history of SOT (yes vs. no), vaccination status, treatment strategies ($2 \times$ DAAs $\pm 1 \times$ mAB vs. $1 \times$ DAA plus $1 \times$ mAB), time of treatment initiation (≤ 5 days vs. > 5 days after diagnosis), WHO ordinal clinical severity scale score (1–3 vs. 4–7), and complicated course of COVID-19 (yes vs. no). Immunodeficiency was presumed in patients with a medical history of organ transplantation or autoimmune disease under immunosuppressive therapy, with underlying HM and/ or with advanced HIV-infection ($CD4^+$ cell count $< 200/\mu l$). Patients who received the basic immunization and a first booster vaccination were considered fully vaccinated. Patients who had received less or no vaccinations were grouped as only partially vaccinated or not vaccinated, respectively.

Clinical data were anonymized and entered into an electronic case report form.

Patients reaching a SARS-CoV-2 viral load $< 10^6$ RNA copies/ml within the first three days after treatment initiation were excluded from the final analysis as the success of

treatment could not be clearly attributed ($n=8$). The same was applied when neutralizing capacity of mABs could not be assumed for a mismatch of virus variant versus mAB chosen ($n=8$). Furthermore, cases with a second course of antiviral therapy due to lacking or insufficient clinical and/or virological response were excluded ($n=10$). Inclusion and exclusion criteria are summarized in Fig. 1.

Statistical analysis was carried out using R version 4.2.3 (R Core Team 2023: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria). Descriptive analyses included the calculation of medians and interquartile ranges for continuous variables and frequencies (absolute and relative) for categorical variables. Prolonged viral shedding was compared between treatment strategies and the patients' characteristics mentioned above in univariable analyses using odds ratios and Fisher's exact tests. Days with SARS-CoV-2 viral load $\geq 10^6$ RNA copies/ml were illustrated with Kaplan – Meier plots and compared between treatment strategies as well as patient characteristics using by *t* tests. Analogously, the days with SARS-CoV-2 viral load $\geq 10^6$ RNA copies/ml were analysed among patients who were excluded from the initial analysis, because they received a second round of antiviral therapy. Moreover, a sub-analysis on combinations of DAAs and mABs was performed using ANOVA. Finally, a multivariable linear regression analysis was carried out including age, sex and those predictors that were significant in univariable analyses. *P* values ≤ 0.05 were regarded as statistically significant.

Results

Study population

Of the 170 patients screened, who received first-line combined, dual anti-SARS-CoV-2 therapies, 144 were eligible for analysis (Fig. 1).

Immunodeficiency was present in 85.4% ($n=123/144$), mostly due to immunosuppression following SOT (52.8%; $n=76/144$) or underlying HM (28.5%; $n=41/144$). In the 21 formally immunocompetent patients (14.6%), age > 65 years and > 2 comorbidities, representing ≥ 3 risk factors for severe COVID-19, were reasons for concern for a severe course of COVID-19. Virus genomic sequencing was conducted in 59.0% of patients ($n=85/144$), with Omicron variants detected in 84 cases and a delta variant in only one. In all 85 patients, potential efficacy of the applied mAB could be

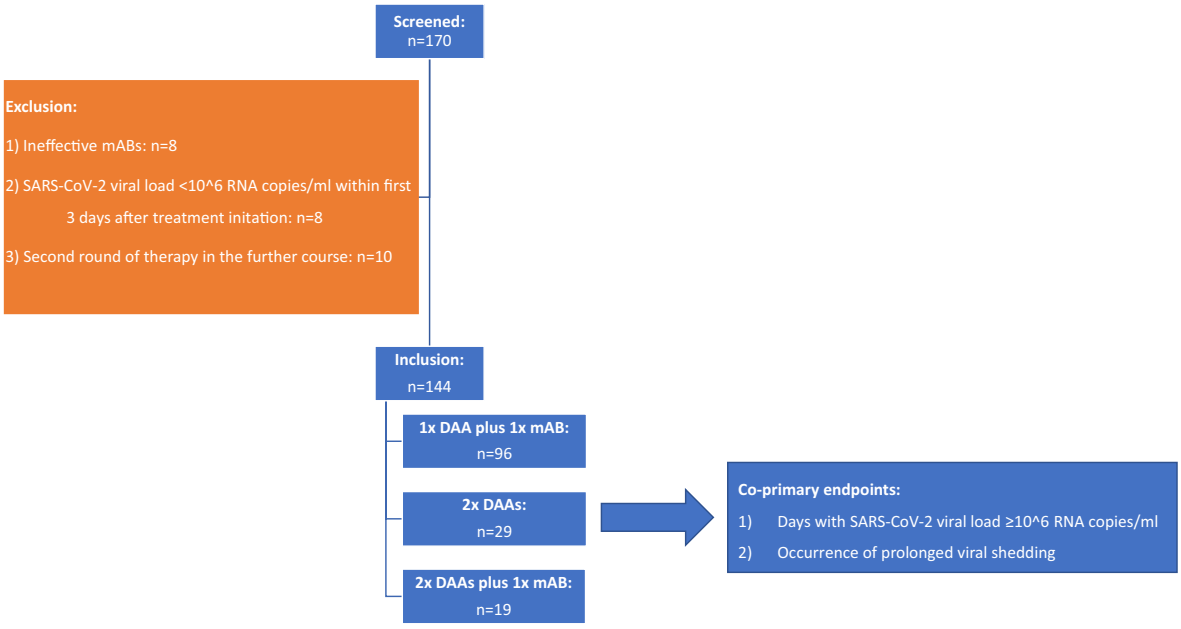

Fig. 1 Subject disposition. 170 patients received primarily combined dual anti-SARS-CoV-2 therapies. Patients having received ineffective mABs or having highlighted a SARS-CoV-2 viral load < 10^6 RNA copies within the first three days were excluded. Furthermore, patients having received a second round of therapy due to clinical and/ or virological deterioration were excluded from the initial analysis. The latter were examined in a sub-analysis with regard to the effect of this second round of therapy on days with SARS-CoV-2 ≥ 10^6 RNA copies/ml Finally, 144 patients were included in the initial analysis having received 1 × DAA plus 1 × mAB, 2 × DAAs or 2 × DAAs plus 1 × mAB. *DAA* direct acting antiviral, *mAB* monoclonal antibody

assumed according to the virus variant [16]. Median treatment initiation was day 0 (range: 0–56; IQR 0–1) after first positive PCR test for SARS-CoV-2. Treatment categories included one DAA plus one mAB (66.7%; $n = 96/144$), two DAAs (20.1%; $n = 29/144$), or two DAAs plus one mAB (13.2%; $n = 19/144$). As there were no significant differences in viral shedding between the latter two groups (8.0 days [IQR 6.0–14.0] vs. 6.0 days [IQR 5.0–8.0]; $p = 0.99$), both groups were combined for further analyses ($2 \times \text{DAAs} \pm 1 \times \text{mAB}$). Patient baseline characteristics are detailed in Table 1.

Days with SARS-CoV-2 viral load ≥ 10^6 copies/ml

Median SARS-CoV-2 viral shedding ≥ 10^6 copies/ml lasted for 8.0 days (IQR 6.0–15.3) following initiation of dual anti-SARS-CoV-2 treatment.

In univariable analysis, patients < 65 years had significantly longer viral shedding (10.4 vs. 15.0 days; $p = 0.03$). The time to viral load < 10^6 copies/ml was significantly longer in patients with late treatment initiation (30.7 vs. 12.3 days; $p < 0.01$) and in immunocompromised patients (13.9 vs. 8.0 days; $p = 0.04$), especially in the subgroup of patients with underlying HM (18.1 vs. 11.0 days; $p < 0.01$). In contrast, patients receiving immunosuppressives post SOT more readily experienced a viral load < 10^6 copies/ml (11.7 vs. 14.5 days; $p = 0.17$). Neither the different treatment strategies ($2 \times \text{DAA} \pm 1 \times \text{mAB}$ vs. $1 \times \text{DAA} + 1 \times \text{mAB}$) nor the number of risk factors (< 5 vs. ≥ 5) resulted in statistically significant differences regarding that endpoint (Table 2). Kaplan – Meier plots were added to visualize the results (Fig. 2a–e).

In multivariable analysis, underlying HM ($p = 0.03$) and treatment initiation > 5 days after diagnosis of COVID-19 ($p < 0.01$) were significantly associated with longer viral shedding (Fig. 3).

Prolonged viral shedding

The second primary endpoint, i.e., achieve a SARS-CoV-2 viral load < 10^6 copies/ml within 21 days, was achieved by 85.4% ($n = 123/144$) of patients (Table 1). Patients with HM had the highest rate of prolonged viral shedding (26.8%; $n = 11/41$; $p = 0.02$). In comparison, the risk for prolonged viral shedding was substantially lower in patients on immunosuppressive medication following SOT (11.8%; $n = 9/76$; $p = 0.35$).

Prolonged viral shedding was significantly more frequent in immunocompromised patients ($p = 0.04$) and in those with late treatment initiation ($p = 0.04$). Within the group of immunocompromised, patients with underlying HM (OR 3.5; 95% CI 1.2–9.9; $p = 0.02$) and patients on immunosuppressive medication following allogenic stem cell transplantation (OR 4.5; 95% CI 0.8–21.4; $p = 0.04$) were at highest risk for prolonged viral shedding. Of note, this led to a delay in cancer therapy in 12.2% ($n = 5/41$) of all patients with HM. In contrast, in recipients of immunosuppressives following SOT, dual anti-SARS-CoV-2 therapy was associated with lower rates of prolonged viral shedding (OR 0.6; 95% CI 0.2–1.8; $p = 0.35$). Patients < 65 years had a higher risk for prolonged viral shedding (OR 3.9; 95% CI 1.2–16.8; $p = 0.02$). Regarding the treatment strategies ($2 \times \text{DAA} \pm 1 \times \text{mAB}$ vs. $1 \times \text{DAA}$ plus $1 \times \text{mAB}$), no significant difference was observed.

Sub-analysis on combinations of DAAs and mABs

Regarding combinations with mABs, there was no significant difference in time with SARS-CoV-2 viral load ≥ 10^6 RNA copies/ml depending on the DAA partner (Fig. 4a). In contrast, the combination of remdesivir with molnupiravir appeared to be beneficial as compared to the combination of remdesivir and nirmatrelvir/ritonavir (8.9 vs. 21.8 days; $p < 0.01$; Fig. 4b). Of note, nirmatrelvir/ritonavir was not used following SOT to avoid drug-drug interactions. As stated above, these patients had shorter viral shedding when compared with other subgroups of patients (Fig. 2e).

Clinical course of COVID-19 and safety of dual anti-SARS-CoV-2 therapies

Patients had a median WHO ordinal clinical severity scale score of 3 (range: 1–5; IQR 3–3), i.e., having been hospitalized with no oxygen therapy (Table 1). A higher score (> 3) was not associated with longer viral shedding (Table 2). Most common complications were COVID-19 pneumonia (13.2%; $n = 19/144$) and bacterial superinfections (8.3%; $n = 12/144$). Most of the patients who required oxygen therapy were stable under oxygen by nasal or mask prongs (13.8%; $n = 20/144$), i.e., a WHO ordinal clinical severity scale score of 4. Only two patients (1.4%) required intensive care with high-flow oxygen (score of 5). No deaths were reported.

Diarrhoea and nausea with vomiting (2.1%; $n = 3/144$ and 0.7%; $n = 1/144$, respectively) were documented as potential adverse effects of dual anti-SARS-CoV-2 treatments. Temporary elevation of amino-transferases (2.1%; $n = 3/144$), acute on chronic kidney failure (1.4%; $n = 2/144$), and renal transplant functional deterioration (0.7%; $n = 1/144$) were considered as complications of COVID-19 rather than drug-induced.

Impact of a second course of antiviral treatment

Among the 10 patients who were excluded from the analysis because they received a second round of

Table 1 Baseline characteristics and clinical parameters

Participants	144
Age category	
< 65 years [%]	65.3 (n = 81/144)
≥ 65 years [%]	43.7 (n = 63/144)
Sex category	
Male [%]	68.8 (n = 99/144)
Female [%]	31.2 (n = 45/144)
Risk factors for severe COVID-19	
Immunodeficiency*	85.4 (n = 123/144)
Solid organ transplantation with drug immunosuppression**	52.8 (n = 76/144)
Underlying haematological malignancies***	28.5 (n = 41/144)
Allogenic bone marrow transplantation with drug immunosuppression	6.9 (n = 10/144)
Chronic variable immunodeficiency	1.4 (n = 2/144)
HIV infection with CD4 + -cell count < 200/ µl	1.4 (n = 2/144)
Rheumatological diseases/ collagenoses/ vasculitides	3.5 (n = 5/144)
Solid cancer	9.0 (n = 13/144)
Chronic heart failure	9.7 (n = 14/144)
Coronary heart disease	12.5 (n = 18/144)
Atrial fibrillation	9.7 (n = 14/144)
Chronic liver disease	13.9 (n = 20/144)
Chronic kidney failure	12.5 (n = 18/144)
Dialysis	0.7 (n = 1/144)
Chronic pulmonary disease	13.9 (n = 20/144)
Diabetes mellitus	15.3 (n = 22/144)
Obesity	6.3 (n = 9/144)
Chronic inflammatory bowel disease	2.1 (n = 3/144)
Chronic neurologic diseases	9.7 (n = 14/144)
Cerebrovascular disease	2.1 (n = 3/144)
Chronic psychiatric diseases	1.4 (n = 2/144)
SARS-CoV-2 vaccination status	
Basic vaccination plus first booster vaccination [%]	72.9 (n = 105/144)
Not/partly vaccinated [%]	18.8 (n = 27/144)
Not documented [%]	8.3 (n = 12/144)
Variant of SARS-CoV-2	
Omicron [%]	58.3 (n = 84/144)
Delta [%]	0.7 (n = 1/144)
No sequencing [%]	41.0 (n = 59/144)
Therapeutical agents	
DAAs [absolute]	192
Remdesivir [%]	57.3 (n = 110/192)
Molnupiravir [%]	28.1 (n = 54/192)
Nirmatrelvir/ritonavir [%]	14.6 (n = 28/192)
mABs [absolute]	115
Sotrovimab [%]	74.8 (n = 86/115)
Tixagevimab/cilgavimab [%]	23.5 (n = 27/115)
Casirivimab/imdevimab [%]	1.7 (n = 2/115)
Days of treatment initiation after first positive PCR for SARS-CoV-2 [median]	0 (range: 0–56; IQR 0–1)
Combined therapy strategies	
1 × DAA plus 1 × mAB [%]	66.7 (n = 96/144)
Remdesivir + mAB [%]	66.7 (n = 64/96)
Nirmatrelvir/ritonavir + mAB [%]	19.8 (n = 19/96)
Molnupiravir + mAB [%]	13.5 (n = 13/96)

Table 1 (continued)

2 × DAAs [%]	20.1 ($n = 29/144$)
Remdesivir + molnupiravir [%]	79.3 ($n = 23/29$)
Remdesivir + nirmatrelvir/ritonavir [%]	17.3 ($n = 5/29$)
Molnupiravir + nirmatrelvir/ritonavir [%]	3.4 ($n = 1/29$)
2 × DAAs plus 1 × mAB [%]	13.2 ($n = 19/144$)
Remdesivir + molnupiravir + mAB [%]	84.2 ($n = 16/19$)
Remdesivir + nirmatrelvir/ritonavir + mAB [%]	10.5 ($n = 2/19$)
Molnupiravir + nirmatrelvir/ritonavir + mAB [%]	5.3 ($n = 1/19$)
WHO ordinal clinical severity scale [median score]	3 (range: 1–5; IQR: 3–3)
Complications of SARS-CoV-2 infection	
COVID-19 pneumonia [%]	13.2 ($n = 19/144$)
Oxygen therapy by nasal or mask prongs [%]	13.9 ($n = 20/144$)
Non-invasive ventilation or high-flow oxygen [%]	1.4 ($n = 2/144$)
Additional therapy with dexamethasone [%]	6.3 ($n = 9/144$)
Bacterial superinfection [%]	8.3 ($n = 12/144$)
Temporary elevation of amino transferases [%]	3.5 ($n = 5/144$)
Acute on chronic kidney failure [%]	2.1 ($n = 3/144$)
Temporary renal transplant deterioration [%]	1.4 ($n = 2/144$)
Hepatic encephalopathy [%]	0.7 ($n = 1/144$)
Delirium [%]	0.7 ($n = 1/144$)
Post COVID-19**** [%]	0.7 ($n = 1/144$)
Delay of oncological therapy [%]	12.2 ($n = 5/41$)
Possible treatment side effects	
Diarrhoea [%]	2.1 ($n = 3/144$)
Nausea and vomiting [%]	0.7 ($n = 1/144$)
SARS-CoV-2 RNA < 10^6 copies at day 21 after treatment initiation	
Yes [%]	85.4 ($n = 123/144$)
No [%]	14.6 ($n = 21/144$)
Days with SARS-CoV-2 RNA ≥ 10^6 copies	
All [median]	8.0 (IQR 6.0–15.3)
Under 1 × DAA plus 1 × mAB [median]	9.5 (IQR 6.0–14.0)
Under 2 × DAAs [median]	8.0 (IQR 6.0–14.0)
Under 2 × DAAs plus 1 × mAB [median]	6.0 (IQR 5.0–8.0)

DAA direct acting antiviral, *IQR* interquartile range, *mAB* monoclonal antibody

*Suspected in patients with a medical history of organ transplantation or autoimmune disease with concomitant immunosuppressive therapy, with underlying haematological malignancies and/ or with uncontrolled HIV-infection ($CD4^+$ cell count < 200/µl)

**kidney transplantation (75·0%; $n = 57/76$), heart transplantation (15·8%; $n = 12/76$), liver transplantation (4·0%; $n = 3/76$), kidney and heart transplantation (2·6%; $n = 2/76$), kidney and pancreas transplantation (1·3%; $n = 1/76$), lung transplantation (1·3%; $n = 1/76$)

***Non-Hodgkin lymphoma (36·6%; $n = 15/41$), acute myeloid leukaemia (26·8%; $n = 11/41$), multiple myeloma (9·8%; $n = 4/41$), myeloproliferative neoplasms (9·8%; $n = 4/41$), myelodysplastic syndromes (7·3%; $n = 3/41$), acute lymphoblastic leukaemia (4·9; $n = 2/41$), undifferentiated leukaemia (2·4%; $p = 1/41$), aplastic anaemia (2·4%; $p = 1/41$)

****The rate might be higher as a follow-up time > 3 months was not given in all cases

antiviral therapy due to lack of clinical and/ or virological response, 8 patients fulfilled the criteria for prolonged viral shedding. To evaluate the potential impact of this second round of therapy, we compared the time to achieve a SARS-CoV-2 viral load < 10^6 RNA copies/ml in these patients with the time in the 21 patients with prolonged viral shedding undergoing one course of dual therapy. The time was numerically reduced by 2.2 days in patients receiving a second round of therapy ($p = 0.71$) as shown in Fig. 1f.

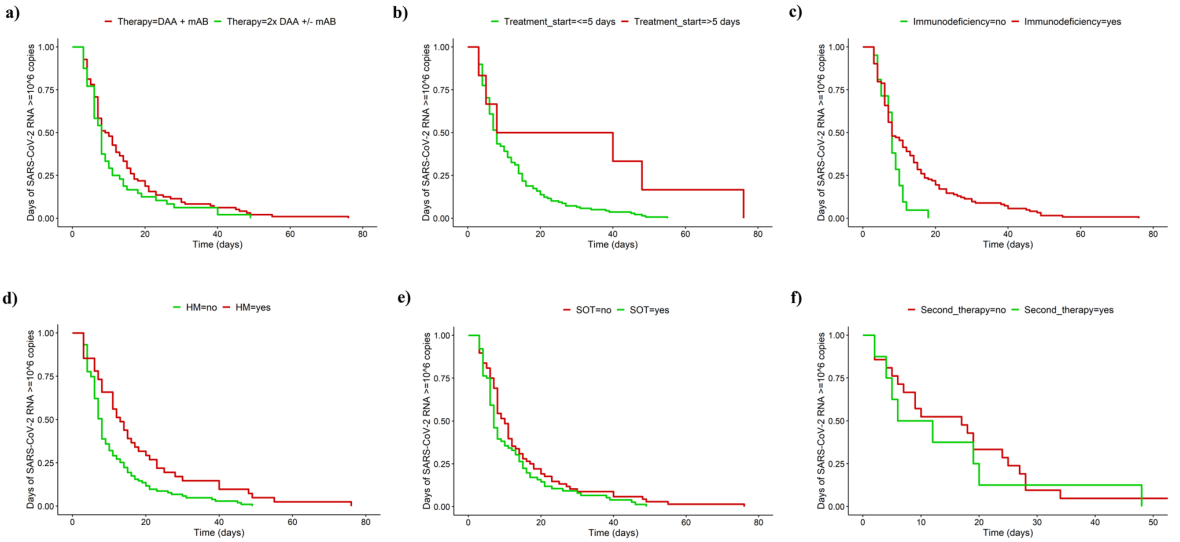

Fig. 2 Viral Shedding. Kaplan–Meier plots showing the probability of SARS-CoV-2 RNA ≥ 10^6 copies/ml set in relation to time in days. The time of viral shedding was not significantly different between the treatment strategies (a: 1 × DAA + 1 × mAB: 14·0 days vs. 2 × DAA ± 1 × mAB: 11·0 days; $p = 0{\cdot}17$). The period was longer in b) patients with a late treatment initiation (> 5 days after diagnosis: 30·7 days vs. ≤ 5 days after diagnosis: 12·3 days; $p < 0{\cdot}01$), c) immunocompromised patients (13·9 days vs. 8·0 days in immunocompetent patients; $p = 0{\cdot}04$) and/ or d) especially in patients with HM (17·1 days vs. 11·0 days in patients without HM; $p < 0{\cdot}01$). In comparison, in patients under immunosuppressive medication following SOT the time was even shorter (11·7 days vs. 14·5 days in patients with no history of solid organ transplantation; $p = 0{\cdot}18$). *DAA* direct acting antiviral, *HM* haematological malignancies, *mAB* monoclonal antibody, *SOT* solid organ transplantation

Discussion

In our cohort of 144 mainly immunocompromised patients receiving first-line combination therapies for COVID-19, clinical courses were mild to moderate and prolonged viral shedding was effectively prevented in 85.4% of these cases. Only two patients required intensive care and no deaths were documented. Our results are encouraging as they exceed those reported with monotherapy in vulnerable populations [6]. Clinical outcomes in patients infected with the SARS-CoV-2 Omicron variant are generally more favourable as compared to earlier virus variants, but still a proportion of 7.8% with treatment failure—defined as severe COVID-19 or COVID-19-related death—has been shown in patients with HM under monotherapy [19]. In our study, prolonged viral shedding was also observed primarily in patients with HM despite dual therapies (14.6%; $n = 11/144$), which once again reveals this group as particularly vulnerable.

SOT patients are also prone to prolonged viral shedding [20]. In our study, persistent viral shedding was less common in this group of patients. The benefit of combined antiviral therapy in these patients appeared to be less pronounced than in patients with HM.

Interestingly, the total number of risk factors for severe COVID-19 had no effect on viral shedding, suggesting that the underlying disease itself has a greater impact than the number of different conditions. Moreover, no association between the clinical course and viral shedding was found.

While the Omicron sub-lineages are associated with milder COVID-19 courses, yet a significant proportion of immunocompromised and older patients are still at risk for severe COVID-19 including death [21].

Prolonged viral shedding delayed antineoplastic treatment in 12.2% of the patients included in this study. This is in line with a study in patients with simultaneous diagnosis of HM and COVID-19 observing frequent (17.2%) substantial delays resulting in significantly higher 30-day mortality [8].

The benefit of DAA in combination with a mAB found in our cohort is consistent with other case reports and small series [14, 22].

However, escape mutations have been observed during mAB exposure, and viral evolution has eventually made all available mABs ineffective, while DAAs retained efficacy [7, 23]. Therefore, treatment combinations including two DAAs seem to be promising and have already shown favourable results in a small case series with patients suffering mostly from HM and/ or receiving anti-CD20 treatment [15]. The case series reports a 30-day virological and clinical response of 73% and a low rate of adverse side effects. In some cases of failure of combined treatment, a repeated course with longer duration was successful. Moreover, the addition of mABs in 18 out of 22 patients was associated with improved

Table 2 Factors influencing viral shedding (univariable analysis)

	Days with SARS-CoV-2 viral load ≥ 10^6 RNA copies/ml	95% CI	*p* value	Prolonged viral shedding	OR	95% CI	*p* value
Age category							
< 65 years	15.0	12.4–17.7	**0·03**	21.0 (*n* = 17/81)	3.9	1.2–16.8	**0.02**
≥ 65 years	10.4	7.5–13.4		6.3 (*n* = 4/63)			
Sex category							
Male	12.8	10.4–15.2	0.76	13.1 (*n* = 13/99)	0.7	0.2–2.1	0.46
Female	13.5	9.9–17.1		17.8 (*n* = 8/45)			
Number of risk factors for severe COVID-19							
< 5	13.5	11.4–15.6	0.14	15.9 (*n* = 21/132)	NA	NA	0.21
≥ 5	8.0	1.1–14.9		0 (*n* = 0/12)			
Immunodeficiency							
Yes	13.9	11.7–16.0	**0.04**	17.1 (*n* = 21/123)	NA	NA	**0.04**
No	8.0	2.9–13.2		0 (*n* = 0/21)			
Haematological malignancies							
Yes	18.1	14.5–21.7	**< 0.01**	26.8 (*n* = 11/41)	3.4	1.2–9.9	**0.02**
No	11.0	8.7–13.3		9.7 (*n* = 10/103)			
Allogenic bone marrow transplantation							
Yes	18.6	10.6–14.7	0·14	40.0 (*n* = 4/10)	4.5	0.9–21.4	**0.04**
No	12.6	11.0–26.2		12.7 (*n* = 17/134)			
Solid organ transplantation							
Yes	11.7	9.0–14·5	0.18	11.8 (*n* = 9/76)	0.6	0.2–1.8	0.35
No	14.5	11.6–17·4		17.6 (*n* = 12/68)			
Fully vaccinated (if documented)							
Yes	12.0	9.7–14.4	0.09	12.4 (*n* = 13/105)	0.5	0.2–1.8	0.22
No	16.6	12.0–21.3		22.2 (*n* = 6/27)			
Therapy strategies							
1 × DAA plus 1 × mAB	14.0	11.6–16.5	0.17	15.6 (*n* = 15/96)	1.3	0.4–4.4	0.80
2 × DAA ± 1 × mAB	11.0	7.6–14.5		12.5 (*n* = 6/48)			
Time of treatment initiation							
Early (≤ 5 days)	12.3	10.3–14.2	**< 0.01**	13.0 (*n* = 18/138)	0.2	< 0.1–1·2	**0.04**
Late (> 5 days)	30.7	21.3–40.0		500 (*n* = 3/6)			
Complicated course of COVID-19							
Yes	11.6	7.8–15.4	0·38	10.0 (*n* = 4/40)	0.6	0.1–1.9	0.43
No	13.6	11.2–15.9		16.3 (*n* = 17/104)			
WHO ordinal clinical severity scale							
1–3	13.2	11.0–15.4	0.67	14.8 (*n* = 18/122)	1.1	0.3–6.4	1.00
4–7	12.0	6.9–17.1		13.6 (*n* = 3/22)			

DAA direct acting antiviral, *mAB* monoclonal antibody, *NA* not applicable

Time with SARS-CoV-2 viral load ≥ 10^6 RNA copies/ml was significantly longer in patients aged < 65 years, patients under immunodeficiency, patients with underlying haematological malignancies and in cases with a late treatment initiation

Prolonged viral shedding (SARS-CoV-2 viral load ≥ 10^6 RNA copies/ml > 21 days) was significantly more frequently observed in patients aged < 65 years, immunocompromised patients, patients with underlying haematological malignancies, patients following a bone marrow transplantation and in cases with a late treatment initiation

therapeutic response. In a recently presented (yet not published as a journal article) study, four of 15 patients with underlying HM experienced a relapse or re-infection despite a five-day course of nirmatrelvir/ritonavir plus remdesivir. In six patients with additional tixagevimab/cilgavimab, no relapse or re-infection was observed [24]. Other than in these studies, we did not see a significant impact of a triple therapy with an additional mAB ($p = 0.99$) (Table 1).

According to the pivotal studies, nirmatrelvir/ritonavir and remdesivir as single compounds had higher efficacy than molnupiravir [2–4]. Therefore, it is conceivable, that combination therapies consisting of nirmatrelvir/ritonavir

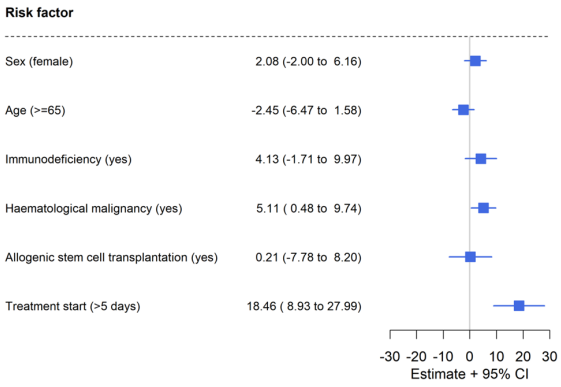

Fig. 3 Factors influencing viral shedding (multivariable analysis). Forest plot visualizing the results of the multivariable analysis regarding factors being associated with longer viral shedding. Despite dual anti-SARS-CoV-2 therapy, viral shedding was significantly longer when treatment start was > 5 days after diagnosis of COVID-19 and/ or in patients with underlying haematological malignancies. The effects of sex, age, immunodeficiency, and/ or allogenic bone marrow transplantation were minor and insignificant

and remdesivir may be more effective than combinations of any of the two compounds with molnupiravir. The use of nirmatrelvir/ritonavir, however, is limited due to a large number of drug-drug interactions [25]. This applies especially for patients after SOT who frequently use calcineurin-inhibitors, such as tacrolimus, to prevent graft rejection. Co-administration with ritonavir may result in a severe increase in tacrolimus serum levels [26]. Thus, in our study with a high proportion of SOT patients ($n = 76/144$, 52.8%), the combination of remdesivir and molnupiravir was most frequently chosen ($n = 39$, additional mAB in 16 cases)—especially in SOT patients. The second most frequent combination was nirmatrelvir/ritonavir and remdesivir ($n = 7$), including two cases with additional mAB, followed by nirmatrelvir/ritonavir plus molnupiravir ($n = 2$, including one case with additional mAB). Therefore, the observed benefit of remdesivir combined with molnupiravir as compared to remdesivir and nirmatrelvir/ritonavir most likely reflects a difference in the groups treated. The same is true for the finding that younger patients were more likely to have prolonged viral shedding: treatment in this group is often more aggressive and immunosuppressive.

In summary, none of the different treatment strategies ($1 \times \text{DAA} + 1 \times \text{mAB}$ vs. $2 \times \text{DAA} \pm 1 \times \text{mAB}$) proved to be superior, while an early treatment initiation was proofed to be favourable.

According to the previously described study and the case reports, in some cases of treatment failure, repeated or extended courses of treatment were ultimately successful in stopping viral shedding [11–13]. In eight patients out of our subgroup of 11 patients with repeated therapies due to initial treatment failure, viral shedding was still observed 21 days after initial treatment initiation, and the duration of viral shedding was not significantly reduced. Nevertheless, viral clearance was ultimately achieved in all patients enrolled.

The low number of adverse effects attributed to the drugs used in this study suggests good tolerability and safety of the combinations used. However, it should be noted that other

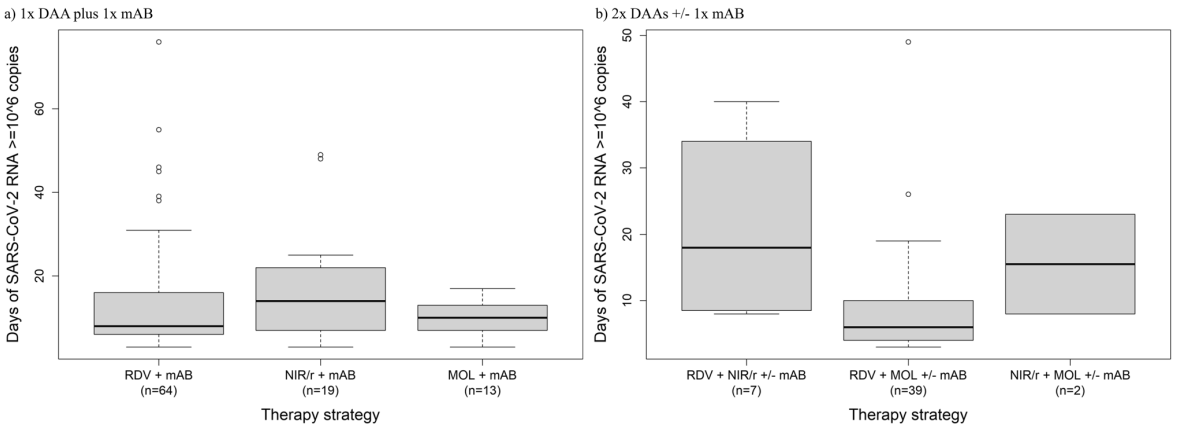

Fig. 4 Sub-analysis on therapy strategies. Using an effective mAB, there was no difference in time with SARS-CoV-2 viral load ≥ 10^6 RNA copies/ml whether combining it with remdesivir (14.1 days), molnupiravir (9.9 days; $p = 0.44$) or nirmatrelvir/ritonavir (16.7 days; $p = 0.30$) (**a**). In contrast, the combination of remdesivir with molnupiravir appeared to be beneficial compared to the combination of remdesivir and nirmatrelvir/ritonavir (8.9 vs. 21.8 days; $p < 0.01$; **b**). Of note, nirmatrelvir/ritonavir could not be used in patients under immunosuppressive medication following organ transplantation due to potential drug-drug-interactions and was therefore rarely administered. The significance is thus weakened. *DAA* direct acting antiviral, *mAB* monoclonal antibody, *MOL* molnupiravir, *NIR/r* nirmatrelvir/ritonavir, *RDV* remdesivir

reports described cardiac events (one myocardial infarction, one bradycardia) that may be related to treatment [15]. In our study, no treatment was discontinued because of adverse effects.

Some of the patients treated in our departments presented with impaired kidney function at baseline. Renal function rarely deteriorated during dual treatment. This was even true in treatment initiated at glomerular filtration rates below 30 ml/min*1.73 m^2. In the meantime data indicate that above all remdesivir can be used in reduced kidney function [27].

Limitations of our study are inherent to its retrospective design. Treatment algorithms differed between the four participating centres, reflecting the lack of knowledge at the time. Our patient group is heterogeneous by baseline factors including comorbidities and causes of immunodeficiency. In some SOT patients, medical immunosuppression was temporarily reduced to improve immune response to SARS-CoV-2 infection. A potential effect could not be discriminated from the effect of antiviral therapy.

A control group is missing. This is mainly due to the fact that at a certain point in time, high-risk patients were regularly treated with combination therapies at the centres. Depriving some patients of this option did not seem to be ethically justifiable. In particular, because of other prevalent virus variants at other times, it was decided against recording a retrospective control group with an assumed bias that was too large.

Due to the defined primary endpoints of time to virus elimination and prolonged viral shedding, patients who had COVID-19 but died soon after diagnosis for any reason and/or did not receive any antiviral therapy were not included in this study, displaying a certain selection bias. The same applies for patients who did not respond to monotherapy and were therefore secondarily treated with combined antiviral regimens. Since the mABs in our study lost efficacy due to viral evolution, treatment combinations involving mABs can currently not be relied on.

In recipients of drugs interacting with ritonavir metabolism pathway, nirmatrelvir use may be limited. The use of nirmatrelvir/ritonavir in these patients would require a high level of expertise in complex interaction management, including close monitoring of immunosuppressant levels. Molnupiravir is no longer available in the European Union, but remains a treatment option in other world regions [28]. Overall, the suitable drug combinations are thus less numerous than at the time of our study, leaving a highlight on the combination of remdesivir with nirmatrelvir/ritonavir.

In conclusion, patients with HM benefited most from early dual anti-SARS-CoV-2 treatment. Combination therapy may also be beneficial in other immunocompromised patients as toxicity was low and viral clearance rates were high. For the time being, cautious individualized approaches should consider dual therapy.

Immunocompromised carriers of SARS-CoV-2 are considered a major factor in viral evolution as well as in maintaining epidemic outbreaks, especially under insufficient treatment [29]. Additionally to the individual benefits in these vulnerable patient group by reducing the risk of progression to severe COVID-19, by enabling participation in social life, and by allowing timely application of scheduled therapies, combined antiviral therapies may thus have additional positive effects on public health by decelerating viral evolution.

Author contributions HMO: study design, data collection, data analysis, writing of the original draft & editing. CF: data collection, data analysis, writing of the original draft & editing. MB: data analysis, review. STH: data collection, review. RM: data collection, review. KB: data collection, review. UK: data collection, review. JS: data collection, review. MF: data collection, review. AD: data collection, review. BNB: data collection, review. EK: data collection, review. OT: data collection, review. AK: data collection, review. CA: data collection, review. SG: data collection, review. JS: data collection, review. NS: data analysis, review. GS: data collection, review. JR: data collection, review. CS: data collection, review. PB: data collection, review. JP: data collection, review. B-EJ: data collection, review. TL: data collection, review. CB: data collection, review. AH: data collection, review. OC: data analysis, writing of the original draft & editing. MM: study design, data collection, data analysis, writing of the original draft & editing.

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Data availability The datasets analysed in this study are available for research purposes on reasonable request from the corresponding author. This applies for a maximum of ten years and only includes anonymised data whose disclosure does not interfere with the patients' interest.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest H.M.O. received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Merck Serono GmbH. C.F. declares no conflicts of interest. M.B. declares no conflicts of interest. S.T.H. received support for attending meetings and/or travel from Takeda and Jansen and participated on a Data Safety Monitoring Board or Advisory Board from Janssen. R.M. received support for attending meetings and/or travel from Takeda and Jansen and participated on a Data Safety Monitoring Board or Advisory Board from Janssen. K.B. declares no conflicts of interest. U.K. declares no conflicts of interest. J.S. received Grants or contracts from Basilea Pharmaceuticals and the German Federal Ministry of Education and Research (BMBF), consulting fees from Gilead and Alvea Vax, payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Gilead, AbbVie, and Pfizer, support for attending meetings and/ or travel from the German Society for Infectious Diseases and the Meta-Alexander Foundation. He participated on a Data Safety Monitoring Board or Advisory Board by Alvea Vax and Micron Research. M.F. declares no conflicts of interest. A.D,D, declares no conflicts of interest. B-N.B. received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from from incyte and support for attending meetings and/or travel from Kite/Gilead and Medac. E.K. declares no conflicts of interest. O.T. declares

no conflicts of interest. A.K. received support for attending meetings and/or travel from Gilead and AbbVie and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Gilead. C.dA. declares no conflicts of interest. S.G. declares no conflicts of interest. J.S. declares no conflicts of interest. N.S. declares no conflicts of interest. G.S. declares no conflicts of interest. J.K.R. received Consulting fees from AbbVie, Boehringer, Gilead, Merck, and ViiV, payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Gilead, Janssen, Merck, and ViiV; he has Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid for EACS. C.P.S declares no conflicts of interest. P.B. declares no conflicts of interest. J.P. declares no conflicts of interest. B-E.O.J. received Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Gilead, ViiV, GSK, Pfizer, Janssen-Cilag, Fresenius Medical care, and the Falk Foundation, support for attending meetrings and/ or travel from Gilead; he participated on a Data Safety Monitoring Board or Advisory Board from Gilead, ViiV, and Theratechnologies; he has Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid for Scientific Secretary for German AIDS Society and member of STAKOB and COVRIIN working groups at Robert-Koch-Institute. T.L. declares no conflicts of interest. C.B. received Grants or contracts from DZIF, DFG, and NEAT ID, Consulting fees from Abbvie, Gilead, JnJ, MSD, and ViiV, Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Abbvie, Gilead, JnJ, MSD, and ViiV, support for attending meetings and/ or travel from Abbvie, Gilead, JnJ, MSD, and ViiV; he participated on a Data Safety Monitoring Board or Advisory Board from Mavmet Study. A.H. declares no conflicts of interest. O.A.C. reports grants or contracts from Amplyx, Basilea, BMBF, Cidara, DZIF, EU-DG RTD (101037867), F2G, Gilead, Matinas, MedPace, MSD, Mundipharma, Octapharma, Pfizer, Scynexis; Consulting fees from Abbvie, Amplyx, Biocon, Biosys, Cidara, Da Volterra, Gilead, IQVIA, Janssen, Matinas, MedPace, Menarini, Molecular Partners, MSG-ERC, Noxxon, Octapharma, Pfizer, PSI, Scynexis, Seres; Honoraria for lectures from Abbott, Abbvie, Al-Jazeera Pharmaceuticals, Astellas, Gilead, Grupo Biotoscana/United Medical/Knight, Hikma, MedScape, MedUpdate, Merck/MSD, Mylan, Noscendo, Pfizer, Shionogi; Payment for expert testimony from Cidara; Participation on a Data Safety Monitoring Board or Advisory Board from Actelion, Allecra, Cidara, Entasis, IQVIA, Janssen, MedPace, Paratek, PSI, Pulmocide, Shionogi, The Prime Meridian Group; A patent at the German Patent and Trade Mark Office (DE 10 2021 113 007.7); Stocks from CoRe Consulting, EasyRadiology; Other interests from DGHO, DGI, ECMM, EHA, ISHAM, MSG-ERC, Wiley. M.B.M. received Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Gilead, Pfizer, Gilead Sciences, AstraZeneca, and Virology Education, and support for attending meetings and/or travel from Gilead.

Ethical approval Due to the retrospective study design, ethical approval and/or patient information was not required according to the North Rhine-Westphalian legislation.

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3 Diskussion:

Die vorliegende kumulative Habilitationsschrift adressiert, wie einleitend dargelegt, offene Fragen nationaler und internationaler Leitlinien zur Impfprävention und zu antiviralen Therapien von Infektionen mit SARS-CoV-2. Dabei werden für vulnerable Subgruppen mit erhöhtem Risiko für komplizierte klinische und/oder prolongierte virologische Verläufe von COVID-19 individualisierte Impf- und Therapieoptionen diskutiert, die bei limitierter Datenlage auf vergleichbare und/oder übergeordnete Patientenpopulationen übertragen werden können.

3.1 Daten zur Förderung der Impfmotivation

Die deutschen und amerikanischen Leitlinien zu Impfungen von hämatologischen/ onkologischen PatientInnen verdeutlichen die limitierte Datenlage zu Impfungen von PatientInnen mit soliden Krebserkrankungen[63,128]. Speziell zu Impfungen gegen SARS-CoV-2 liegen im Vergleich zu anderen impfpräventablen Infektionskrankheiten aufgrund des pandemiebedingten hohen klinischen und wissenschaftlichen Interesses zwar fundiertere Daten vor, dennoch sind Auswertungen zu PatientInnen mit hämatologischen Neoplasien umfangreicher[62,63]. Dadurch bleibt die Evidenz bezüglich der Empfehlungen für PatientInnen mit soliden Krebserkrankungen qualitativ eingeschränkt und Daten von Vergleichsgruppen müssen transferiert werden. Auf der einen Seite birgt die üblicherweise stattfindende Übertragung der Daten von PatientInnen mit hämatologischen Neoplasien mit systemischer Immundefizienz auf PatientInnen mit soliden Tumoren das Risiko zu gering geschätzter Impfantworten[62,63,128]. Dabei ist eine effektivere Immunantwort bei PatientInnen mit soliden Tumoren im Vergleich zu PatientInnen mit hämatologischen Neoplasien belegt[49]. Auf der anderen Seite werden einzelne Entitäten solider Karzinome mit einem besonders hohen Risiko für eingeschränkte Immunantworten auf Impfungen nicht identifiziert. Differenzierte Daten sollten daher in Subgruppen von PatientInnen mit soliden Tumoren generiert werden, die besser auf PatientInnen mit anderen soliden Tumoren transferiert werden können. Longitudinale Betrachtungen sind notwendig, um zu verstehen, wie effektive Immunantworten über die Zeit aufrechterhalten werden können und wie gut PatientInnen vor Infektionen mit neuen Virusvarianten geschützt sind.

In unserer initialen Kohorte von PatientInnen mit gastrointestinalen Tumorerkrankungen wurden a) der Tumortyp, b) eine systemische Immundefizienz und/oder c) tumorspezifische Therapien, insbesondere Chemotherapien, als Faktoren identifiziert, die mit besonders eingeschränkten Immunantworten auf die Primärimpfserie gegen SARS-CoV-2 assoziiert waren. Dabei konnte erstmals belegt werden, dass im Vergleich vor allem PatientInnen mit hepatozellulären, pankreatikobiliären und/oder kolorektalen Karzinomen vergleichsweise niedrige Titer nach der Primärimpfserie aufwiesen[59]. Dies unterstützt die Annahme, dass zur Identifikation von HochrisikopatientInnen sehr differenzierte Daten in Subgruppen von Patient-Innen mit soliden Tumorerkrankungen notwendig sind. PatientInnen, die eine Infektion mit SARS-CoV-2 durchgemacht hatten und somit eine hybride Immunität aufwiesen, hatten tendenziell höhere Werte von SARS-CoV-2 anti-spike IgG. Bei initial insgesamt wenig stattgehabten Infektionen mit SARS-CoV-2 in unserer Kohorte, am ehesten aufgrund einer guten Adhärenz im Hinblick auf basale hygienische Maßnahmen (Tragen von Mund-Nasen-Masken, *social distancing*, etc.), konnte jedoch kein signifikanter Vorteil einer hybriden Immunität, wie in der Literatur beschrieben[36–39], belegt werden.

Wie in Kapitel 2.1 dargestellt gingen wir ab einem absoluten SARS-CoV-2 anti-spike IgG-Spiegel von 482,0 BAU/ml von einem Schutz vor schweren Verläufen von COVID-19 aus[59]. Dabei lag der Wert deutlich über dem eingangs diskutierten Zieltiter von 264,0 BAU/ml[43]. Für die Auto-COVIDVACC-Studie zur Impfung gegen SARS-CoV-2 von PatientInnen nach auto-loger Stammzelltransplantation und/oder CAR-T-Zelltherapie werden sogar noch höhere Titer von 847,0 BAU/ml angestrebt[130]. Der Wert von 847,0 BAU/ml lag oberhalb der 20. Perzentile der SARS-CoV-2 anti-spike IgG-Spiegel von immunkompetenten PatientInnen, die sich in verschiedenen Notaufnahmen in Nordrhein-Westfalen vorgestellt hatten und in eine Sero-prävalenzstudie aufgenommen wurden. Zuvor war gezeigt worden, dass Werte unterhalb der 20. Perzentile mit einer höheren Mortalitätsrate bei Durchbruchinfektionen assoziiert waren[131,132]. Ein möglicher Zieltiter wird in Zusammenschau am ehesten in einem hohen dreistelligen Bereich liegen.

In der Folgearbeit untersuchten wir speziell bei PatientInnen mit hepatobiliären Karzinomen den Effekt einer ersten Auffrischungsimpfung gegen SARS-CoV-2. Die noch wildtypbasierte Auffrischungsimpfung stabilisierte die zuvor weiter regredienten Impftiter und glich die Höhe der Titer zwischen der Referenz- und der Kontrollgruppe aus. Bei allen PatientInnen, bei denen nach der Primärimpfserie gegen SARS-CoV-2 noch keine humorale Immunantwort detektiert werden konnte, lag nach der Auffrischungsimpfung in unserer Kohorte eine Serokonversion

vor. Außerdem bestanden keine Unterschiede bezüglich der zellulären Impfantwort zwischen den Gruppen[60,133]. Im zeitlichen Verlauf sanken die Antikörpertiter aller in die Studie aufgenommener PatientInnen bei Ausbleiben eines weiteren SARS-CoV-2-Antigenkontaktes sowohl nach der Primärimpfserie als auch später nach der ersten Auffrischungsimpfung wieder ab. Diese Entwicklung war dabei in der Gruppe der PatientInnen mit aktiven Tumorerkrankungen ausgeprägter[59–61,133]. Ein stärkerer Abfall der IgG-Titer bei PatientInnen mit soliden Tumorerkrankungen (1,78-fach) im Vergleich zu Beschäftigten im Gesundheitswesen (1,30-fach) ist beschrieben worden, ohne dass die Rate an Durchbruchinfektionen zunahm. Dabei führten eine immunsuppressive Therapie mit Steroiden und/oder chemotherapeutische Behandlungen, wie in unserer Studie, zu signifikant niedrigeren Titern über alle Zeitpunkte hinweg. Der Abfall der Titer war jedoch bei PatientInnen ohne immunsuppressive Dauertherapie und bei PatientInnen unter Immuntherapie schneller, was wiederum für eine immunologisch-getriggerte Ursache des Abbaus der Immunglobuline spricht[134]. Innerhalb der Gruppe von PatientInnen mit soliden Tumoren war der Immunglobulin-Abbau nach Impfungen gegen verschiedene Erreger bei PatientInnen mit zunehmendem Alter, solchen unter Chemotherapie und/oder unter immunsuppressiver Therapie am stärksten ausgeprägt[135–139]. Auch wenn in unserer Studie die Auffrischungsimpfung die Immunantwort also nur temporär zu stabilisieren vermochte, war dennoch durchgehend eine humorale und eine zelluläre Immunantwort auf SARS-CoV-2-Impfungen und/oder Infektionen nachweisbar. Somit kann von einem immunologischen Basisschutz ausgegangen werden.

In unserer Kohorte wurde eine abnehmende Impfbereitschaft beobachtet. Bei nur 29,5% der PatientInnen erfolgte ein erster Omikron-Antigenkontakt über eine von den Experten der STIKO und der Fachgesellschaften für PatientInnen mit soliden Tumorerkrankungen empfohlene jährliche Impfung mit einem Omikron-angepassten Impfstoff. Erfreulicherweise verlief bei den übrigen 70,5% der PatientInnen, deren erster Omikron-Antigenkontakt durch eine SARS-CoV-2-Infektion zustande kam, COVID-19 klinisch mild bis moderat. In keinem Fall war eine intensivmedizinische Versorgung notwendig und kein Studienteilnehmer verstarb an einer Omikron-Infektion[61]. Diese Daten sind ermutigend, weil sie zusätzlich einen klinischen Basisschutz vor schweren Infektionsverläufen auch mit neuen, in diesem Fall den Omikron-Varianten von SARS-CoV-2 belegen. Vergleichbare Daten aus der prä-Omicron-Ära zeigen, dass bei hämatologischen/onkologischen PatientInnen insgesamt von einer 80- bis 90-prozentigen Präventionsrate von symptomatischen Verläufen von COVID-19 ausgegangen werden kann[140]. Wie eingangs dargelegt wird als ursächlich für den Schutz vor schweren Verläufen von

Omikron-Infektionen von kreuzreaktiven Immunantworten ausgegangen[110–116]. Tatsächlich konnten auch wir entsprechend eine im Vergleich zur Wildtyp-Neutralisation signifikant eingeschränktere, aber immerhin 70-prozentige Neutralisation der Omikron-Varianten nach prä-Omikron-Antigenkontakten belegen[61].
Auch wenn in unserer Studie keine schweren Verläufe von COVID-19 beobachtet wurden zeigten andere Studien dennoch, dass PatientInnen mit soliden Tumoren unter Chemotherapie häufiger mit SARS-CoV-2-Durchbruchinfektionen konfrontiert waren und dass deren Infektionsverläufe schwerwiegender waren[57]. Dies ist bei der Aktualisierung von Impf- und Therapieempfehlungen für PatientInnen mit Karzinomen trotz unserer ermutigenden Daten zu berücksichtigen.

Ein erster Omikron-Antigenkontakt führte bei den PatientInnen in unserer Kohorte zu einer signifikanten Stabilisierung der Gesamtimmunantwort und insbesondere zu einer Verbesserung der Omikron-Neutralisation. Dabei konnte erneut kein Vorteil einer hybriden Immunität belegt werden. Einschränkend fiel auf, dass ein erster Omikron-Antigenkontakt bei PatientInnen mit aktiver gastrointestinaler Tumorerkrankung, insbesondere bei PatientInnen mit pankreatikobiliären Karzinomen, mit systemischer Immundefizienz und/oder unter Chemotherapie erneut weniger immunogen war[61]. Diese Faktoren waren bereits nach der Primärimpfserie mit einem schlechteren Ansprechen auf die wildtypbasierten Impfungen assoziiert[59]. Somit scheint in der longitudinalen Betrachtung ein jeweils erster Antigenkontakt mit einem neuen Virus/einer neuen Virusvariante weniger immunogen zu sein. Dabei brachte die Auffrischungsimpfung nur vorübergehend eine Stabilisierung[60,133].

In Zusammenschau stützen unsere longitudinalen Daten die Empfehlungen der STIKO sowie der nationalen und internationalen Leitlinien der Fachgesellschaften für Hämatologie und Onkologie, dass bis auf Weiteres jährliche Auffrischungsimpfungen mit variantenadaptierten Vakzinen für die PatientInnen unserer Kohorte und vergleichbare Patientengruppen notwendig sein werden[41,62,63]. Die bisher gesondert erschienenen DGHO-Impfempfehlungen zu SARS-CoV-2-Infektionen werden während der aktuell laufenden Aktualisierung in die Leitlinie zu anti-infektiven Impfstrategien für hämatologische/onkologische PatientInnen integriert. Im Zuge dieses Prozesses, an dem der Autor der vorliegenden Habilitationsschrift beteiligt ist, werden auch die hier vorgelegten Daten berücksichtigt[128]. Individuelle Beratungen und öffentliche Impfkampagnen sind notwendig, um Impflücken zu schließen. Zu Beginn unserer Auswertungen haben wir uns auf die relative und absolute Höhe der humoralen Impftiter fokussiert. Ziel einer Impfung sollte aber vor allem eine Risikoreduktion bezüglich

Durchbruchinfektionen, schwerer Verläufe und möglicher Folgeschäden von COVID-19 sein. Den Erfolg einer Impfung allein anhand absoluter oder relativer Impftiter zu beurteilen, schränkt die Qualität zahlreicher Impfstudien, in denen die klinischen Verläufe im Falle von Durchbruchinfektionen nicht betrachtet wurden, ein. Unsere Studie hat neben der Immunogenität auch die klinische Wirksamkeit von Impfungen gegen SARS-CoV-2 belegt. Die Messung und Bewertung der humoralen und zellulären Immunantworten müssen ergänzend zur Anpassung der Impfstrategien für vulnerable Patientengruppen herangezogen werden und können auch für individuelle Risikoeinschätzungen dienlich sein. Klinische Aspekte, also vor allem, ob ein Schutz vor einer schweren Infektion durch Impfungen gegeben ist, haben in der Routineversorgung von PatientInnen mit COVID-19 jedoch eine unmittelbarere Bedeutung.

3.2 Klinische Aspekte für die Bewertung antiviraler Monotherapien

Bei der Auswahl und insbesondere der Bewertung antiviraler Monotherapien zur Vermeidung schwerer Verläufe von COVID-19 stand in vielen Studien eher der virologische statt des klinischen Verlaufs im Vordergrund. So waren in den Zulassungsstudien zum Einsatz der DAAs (RDV, NIR/r und Mol) in der Frühen Phase von Infektionen mit SARS-CoV-2 Hospitalisierung und Tod der kombinierte primäre Endpunkt und in deren Folgestudien in geimpften Kollektiven zusätzlich Vorstellungen in der Notaufnahme. Der klinische Verlauf wurde nicht detaillierter betrachtet[64,65,68–70]. Dies trifft ebenfalls auf die Studien zum Einsatz von RDV in der Pulmonalen Phase der Infektion zu[66,67]. Innerhalb des LEOSS-Registers wurde das Ende der akuten Erkrankung bei stationären PatientInnen durch die Entlassung aus dem Krankenhaus und bei ambulanten PatientInnen durch das Fehlen weiterer klinischer Maßnahmen in einem Beobachtungszeitraum von 14 Tagen bzw. für beide Gruppen durch den Tod definiert[67]. Unsere Daten ergänzten die LEOSS-Daten als Kommentar mit einer umfassenderen klinischen Betrachtung von PatientInnen unter antiviraler Monotherapie anhand der WHO-Ordinalskala mit Dokumentation des jeweils schlechtesten Scores[33].

Wir untersuchten hierfür ein Kollektiv von älteren, multimorbiden PatientInnen mit einem hohen Risiko für einen schweren klinischen Verlauf von COVID-19 und somit einer Indikation für eine antivirale Monotherapie im Falle einer Infektion mit SARS-CoV-2. Durch einen sehr kleinen Anteil von PatientInnen mit systemischer Immundefizienz war das Risiko für einen prolongierten virologischen Verlauf in diesem Patientenkollektiv gering. Zunächst bestätigten wir, dass der Zeitpunkt der Einleitung einer RDV-Monotherapie anhand des klinischen

Verlaufs festgelegt werden kann, nämlich zu Beginn einer Sauerstoffpflichtigkeit in der Pulmonalen Phase von COVID-19 bei allen PatientInnen, bei denen keine antivirale Frühtherapie initiiert wurde[33]. Der Einsatz von RDV in der frühen Pulmonalen Phase wird in den nationalen und internationalen Leitlinien angeraten[66,67]. In den Empfehlungen der DGHO werden SARS-CoV-2-Therapieempfehlungen bereits anhand einer modifizierten WHO-Ordinalskala mit Scores von 0-10 geordnet[62]. In der modifizierten Skala wird eine asymptomatische Infektion (Score=1) ergänzt. Außerdem wird im Bereich des schweren Verlaufs von COVID-19 anhand der Höhe des Horovitz-Index, also des Verhältnisses von arteriellem Sauerstoffpartialdruck und der inspiratorischen Sauerstoffkonzentration, sowie anhand des Einsatzes von Vasopressoren, Dialyseverfahren und/oder einer ECMO (*extracorporeal membrane oxygenation*) weiter differenziert (Scores 7-9)[141].

In unserer Studie wurden neben der Therapieauswahl auch das Therapieansprechen unter RDV oder CVIV im Weiteren klinisch anhand der ursprünglichen WHO-Ordinalskala beurteilt. Dabei waren die Monotherapien mit einem geringeren Fortschreiten der Erkrankung auf der WHO-Ordinalskala und einer verkürzten Hospitalisierungszeit assoziiert, was einem Therapieerfolg entsprach[33]. Die Skala ist damit für klinisch arbeitende ÄrztInnen mit geringerer infektiologischer Expertise ein leicht zu handhabendes Instrument zur Beurteilung von PatientInnen mit COVID-19 in der Routineversorgung. Da ein zunehmendes Patientenalter als einer der zentralen Risikofaktoren für schwere klinische Verläufe von COVID-19 – auch unter antiviraler Monotherapie – in unserer Studie bestätigt werden konnte[33], sollte die Skala insbesondere zur Überwachung älterer PatientInnen (>65. Lebensjahr) angewandt werden.

Um die klinische Anwendbarkeit der WHO-Ordinalskala als Steuerungselement zu optimieren, ist eine Korrelation mit einer potenziell fortbestehenden relevanten Infektiosität sinnvoll und notwendig. In Arbeiten zur Bewertung der Effektivität antiviraler Therapien von COVID-19 steht häufig der Abfall der Viruslast in sequenziellen PCR-Testungen als vermeintlicher Surrogatparameter einer potenziell fortbestehenden Infektiosität eher im Vordergrund als der klinische Verlauf. Dabei ist die PCR aus einem nasopharyngealen Abstrich anwenderbedingt eine fehleranfällige Messmethode. Außerdem muss vor allem bei immundefizienten Patient-Innen das Phänomen prolongierter Virusnachweise berücksichtigt werden, sodass die Aussage-kraft einer positiven PCR hinsichtlich der Infektiosität der PatientInnen begrenzt ist. Bei PatientInnen mit systemischer Immundefizienz und sehr langem Nachweis von SARS-CoV-2 wurde eine fortbestehende Infektiosität durch positive Viruskulturen belegt[17–23]. Eine hohe Viruslast in der PCR ist dabei mit einer höheren Wahrscheinlichkeit einer positiven Viruskultur

assoziiert, ohne dass jedoch eine Viruskopienzahl definiert werden konnte, ab der eine Infektiosität ausgeschlossen werden kann[142]. Allgemeinhin wird bei Nachweis von >10^6 SARS-CoV-2-Kopien/ml mittels PCR aus einem nasopharyngealen Abstrich von Infektiosität ausgegangen[16]. Die Datenlage reicht aktuell nicht aus, um von einer SARS-CoV-2-positiven PCR auf Infektiosität zu schließen. Damit ist derzeit die Sinnhaftigkeit sequenzieller PCR-Testungen fraglich. Auf Grundlage der dargestellten Daten empfiehlt die Kommission für Krankenhaushygiene und Infektionsprävention beim Robert Koch-Institut (KRINKO) eine Isolierung von PatientInnen ohne systemische Immundefizienz mit milden/moderaten Verläufen von COVID-19 für sieben Tage ohne erneute PCR-Testung wie auch bei anderen Infektionskrankheiten üblich. Bei PatientInnen mit schweren Verläufen und/oder bei bekannter systemischer Immundefizienz ist jedoch weiterhin eine Isolierung bis zum Erreichen einer Viruslast <10^6 SARS-CoV-2- Kopien/ ml empfohlen[143]. Es gibt bereits Ansätze, die WHO-Ordinalskala mit laborchemischen Parametern zu erweitern. Ein Vorschlag ist, den Inflammationsgrad anhand der Lymphozytenzahl, des Wertes des C-reaktiven Proteins, der Laktatdehydrogenase, des Ferritins und der D-Dimere neben der respiratorischen Verschlechterung zu berücksichtigen[15]. Weitere Studien sind notwendig, um offene Fragen zur Korrelation des virologischen und/oder klinischen Verlaufs mit einer relevanten Infektiosität zu adressieren und diese in die WHO-Skala zu transferieren.

In Zusammenschau der KRINKO-Empfehlungen und unserer Daten kann die WHO-Ordinalskala bis auf Weiteres zur klinischen Verlaufskontrolle insbesondere von PatientInnen ohne systemische Immundefizienz mit COVID-19 unter Therapie zur Verlaufsbeurteilung in der Routineversorgung genutzt werden. Bei klinisch untypischen Fällen und/oder bei PatientInnen mit systemischer Immundefizienz kann der Virusnachweis mittels PCR sekundär herangezogen und unter Berücksichtigung des klinischen Verlaufs interpretiert werden. Für Studien zu COVID-19 empfiehlt die WHO drei Aspekt zu beleuchten: die Viruslast (PCR-basiert), das Überleben der PatientInnen und die Krankheitsprogression anhand der WHO-Ordinalskala[141], was durch unsere Daten gedeckt wird.

3.3 Anwendung etablierter infektiologischer Therapieprinzipien bei COVID-19

Die klinischen Verläufe von Infektionen mit vornehmlich Omikron-Varianten von SARS-CoV-2 bei HochrisikopatientInnen mit systemischer Immundefizienz waren in unserer Studie durch den Einsatz antiviraler Kombinationstherapien in der Erstlinie gemessen an der WHO-Ordinalskala überwiegend mild bis moderat ohne Hinweise auf erhöhte Toxizität[27]. Bei

7,8% von PatientInnen mit hämatologischen Neoplasien sind im Falle einer Omikron-Infektion weiterhin schwere Verläufe von COVID-19 mit teils letalem Ausgang trotz antiviraler Monotherapie beschrieben worden. Dabei wurde bei diesen PatientInnen eine funktionell eingeschränkte Immunantwort auf SARS-CoV-2-Impfungen und/oder Infektionen angenommen[120]. Gerade diese Subpopulation profitierte in unserer Studie klinisch von einer Kombinationstherapie in der Erstlinie mit einem medianen Score von 3 (hospitalisiert, keine Sauerstofftherapie) und einem maximalen Score von 5 (hospitalisiert, nicht-invasive Beatmung oder *high-flow* Sauerstofftherapie) auf der WHO-Ordinalskala. Wir empfahlen den Einsatz von Kombinationstherapien in der Erstlinie daher insbesondere bei PatientInnen mit hämatologischen Neoplasien, diskutierten aber auch einen Einsatz in vergleichbaren Populationen[27].

Prolongierte Virusnachweise trotz klinischer Besserung sind bei PatientInnen mit systemischer Immundefizienz insbesondere aufgrund der potenziell fortbestehenden Kontagiosität und des Risikos von Mutationen des viralen Genoms relevant und müssen neben dem klinischen Verlauf therapeutisch berücksichtigt werden (vgl. Kapitel 1.6). In unserer Studie konnten prolongierte Virusnachweise unter Kombinationstherapien in der Erstlinie in 85,4 % der Fälle verhindert werden[27]. In einem nach unserer Publikation erschienenen systematischen Review zu persistierenden Infektionen mit SARS-CoV-2 errechneten die WissenschaftlerInnen, dass Kombinationstherapien aus zwei DAAs prolongierte Virusnachweise zu 79,0% (in 53 von 67 betrachteten Fällen) und Kombinationstherapien aus einem DAA und einem mAB zu 89,0 % (in 17 von 19 betrachteten Fällen) verhindern konnten. Trotz des Fehlens von Kontrollgruppen in allen Studien, die in das Review eingegangen sind, kommen die KollegInnen analog zu uns zu dem Schluss, dass man antivirale Kombinationstherapien bei immunkompromittierten PatientInnen in Erwägung ziehen kann[144].

Antivirale Monotherapien waren bei hochgradig immunsupprimierten PatientInnen zuvor als nicht ausreichend effektiv beschrieben worden, um schwere klinische und vor allem prolongierte virologische Verläufe sicher zu verhindern. Hierdurch entstand ein Selektionsdruck mit Nachweis von Mutationen im viralen Genom und damit dem Risiko der Entwicklung neuer Varianten und Resistenzen insbesondere gegen die therapeutisch eingesetzten mABs[99–103]. Die Verabreichung von Monotherapien gegen SARS-CoV-2 bei PatientInnen mit systemischer Immundefizienz und einem hohen Risiko für prolongierte Virusnachweise wurde daher kritisch betrachtet und antivirale Kombinationstherapien wurden stattdessen diskutiert. Fallberichte und kleinere Fallserien zu Kombinationstherapien bei COVID-19 hatten bisher deren Einsatz bei PatientInnen dargestellt, bei denen es bereits zu einem schwereren klinischen

Verlauf und/oder einem prolongierten SARS-CoV-2-Nachweis gekommen war. Eine antivirale Dual- oder Trippeltherapie im Verlauf der Infektion zeigte hierbei positive Signale für eine schnellere klinische Genesung und virale Eliminierung[83–94]. Im Gegensatz zu diesem reaktiven Einsatz werden Kombinationstherapien bei anderen Infektionskrankheiten wie der HIV-Infektion oder der Tuberkulose bereits in der Erstlinie genutzt. Ziel hierbei ist es, die Wirksamkeit der Behandlung zu maximieren und potenzielle Resistenzentwicklungen durch unzureichende Monotherapien *upfront* zu verhindern. In unserer Studie, die derzeit durch Einschluss von 144 PatientInnen im Vergleich zu den bisher publizierten Fallserien mit Abstand die Größte ist, wurden Kombinationstherapien bei COVID-19 daher präemptiv in der Erstlinie eingesetzt. Eine antivirale Trippeltherapie erwies sich dabei als nicht vorteilhaft im Vergleich zu einer dualen Therapie. Außerdem konnte die virale Elimination durch eine erneute reaktive antivirale Mono- oder Kombinationstherapie in Fällen mit prolongiertem Virusnachweis trotz initialer Kombinationstherapie nicht beschleunigt werden. In einer multivariablen Analyse konnten wir zeigen, dass in Fällen mit später Therapieeinleitung signifikant längere Virusnachweise gefunden wurden als in Fällen mit früher Therapieeinleitung[27]. Die Daten unterstützen unsere Hypothese, dass eine frühzeitige duale Kombinationstherapie in der Erstlinie (innerhalb von 5 Tagen nach der ersten SARS-CoV-2-positiven PCR) effektiv ist, um die Dauer des Virusnachweises zu verkürzen. Dabei scheint ein Erstlinieneinsatz sinnvoller zu sein, als eine antivirale Kombinationstherapie reaktiv nach dem Auftreten eines prolongierten Virusnachweises analog zu unseren Vorläuferstudien einzusetzen. Unsere Daten sind insbesondere aufgrund des retrospektiven Charakters der Studie und des Fehlens einer Kontrollgruppe limitiert. Die Möglichkeit einer Kombinationstherapie in der Erstlinie wird aber in den COVRIIN-Empfehlungen und der Leitlinie der DGHO zum Management von COVID-19 bei hämatologischen/onkologischen PatientInnen bereits diskutiert[62,95]. Außerdem wird die Empfehlung hierzu in der aktuell laufenden Aktualisierung der DGHO-Leitlinie zu respiratorischen Virusinfektionen[145], an der der Autor dieser Habilitationsschrift mitarbeitet, im Wesentlichen auf Grundlage unserer Daten gestärkt.

In unserer Studie führten Infektionen mit SARS-CoV-2 trotz Kombinationstherapien noch zu Therapieverzögerungen bei gut 12% der PatientInnnen[27]. Verzögerungen tumorspezifischer Therapieeinleitungen von über einem Monat sind bei 17,2% von PatientInnen mit gleichzeitiger Diagnose einer hämatologischen Neoplasie und einer Infektion mit SARS-CoV-2 beschrieben worden. Die Autoren wiesen darauf hin, dass Therapieverzögerungen so kurz wie möglich gehalten werden sollten, um die Prognose der Grunderkrankung nicht zu verschlechtern[4].

Fallberichte belegen, dass Therapieeinleitungen bezüglich anderer Grunderkrankungen bei dringlichen Indikationen auch bei PatientInnen mit hochaktiven und/oder kurz nach Infektionen mit SARS-CoV-2 erfolgreich waren, ohne dass es zu einem klinisch kritischen Verlauf und/ oder einer viralen Reaktivierung kam[146–149]. Die unkomplizierten Verläufe von COVID-19 ohne Hinweise auf erhöhte Toxizität bei den PatientInnen unserer Studie befürworten den Einsatz antiviraler Kombinationstherapien insbesondere zur Reduktion von Therapieverzögerungen aufgrund prolongierter Infektionen mit SARS-CoV-2 bereits in der Erstlinie.

Insgesamt sind in unserer Analyse verschiedenste Kombinationen von DAAs und/oder mABs berücksichtigt worden, ohne dass ein spezielles Regime überlegen war. Auch für den Einsatz von MOL, das während des Erhebungszeitraums in Europa noch bedingt zugelassen war, konnten wir einen Nutzen in Kombinationstherapieregimen belegen[27]. Studien zum Einsatz von MOL in Kombination mit RDV, NIR/r und/oder einem mAB in Fällen, bei denen ein prolongierter Virusnachweis bereits eingetreten war, stützen unsere Daten bezüglich der viralen Elimination[80,81,85,88]. Beim Einsatz von MOL als Monotherapeutikum bei immungeschwächten PatientInnen wurde zuletzt eine Anhäufung von Mutationen beobachtet mit dem Risiko der Entstehung neuer Virusvarianten[104]. Folgestudien zu Kombinationstherapien müssten dies beim Einsatz von MOL durch regelmäßige Mutationsanalysen berücksichtigen. Außerdem müssten etwaige neue DAAs und/oder mABs, wie Ibuzatrelvir oder Sipavibart[150,151], im Falle einer Zulassung durch die EMA als Kombinationstherapeutika evaluiert werden.

Das Post COVID-19-Syndrom ist mit einem längeren Virusnachweis assoziiert[25]. Dabei wiesen speziell PatientInnen mit hämatologischen/onkologischen Grunderkrankungen Raten von bis zu 15% für das Auftreten von Post COVID-19 auf[152]. In unserer Kohorte wurde in nur einem Fall ein Post COVID-19-Syndrom beschrieben. Da der Nachbeobachtungszeitraum der Studie nicht definiert war, konnte aber keine Aussage getroffen werden, inwieweit antivirale Kombinations-therapien das Auftreten von Post COVID-19 verhindern können[27]. Epidemiologisch ist auch die Entstehung neuer Varianten von SARS-CoV-2 mit prolongierten Virusnachweisen, vor allem unter unzureichender Therapie, assoziiert[153]. Auch dieser Aspekt konnte insbesondere aufgrund des Fehlens einer Kontrollgruppe in unserer Studie nicht beleuchtet werden. Der Einfluss antiviraler Kombinationstherapien in der Erstlinie auf die Entwicklung eines Post COVID-19-Syndroms und/oder die Entstehung neuer Virusvarianten als Treiber epidemischer Ausbrüche muss in Folgestudien untersucht werden.

4 Zusammenfassung

Gesamtgesellschaftlich betrachtet besteht bei einem Großteil der Bevölkerung in Deutschland durch SARS-CoV-2-Impfungen und/oder -Infektionen ein effektiver immunologischer Schutz vor klinisch schweren Verläufen von COVID-19. Im Fokus der vorliegenden kumulativen Habilitationsschrift standen Subgruppen von RisikopatientInnen mit weiterhin erhöhtem Risiko für komplizierte Infektionen mit SARS-CoV-2 und/oder prolongierte Virusnachweise.

Angesichts zunehmender Impfmüdigkeit sollten RisikopatientInnen individuell beraten und vor allem auch die Allgemeinheit durch gezielte Impfkampagnen motiviert werden, etwaige Impflücken zu schließen, um vulnerable Gruppen weiterhin zu schützen. Für PatientInnen mit aktiven gastrointestinalen Karzinomen konnten wir zeigen, dass ein Erstkontakt mit SARS-CoV-2 und/oder einer der neuen Omikron-Varianten des Virus weniger immunogen war. Außerdem wurde im zeitlichen Verlauf eine raschere Abnahme der Immunität bei diesen PatientInnen beobachtet. Dabei wiesen PatientInnen mit hepatozellulären, kolorektalen und/oder pankreatikobiliären Karzinomen besonders eingeschränkte Impfantworten auf und waren somit einem anhaltend hohen Risiko schwerer Infektionen ausgesetzt. Durch eine erste Auffrischungsimpfung konnten die Immunantworten temporär stabilisiert werden: Bei allen PatientInnen lag eine Serokonversion vor und die Impftiter waren nicht mehr vermindert, jedoch kam es erneut zu einem Abfall der IgG-Spiegel über die Zeit. Klinisch verliefen Durchbruchinfektionen auch mit einer der Omikron-Varianten von SARS-CoV-2, ohne dass es zuvor zu einem Omikron-Antigenkontakt gekommen war, nach der Auffrischungsimpfung dennoch mild. Ein immunologischer und klinischer Schutz auch vor neuen Virusvarianten durch die Basis-immunität kann somit diskutiert werden. Durch eine Omikron-Infektion und/oder eine Impfung mit einem Omikron-adaptierten Impfstoff wurde die Kapazität zur Neutralisation von Omikron-Varianten signifikant verbessert und die Gesamtimmunantwort erneut stabilisiert. In Zusammenschau unterstützen unsere Daten die aktuellen Empfehlungen der STIKO und verschiedener Fachgesellschaften zu jährlichen Auffrischungsimpfungen, legen aber auch nahe, dass für Subgruppen individualisierte Konzepte notwendig sein können.

Insbesondere bei PatientInnen mit zu erwartender eingeschränkter Impfantwort und/oder unvollständiger Umsetzung der Impfempfehlungen besteht weiterhin die Indikation für antivirale Therapien. Wir konnten zeigen, dass bei PatientInnen ohne systemische Immundefizienz der Infektionsverlauf unter antiviraler Monotherapie klinisch anhand der simplen WHO-Ordinalskala beurteilt werden kann, ähnlich wie bei anderen Infektionskrankheiten. Trotz anti-

viraler Monotherapie waren vor allem PatientInnen im Alter über 65 von schweren klinischen Verläufen betroffen. Bei PatientInnen ohne systemische Immundefizienz kann dennoch auf sequenzielle PCR-Testungen verzichtet werden. Diese sind nur sekundär bei untypischen Krankheitsverläufen oder bei PatientInnen mit systemischer Immundefizienz und dem Risiko prolongierter Virusnachweise mit ggf. persistierender Infektiosität, wie von der KRINKO empfohlen, korrelierend indiziert.

Bei PatientInnen mit systemischer Immundefizienz und somit einem anhaltenden Risiko schwerer klinischer und auch prolongierter virologischer Verläufe scheint der Einsatz antiviraler Kombinationstherapien in der Erstlinie gemäß unseren Daten im Vergleich zu einem abwartenden Konzept mit Einsatz von Kombinationstherapien erst nach Eintritt eines prolongierten Virusnachweises vorteilhaft zu sein. Dabei waren in unserer Kohorte die klinischen Verläufe mild bis moderat und prolongierte Virusnachweise selten ohne Hinweise auf erhöhte Toxizität durch Kombination mehrerer antiviraler Substanzen. Diese Beobachtungen legen nahe, dass Therapieverzögerungen aufgrund von Infektionen mit SARS-CoV-2 durch den Einsatz antiviraler Kombinationstherapieregime vermieden werden könnten. Insgesamt profitierte vor allem die Subgruppe von PatientInnen mit hämatologischen Neoplasien. Der Einfluss von Kombinationstherapien auf die Entwicklung eines etwaigen Post COVID-19-Syndroms und/oder die virale Evolution konnte mit unseren Daten nicht abgeschätzt werden. Folgestudien sind daher erforderlich, weil beide Phänomene mit prolongierten Virusnachweisen assoziiert sind.

Daten zu Subpopulationen sind essenziell, um HochrisikopatientInnen zu identifizieren. Durch den Transfer auf vergleichbare und/oder übergeordnete Populationen kann die Evidenz bei limitierter Studienlage gestärkt werden. Dabei sind klinische Parameter wie die Vermeidung von Durchbruchinfektionen bzw. eines schweren klinischen Verlaufs in der Routineversorgung entscheidender als immunologische und/oder virologische Parameter. Die letztgenannten Parameter können zur individuellen Risikostratifizierung und Anpassung der generellen Impf- und Therapieempfehlungen herangezogen werden. Bei neuen Infektionserkrankungen sollte sich die Strategie zur Impfung und Therapie künftig noch mehr an bewährten Prinzipien anderer Infektionskrankheiten orientieren, um die Wirksamkeit zu steigern und Resistenzentwicklungen zu vermeiden.

Mögliche inhaltliche Überlappung mit anderen kumulativen Habilitationsschriften

Die vorliegende Habilitationsschrift hat fünf publizierte Arbeiten zur Grundlage. Bei der Publikation von drei Arbeiten war ich als Hauptverantwortlicher für die Planung und Durchführung der Studien alleiniger Letztautor (vgl. Kapitel 2.3, 2.4 und 2.5). Zwei Arbeiten habe ich jeweils als geteilter Erstautor mit Herrn Jens Gorny (vgl. Kapitel 2.1) und Frau Leona Baier (vgl. Kapitel 2.1) zusammen veröffentlicht. Beide waren wissenschaftlich sehr engagierte DoktorandInnen, die Frau Prof. Dr. med. Maria Gonzalez-Carmona und ich zusammen betreut haben. Im Rahmen der SARS-CoV-2-Pandemie mussten Datenerfassungen zügig abgeschlossen werden. Gründe hierfür waren unter anderem das hohe gesundheitspolitische und wissenschaftliche Interesse, aber auch virologische Faktoren wie neue Virusvarianten. Entsprechend häufig ergaben sich rasch neue Fragestellungen und wurden Empfehlungen kontinuierlich aktualisiert. Ich war zusätzlich auf der COVID-19-Station eingesetzt mit einer besonderen Arbeitsbelastung. Bei den Arbeiten, die mit geteilten Erstautorenschaften publiziert wurden, war ich federführend zuständig für a) die Studienkonzeptierung und -aufsicht, b) die Analyse und Interpretation der Daten, c) die statistische Analyse und insbesondere d) die Erarbeitung der Manuskripte. Frau Leona Baier und Herr Jens Gorny haben die Datenerfassung in kurzer Zeit bewerkstelligt und waren an der Analyse und Interpretation der Daten beteiligt. Entsprechend ihres Engagements wurde die Autorenschaft geteilt. Eine Überlappung mit anderen Habilitationsschriften ist nicht gegeben.

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Kenntnis der Richtlinien zur guten wissenschaftlichen Praxis der Universität Bonn (§ 3 der Habilitationsordnung)

Hiermit bestätige ich, dass ich die Richtlinien zur guten wissenschaftlichen Praxis der Universität Bonn, laut Habilitationsordnung, zur Kenntnis genommen habe und ich versichere, dass ich sie beim Verfassen der Habilitationsschrift beachtet habe. Insbesondere versichere ich, dass ich alle in der Habilitationsschrift benutzten Quellen und Hilfsmittel angegeben habe.