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Wirtsfaktoren in primären Tumoren der Leber und Gallenwege: Einfluss auf kurative Therapieansätze

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Meinen Eltern

in Liebe und Dankbarkeit

Inhaltsverzeichnis

Abkürzungsverzeichnis

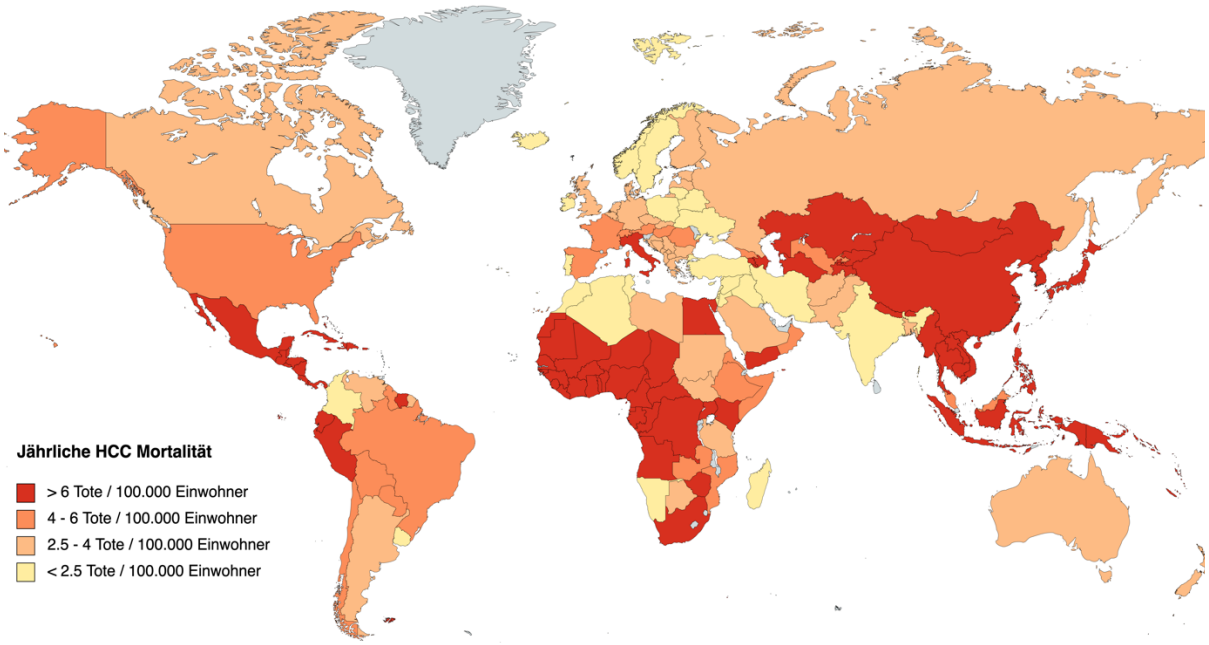
ALPPS	Associating Liver Partition and Portal vein Ligation for Staged Hepatectomy
BCLC	Barcelona Clinic Liver Cancer
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CAF	Tumor-assoziierter Fibroblast (Cancer-Associated Fibroblast)
CCA	Cholangiozelluläres Karzinom
CT	Computertomographie
CXCR	C-X-C Motif Chemokine Receptor
dCCA	Distales Cholangiozelluläres Karzinom
GWAS	Genome-Wide Association Studies
IDH	Isocitratdehydrogenase
iCCA	Intrahepatisches Cholangiozelluläres Karzinom
FGFR	Fibroblast Growth Factor Receptor
HCC	Hepatozelluläres Karzinom
IL	Interleukin
MAFLD	Metabolic (Dysfunction)-Associated Fatty Liver Disease
MASH	Metabolic Dysfunction-Associated Steatohepatitis
MASLD	Metabolic Dysfunction-Associated Steatotic Liver Disease
NAFLD	Non-Alcoholic Fatty Liver Disease
NET	Neutrophile Extrazelluläre Fallen (Neutrophil Extracellular Traps)
pCCA	Perihiläres Cholangiozelluläres Karzinom
PET	Positronen-Emissions-Tomografie
PD-L1	Programmed Death-Ligand 1
PSC	Primär Sklerosierende Cholangitis
SNP	Einzelnukleotidpolymorphismus (Single Nucleotide Polymorphism)
TAM	Tumor-Assoziierte Makrophagen
TME	Tumor-Microenvironment
UICC	Union Internationale Contre le Cancer
VEGF	Vascular Endothelial Growth Factor

1. Einleitung

1.1 Ätiologie und Epidemiologie primärer Lebertumore

Primärer Leberkrebs wurde im Jahr 2020 bei fast einer Million Menschen diagnostiziert und war für über 800.000 Todesfälle weltweit verantwortlich, und damit die dritthäufigste krebsbezogene Todesursache weltweit (1, 2). Ein weltweiter Anstieg der Inzidenz um über 50% wird bis 2040 projiziert (2). Das Zusammentreffen von hoher Inzidenz, persistierenden Risikofaktoren, später Diagnosestellung und limitierten Therapiemöglichkeiten bedingen die weltweit bedrohliche Situation durch Leberkrebs.

Die häufigste primäre Tumorentität in der Leber ist mit etwa 80% der primären Tumoren das Hepatozelluläre Karzinom (HCC), ausgehend von den Hepatozyten, gefolgt vom Cholangiozellulären Karzinom (CCA, 15%) in intrahepatischer Lokalisation (iCCA, intrahepatisches Cholangiozelluläres Karzinom), ausgehend von den Cholangiozyten (3). Bei beiden Tumorentitäten bedingt eine regionale Variation der Risikofaktoren starke Unterschiede der lokalen Inzidenzen (2). Die weltweite altersstandardisierte Inzidenz des HCCs liegt bei etwa 9.3 Fällen/100.000 Einwohnern, entsprechend liegt die weltweite Jahresmortalität des HCCs bei 8.5/ 100.000 Einwohnern pro Jahr (4) (Abbildung 1). Insgesamt gilt in Europa, Nordamerika und Australien das CCA mit einer altersstandardisierten Jahresmortalität von 2-4/ 100.000 Einwohnern als seltene Tumorerkrankung (5). Entsprechend existieren zum CCA im Vergleich zum HCC bedeutend weniger Studien in westlichen Patient*innenkollektiven, wo sich Ätiologie und Tumorbiologie deutlich von den asiatischen unterscheiden (3).



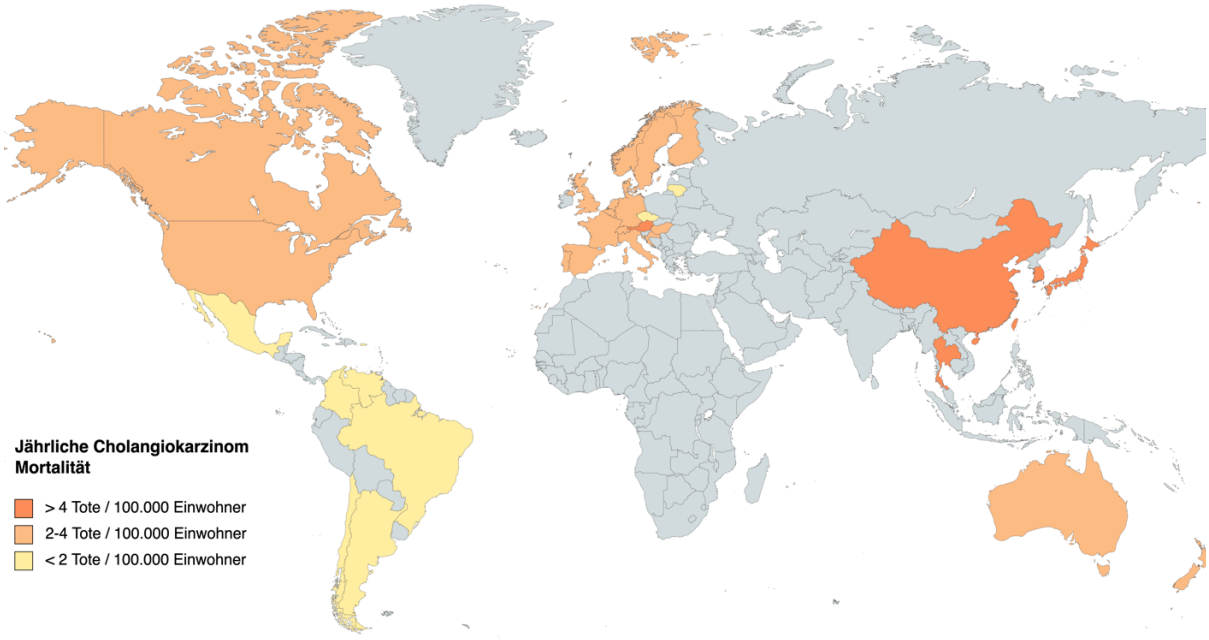


Abbildung 1: Weltweite Mortalität durch HCC und CCA (altersstandardisiert), pro 100.000 Einwohner und Jahr.
Für grau hinterlegte Länder sind nur unzureichende Daten verfügbar.
Modifiziert nach: (5, 6). Karten erstellt mit Mapchart.net (https://www.mapchart.net/world.html)
Abkürzungen: CCA, Cholangiozelluläres Karzinom; HCC, hepatozelluläres Karzinom.

Die wichtigsten Ätiologien des HCCs sind die alkoholische und virale Leberzirrhose durch Hepatitis B und C sowie zunehmend die Fettlebererkrankung (non-alcoholic fatty liver disease, NAFLD, oder neu [seit Juni 2023] Metabolic Dysfunction-Associated Steatotic Liver Disease, MASLD) (7). Weltweit, vor allem aber in Subsahara-Afrika und Zentral- und Südostasien ist die Hepatitis B-Zirrhose die häufigste HCC-Ätiologie, gefolgt von der Hepatitis C-Zirrhose, vor allem vertreten in Ägypten, Pakistan, und mit zahlreichen Fällen auch in den USA und Europa (2). Gleichzeitig vollzieht sich aktuell in Nordamerika, Europa und einigen Ländern des Nahen Ostens ein Wandel der HCC-Ätiologien, mit einer Spitzenstellung der MASLD als HCC-Ätiologie. Gründe hierfür sind einerseits der Rückgang viraler Hepatitiden durch die Implementierung von Hepatitis B-Impfungen und Hepatitis C-Eradikation, sowie andererseits die zunehmende Übergewichtsepidemie der westlichen Welt. So werden aktuell in den USA etwa die Hälfte der Todesfälle durch Leberzirrhose und über ein Drittel der Todesfälle durch HCC einer MASLD-Ätiologie zugeschrieben (8).

Mittlerweile wurde das HCC in MASLD-Zirrhose als eine Tumorentität mit eigenständigen molekularbiologischen und immunologischen Charakteristiken identifiziert, die auch potenzielle therapeutische Relevanz, beispielsweise durch ein geringeres Ansprechen auf Checkpointinhibitoren, hat (9). Das frühe Stadium der MASLD ist weltweit hochprävalent – aktuell sind etwa 30% der Bevölkerung betroffen – und ist gekennzeichnet durch die pathologische Fetteinlagerung im Leberparenchym (10). In bis zu 20% der MASLD-Fälle

kommt hierzu eine inflammatorische Komponente mit begleitendem Hepatozytenschaden und der Einlagerung fibrotischen Bindegewebes, als Metabolic Dysfunction-Associated Steatohepatitis (MASH) definiert (11). Diese Zahlen steigen in zahlreichen Bevölkerungsgruppen (USA, Europa, China, Japan), mit dem Risiko der Progression zum Vollbild der Zirrhose mit fibrotisch-knotigem Umbau der Leber und massiv erhöhtem Risiko für primäre Lebertumoren (11). Bereits die prä-zirrhotische MASH-Erkrankung kann zu einem erhöhten HCC-Risiko beitragen, wodurch ein erheblicher Anteil der Bevölkerung klinisch inapparent einem Malignitätsrisiko ausgesetzt ist (12). Da im Jahr 2023 die nicht-alkoholische Leberverfettung (NAFLD) durch eine neue Konsensus-Terminologie zu einer neuen Definition mit positiven Einschlusskriterien, die die metabolische Dysregulation dieser Patient*innen wiederspiegelt, umgewandelt wurde (MASLD) (13), wird in dieser Arbeit die MASLD-Terminologie verwendet. Erste Analysen zeigen, dass trotz leicht unterschiedlicher diagnostischer Kriterien die Begriffe auch retrospektiv synonym verwendet werden können, sodass in dieser Schrift auch in Bezug auf Studien mit NAFLD-Kriterien die einheitliche MASLD-Terminologie verwendet wird (14).

Weitere Risikofaktoren für das HCC beinhalten toxische Ursachen wie Aflatoxinexposition – hochprävalent im sogenannten Aflatoxin-Gürtel, der weitestgehend deckungsgleich mit den Hepatitis B-Hochrisikogebieten in Afrika und Asien ist (15) – und genetisch-metabolische Erkrankungen wie die Hämochromatose, Morbus Wilson oder der alpha-Antitrypsinmangel (16).

Für das CCA stellen in der westlichen Welt chronisch-inflammatorische Erkrankungen der Gallenwege, vor allem die Primär Sklerosierende Cholangitis (PSC) (17), und biliäre Stase, beispielsweise durch chronische Cholelithiasis, den wichtigsten Risikofaktor dar, wobei über die Hälfte der CCA-Fälle in Abwesenheit anerkannter onkologischer Risikofaktoren entsteht (3, 18). In Südostasien tragen Infektionen mit Leberegeln (*Opisthorchis viverrini, Clonorchis sinensis*) zu wesentlich höheren CCA-Inzidenzen als in Europa und Nordamerika bei (5) (Abbildung 2).

Des Weiteren teilt das iCCA auch die Ätiologien des HCCs, wie chronische Hepatitiden, Zirrhose und die MASLD (5). Letztere Ätiologie ist im CCA – im Gegensatz zum HCC – weitestgehend unerforscht, hauptsächlich aufgrund der relativen Seltenheit der Tumorerkrankung in den USA und Europa (19), wobei bekannt ist, dass die Fettlebererkrankung einhergeht mit einem erhöhten Risiko für sowohl das HCC, als auch für sämtliche andere gastrointestinale Tumorentitäten (20).

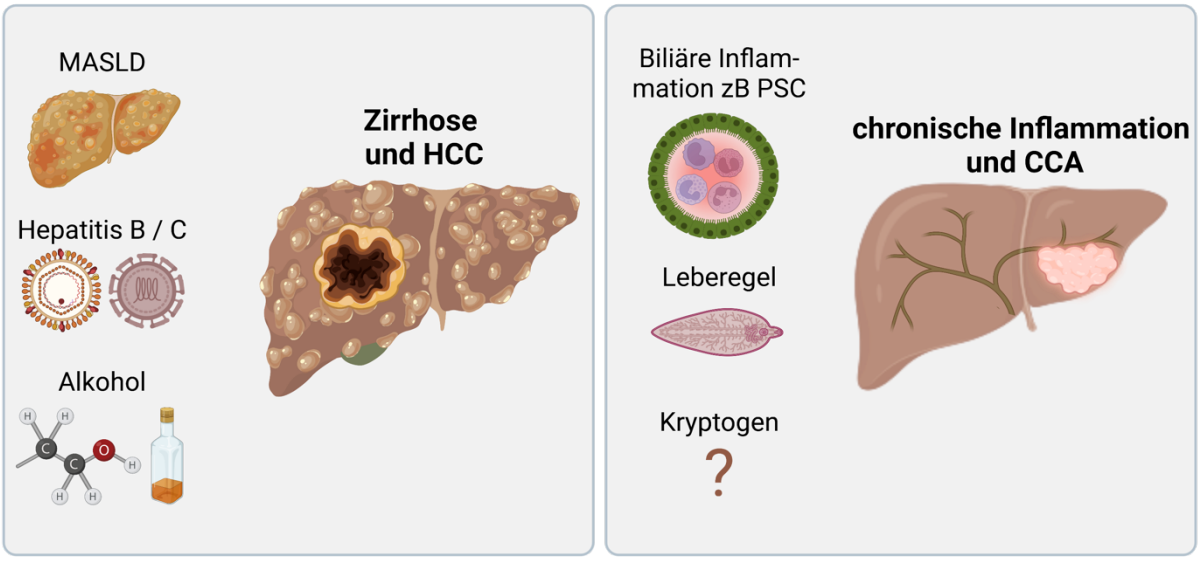


Abbildung 2: Häufigste Risikofaktoren von HCC und CCA

Abkürzungen: CCA, Cholangiokarzinom; HCC, Hepatozelluläres Karzinom; MASLD, Metabolic Dysfunction-Associated Steatotic Liver Disease; PSC, Primär Sklerosierende Cholangitis

Abbildung erstellt mittels Biorender.com

1.2 Stadieneinteilung, Therapie und Prognose

Das HCC wird klinisch im Wesentlichen nach Tumorausdehnung, dem Allgemeinzustand der Patient*innen und der zugrundeliegender Lebererkrankung, beziehungsweise -funktion in Stadien nach dem Barcelona Clinic Liver Cancer (BCLC) Staging eingeteilt (21).

Da das CCA im gesamten Verlauf der Gallenwege auftreten kann, wird zwischen Tumoren proximal der zweiten Hepaticusgabelung, dem intrahepatischen CCA (iCCA), Tumoren distal davon, die die beiden hepaticus communis Äste und den ductus choledochus bis zur Einmündung des ductus cystikus betreffen, perihiläres CCA (pCCA), sowie Tumoren, die distal davon auftreten, distales CCAs (dCCA), differenziert (22). Diese Tumore unterscheiden sich maßgeblich nicht nur in ihrer Lokalisation, sondern auch in ihrer Tumorbiologie, Prognose und Therapie.

Sowohl das HCC als auch das CCA sind hochaggressive Tumore, die wegen ihres zunächst symptom- und schmerzlosen Wachstums oftmals erst in fortgeschrittenen Tumorstadien diagnostiziert werden. Sowohl im Falle des HCCs als auch des CCAs wurden nur etwa 15% der Patient*innen mit der Erstdiagnose des primären Lebertumors laut retrospektiven real-world Analysen einer potenziell kurativen Therapie zugeführt (23, 24), bei hochaggressiver mehrschrittiger chirurgischer Herangehensweise bis zu 30% in ausgewählten Kollektiven, bei hoher Morbidität und Mortalität (25, 26). Entscheidend für die Prognose und Therapie der Wahl sind in beiden Entitäten die zugrundeliegende

Leberfunktion, die Lokalisation und der Invasionsgrad der Tumore, sowie das Vorhandensein von Lymphknoten- und Fernmetastasen. Für eine Übersicht stadiengerechter Behandlung von HCC und CCA soll hier auf frühere Übersichtsarbeiten verwiesen werden (27, 28), und entsprechend dem Fokus dieser Arbeit explizit auf die kurativen Therapieoptionen primärer Leber- und Gallengangstumoren eingegangen werden.

Kurative Therapieansätze für das HCC sind die Leberteilresektion und die Lebertransplantation. Die Lebertransplantation unter adäquater Patient*innenselektion (Tumorgröße-basierte Systeme, beispielsweise Milan-Kriterien) für das lokalisierte HCC hat ein exzellentes Langzeitoutcome, mit über 70% 5-Jahresüberleben (29). Wichtigster Vorteil der Lebertransplantation ist die gleichzeitige Heilung der hepatischen Grunderkrankung, wichtigste Limitation der eklatante Organmangel in Deutschland und Europa und die damit zusammenhängende Wartelistenmortalität, die die Verfügbarkeit dieser lebensrettenden Therapie drosselt (30). Zudem besteht eine deutliche Korrelation zwischen der Tumorlast vor der Lebertransplantation und dem Rezidivrisiko unter Immunsuppression nach der Transplantation, was sich in verschiedenen Organ-Vergabesystemen wie dem Milan-System äußert und Patient*innen mit geringer Tumorlast bevorzugten Zugang zur Warteliste verschafft (31). Die Leberteilresektion hat gerade in frühen Stadien einer zugrundeliegenden Lebererkrankung ein ähnliches Outcome wie die Lebertransplantation, ohne auf lebensrettende Organe aus dem Donorpool zurückgreifen zu müssen (32). Ein zentraler Nachteil der Leberresektion ist, dass das erkrankte, verbleibende Restparenchym eine häufige Ursache rekurrenter Tumore ist, sogar Jahre nach der initialen Tumorresektion (33).

Für das CCA gilt aufgrund von hohen Rezidivraten in initialen Transplantationsstudien und den hochselektiven, zeitlich mehrschrittigen Kriterien für die erfolgsversprechende Lebertransplantation die Leberresektion als am weitesten verbreiteter Goldstandart der kurativen Therapie (34). Im Falle von iCCA und pCCA bedeutet dies oft ausgedehnte Leberresektionen mit großen Parenchymeinbußen und vaskulären Resektionen mit komplizierten Rekonstruktionen im Leberhilus (35). Im Falle distaler Tumoren werden zumeist Resektionen analog zur chirurgischen Therapie von Pankreaskopfkarzinomen angestrebt. Neben einer hohen perioperativen Morbidität und Mortalität – eine 13%-ige perioperative 3-Monatsmortalität wurden kürzlich als „benchmark“, also typisches, anzustrebendes Outcome erfahrener internationaler Zentren in Kollektiven ohne wesentliche präoperative Komorbidität berichtet – ist vor allem die postoperative Rekurrenz ein zentrales und aktuell unzureichend adressiertes Problem (35). Nach erfolgter Resektion in kurativer Intention liegen beim iCCA die Rekurrenzraten bei 50-70%, mit einer hohen Rezidiv-assoziierten Mortalität, die ein 20-40% 5-Jahresüberleben bedingt (36). Erste Erfolge in der Verlängerung des postoperativen Langzeitüberlebens konnten in der BILCAP Studie

verzeichnet werden (37), wodurch die adjuvante Verabreichung von Capecitabine zum allgemeinen Therapiegrundsatz nach Resektion wurde (36).

Die am besten etablierten prognostischen Parameter für die postoperative CCA-Rekurrenz und das Überleben sind das Vorliegen einer Leberzirrhose, positive Resektionsränder, positiver Lymphknotenstatus und vaskuläre Invasion (38). Zunehmend konnten auch molekulare Tumorfaktoren mit prognostischer und prädiktiver Relevanz identifiziert werden: Driver-Mutationen der Isocitratdehydrogenase (IDH) 1 und IDH2 (ca. 15% der iCCAs in Europa und Nordamerika), können gezielt inhibiert werden, mit einer resultierenden Verlängerung des progressionsfreien Überlebens (39). Genfusionen des Fibroblast Growth Factor Receptors (FGFR) 2 und FGFR3, präferenziell vorkommend im nicht-Leberegel assoziierten CCA, können ebenfalls selektiv therapeutisch genutzt werden (40). Hierbei handelt es sich um Studien in hochpalliativen, Chemotherapie-refraktären, systemisch therapierten Patient*innenkollektiven, ohne direkte Rückschlüsse auf kurative Therapiesituationen zu erlauben. Diese tumor-und lebererkrankungsspezifischen Parameter sind somit aktuell unzureichend für eine adäquate präoperative Stratifikation dieser Hochrisiko-Kollektive.

In dieser kumulativen Arbeit werden Wirtsfaktoren von Patient*innen mit primären Lebertumoren als prognostische Einflussgrößen im iCCA untersucht. Allgemein werden Wirtfaktoren in angeborene und akquirierte Faktoren unterschieden. Wichtige akquirierte Risikofaktoren sind Toxinexposition, chronische Infektionen wie Hepatitis B und C sowie der Ernährungsstatus, mit den hochprävalenten Risikofaktoren Unter-und Überernährung als zentrale gesundheitliche Probleme des 21. Jahrhunderts (41). Zu den angeborenen Faktoren zählen hereditäre Erkrankungen sowie Einzelnukleotidpolymorphismen (single nucleotide polymorphism, SNP), also in einer Population prävalente (>1% der Allele) Genvarianten, die sich durch ein Nucleotid im selben Genlocus unterscheiden, und im Gegensatz zur somatischen Tumor-Mutation, durch Vererbung (Keimbahn) weitergegeben werden. Insgesamt sind über 80 Millionen SNPs beschrieben, davon bleiben die meisten aber ohne Effekt auf die translatierte Proteinstruktur oder die Proteinexpression (42). Während somatische Mutationen meist Krebs-spezifische biologische Effekte haben, beeinflussen SNPs direkt die Prozesse der Wirtszellen, und somit immunologische Prozesse, Metabolismus, sowie, im Falle systemischer Therapien, Wirkungskinetik und Bioverfügbarkeit (43). Zuvor konnte für ausgewählte Genvarianten in einigen gastrointestinalen Tumoren, wie dem kolorektalen Karzinom und dem Magenkarzinom eine prognostische Relevanz von Genen, deren Produkte in der Tumorumgebung eine Mediatorrolle, beispielsweise für Immunfunktion und Neovaskularisation haben, aufgezeigt werden (44-46).

Ziel dieser Arbeit war es, für primäre Lebertumoren, insbesondere für das weniger untersuchte CCA, Patienten-zentrierte prognostische Faktoren zu finden.

1.3 Tumor-Microenvironment

Das Tumor-Microenvironment (TME) – also das Zusammenspiel von Tumorzellen, parenchymatösen Zellen wie Endothelzellen und Neuronen, sowie residenten oder infiltrierenden Immunzellen und deren Produkten – ist spätestens seit der breiten Zulassung von Checkpoint-Inhibitoren und Einzelzell-Sequenzierungsmethoden in den Fokus onkologischer Forschung gerückt (47). Das TME beeinflusst das Wachstum, die Invasion und die Metastasierung maligner Tumore und kann Immunantworten und die Wirksamkeit onkologischer Therapien unterdrücken (48). Zwei zentrale Mechanismen tragen entscheidend zur Progression der malignen Erkrankung bei: (i) die direkte Stimulation des Tumorwachstums, beispielsweise durch Förderung der Zellteilung, der Invasion und Metastasierung und (ii) die Inhibition zytotoxischer Immunantworten, beispielsweise durch suppressive myeloide und lymphoide Zellpopulationen (49). Sowohl Mechanismus (i) und (ii) fördern die Tumor-Neoangiogenese – eine essenzielle Voraussetzung für Tumorwachstum – durch Vascular Endothelial Growth Factor (VEGF)-abhängige und unabhängige Pathways, die nicht nur durch maligne Zellen, sondern auch durch akzessorische Zellen, wie Tumor-assoziierte Makrophagen (TAM) gefördert werden (50) (Abbildung 3).

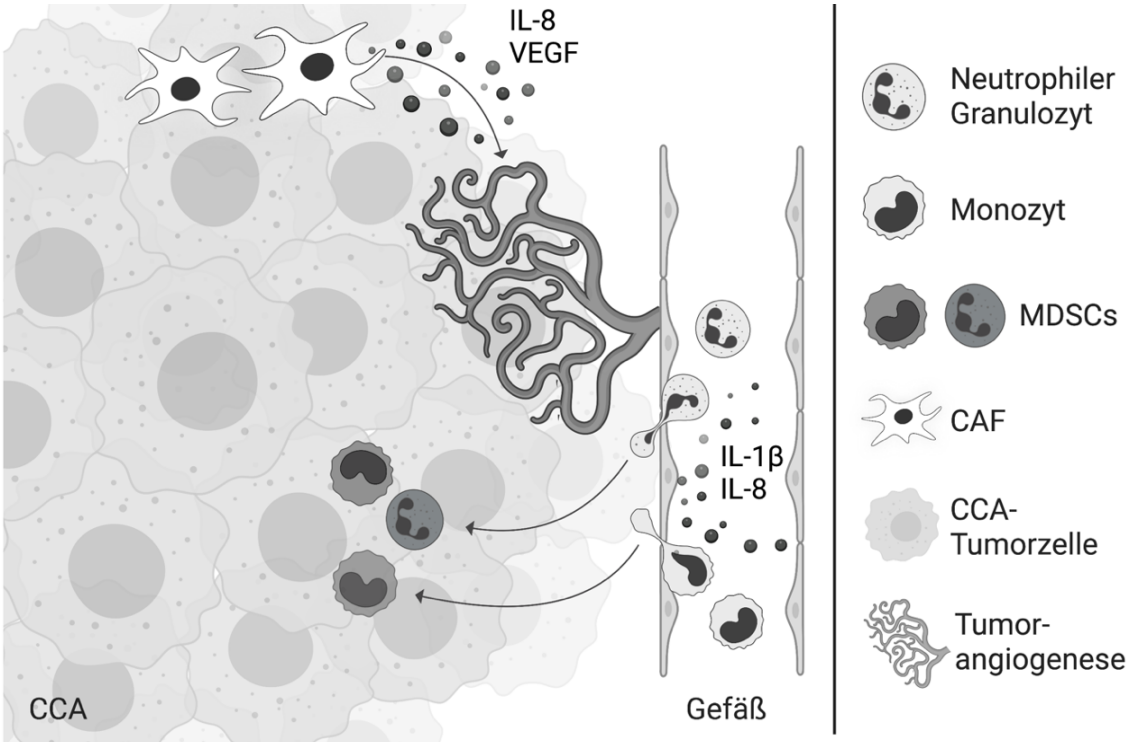


Abbildung 3: Schematische Darstellung der Tumor-assoziierten Inflammation im Cholangiokarzinom mit einem Fokus auf IL-8, IL-1β und Neoangiogenese.
CAFs stimulieren die Neoangiogenese via VEGF und IL-8-Ausschüttung. Zusätzlich setzen tumornahe Endothelzellen IL-8 frei, wodurch zirkulierende neutrophile Granulozyten und Monozyten vermehrt in das Gewebe einwandern und im Tumor-Mikromilieu als sogenannte MDSCs immunsuppressive Funktionen ausüben. *Abbildung erstellt mittels Biorender.com*

Abkürzungen: CAF – Cancer-associated fibroblast (tumor-assoziierter Fibroblast), CCA – Cholangiozelluläres Karzinom, IL – Interleukin, MDSC – myeloid-derived suppressor cell (myeloide Supressor-Zelle), VEGF – Vascular epithelial growth factor.

Typisch für das CCA ist eine starke desmoplastische Umgebungsreaktion aus Tumor-assoziierten Fibroblasten (CAF, cancer-associated fibroblast) und Endothelzellen (51). Sowohl der Tumor selbst als auch CAFs und Endothelzellen beeinflussen das TME durch die Sekretion vasoaktiver und tolerogener Mediatoren wie VEGF und Interleukin (IL)-10, IL-8 und IL-1β (49). Kürzlich wurde außerdem nachgewiesen, dass CAFs speziell im iCCA eine erhöhte Expression angiogenese-fördernder Gene aufweisen (52).

Das TME im HCC ist durch die Tumorbiologie einerseits, und durch die fast immer zugrundeliegende Lebererkrankung andererseits, mit chronisch-inflammatorischen und Prozessen der Neoangiogenese geprägt (53). In diesem Microenvironment können sogar Zellpopulationen, die regulär an zytotoxischen und immunogenen Reaktionen beteiligt sind, durch die Induktion tolerogener T-Zellpopulationen, immunsuppressiv und tumorfördernd wirken, beispielsweise indem infiltrierende Neutrophile oder Monozyten eine Polarisierung hin zu myeloiden Supressor-Zellen entwickeln (myeloid-derived suppressor cell, MDSC), welche Immunantworten, die gegen den Tumor gerichtet sind, verhindern (54). Typisch für das HCC ist eine massive arterielle Neovaskularisation mit einer Überexpression von Angiogenese-Genen wie HIF1α und VEGF, induziert nicht nur über den kanonischen Pathway der Hypoxie, sondern durch pro-inflammatorische Zytokine infiltrierender Immunzellen, Onkogene und Wachstumsfaktoren, was unabhängig vom tatsächlichen lokalen Sauerstoffpartialdruck zu einem Überlebensvorteil der malignen Zellen führt (55). Die aktuelle Leitlinientherapie für das fortgeschrittene und metastasierte HCC – eine Kombination aus Bevacizumab (anti-VEGF) und Atezolizumab (anti-Programmed Death-Ligand 1, PD-L1) unterstreicht die zentrale pathophysiologische Rolle des TME und der Neoangiogenese in dieser Tumorentität (56).

1.4 Body Composition/ Körperzusammenzetzung

1.4.1 Definition und Diagnose von Pathologien der Körperzusammensetzung

In dieser Arbeit soll neben molekularen Tumor-Wirt Wechselwirkungen auch auf bereits makroskopisch erkennbare Wirtsfaktoren eingegangen werden. Body composition, übersetzt, Körperzusammensetzung, beschreibt die absolute und relative Menge und Verteilung von Muskel, Fett, Wasser, Protein und Mineralien im Körper. Im Folgenden wird mit dem Begriff Körperzusammensetzung ausschließlich auf die Muskel-und Fettaspekte dieser breiteren Definition eingegangen, da die Qualität und Quantität von Muskelgewebe und die Quantität und Verteilung von Fettgewebe bei Patient*innen mit fortgeschrittener

Lebererkrankung oder Tumorerkrankungen hochvariabel und oft prognostisch relevant sind (57, 58). Obwohl der Body Mass Index (BMI) immer noch die meistverwendete anthropometrische Einheit im klinischen Alltag ist, schätzt dieser nur unzureichend das Verhältnis der Muskel-zu Fettmasse und die metabolische Gesundheit ein (59).

Zur detaillierten Analyse der Körperzusammensetzung sind verschiedene diagnostische Mittel geeignet. Die Duale-Energie X-ray Absorptiometrie gilt als der Goldstandard zur schnellen Untersuchung von Fettmasse, fett-freier Masse und Knochendichte, und hat eine hohe Ergebniskonkordanz mit der Bioelectrical Impedance Analysis (BIA)-Messung, die als klinisch praktischere Methode breiter implementiert ist (60). Ergänzend kann zur Evaluation der Muskelkraft die Handgrip Strength bestimmt werden, also die Kraft der Unterarmmuskulatur, die hochgradig mit onkologischer und geriatrischer Morbidität und Mortalität korreliert (61, 62). Die Evaluation der Körperzusammensetzung anhand von Computertomographie (CT)- oder Magnetresonanztomographie-Schnittbildgebung hat den Vorteil, dass sie auch im Setting retrospektiver Studien realisierbar ist und erlaubt die quantitative Analyse des Muskel- und Fettkompartiments (Abbildung 4), wobei der Verfettungsgrad der Muskulatur ebenfalls erhoben wird (63, 64).

Sarkopenie ist die am besten charakterisierte Veränderung der Körperzusammensetzung und ist definiert als niedrige Skelettmuskelmasse mit eingeschränkter Kraft. Sarkopenie als Überbegriff für geringe Muskelmasse und -Qualität geht eng einher mit klinischer Gebrechlichkeit und herabgesetzter körperlicher Leistungsfähigkeit (65). Die zunehmende Anerkennung der klinischen und prognostischen Relevanz dieses Krankheitsbildes ist durch die Einführung eines ICD-10 Codes für Sarkopenie 2016 abgebildet (66). Im Kontext konsumierender Tumorerkrankungen, wie gastrointestinalen Tumoren, bestehen große Überlappungen zwischen Sarkopenie und Kachexie, wobei die letztere definiert ist als schwerer, unfreiwilliger Verlust von überwiegend Muskelmasse aufgrund einer systemischen Entzündungsreaktion und metabolischer Dysregulation (67).

Eine pathologische Einlagerung von Fettzellen im Muskelgewebe wird als Myosteatose bezeichnet, wobei eine klare Assoziation dieser Kondition mit metabolischer Dysregulation besteht (68). Ein weiterer Aspekt der Fettverteilung ist die Unterscheidung zwischen subkutanem und intraabdominellen/viszeralem Fett. Hierbei ist das Letztere besonders mit Insulinresistenz und anderen kardiometabolischen Risikofaktoren assoziiert (69). Eine Sonderform der Obesität ist die Kombination von Übergewicht mit einer reduzierten Muskelmasse, die sogenannte sarkopene Obesität, die neben den konventionellen kardiometabolischen Risiken des Übergewichtes zusätzlich mit einer systemischen pro-inflammatorischen Deregulation einhergeht (70).

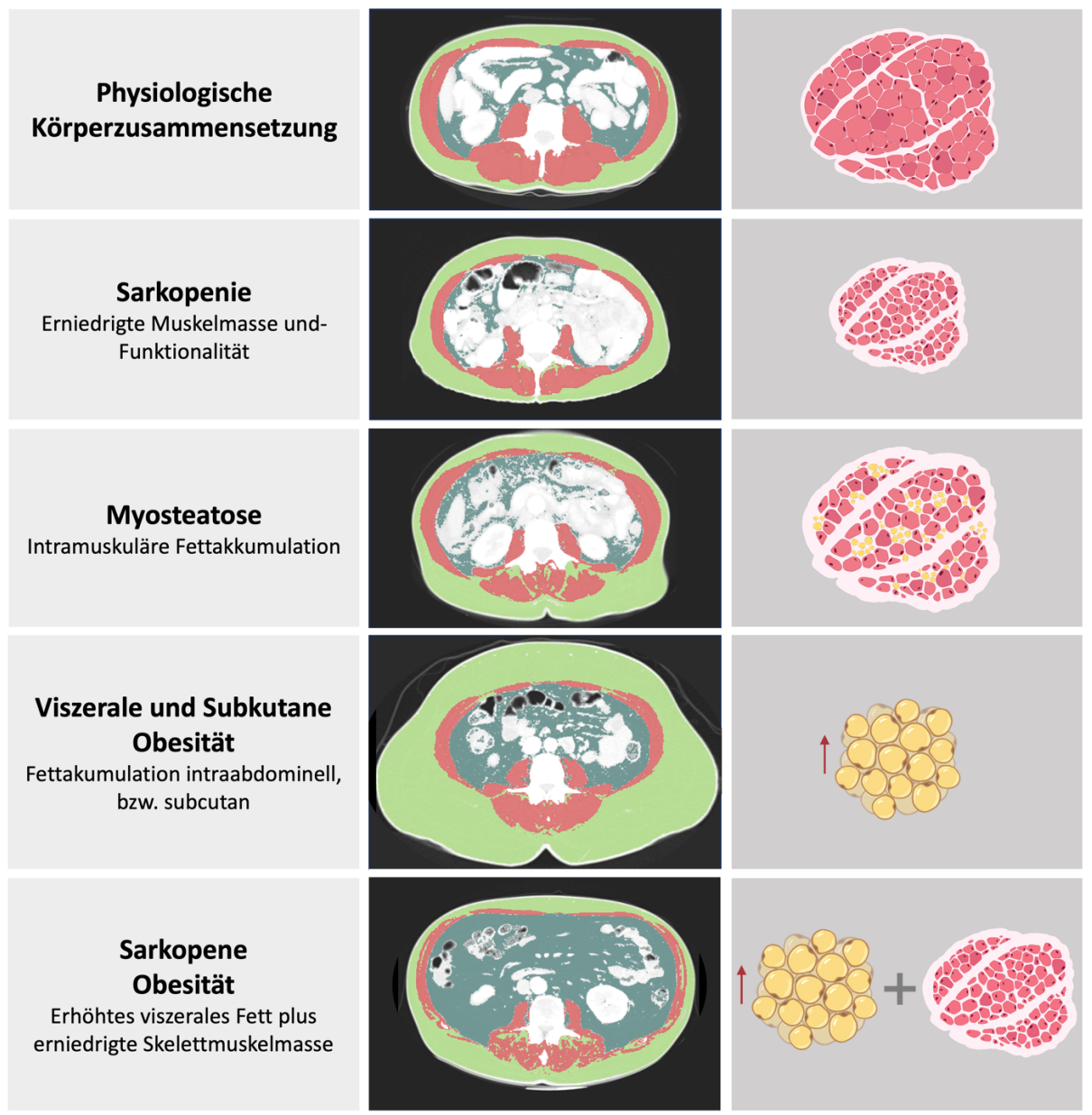


Abbildung 4: Übersicht der häufigsten Body Composition-Veränderungen.
Repräsentative Darstellung der häufigsten Body Composition-Pathologien anhand von CT-Bildern (mittlere Spalte), die auf Höhe des Wirbelkörpers L3 segmentiert wurden. Skelettmuskel ist rot, subkutanes Fett hellgrün, intraabdominelles Fett dunkelgrün visualisiert. In der rechten Spalte sind die Veränderungen der Fett Quantität und Muskel Quantität und Qualität schematisch dargestellt.
Abbildung erstellt mittels 3d Slicer mit body composition module Version 4.1, https://www.slicer.org/ (71) und mittels Biorender.com.

1.4.2 Körperzusammensetzung in chronischen Lebererkrankungen und primären Lebertumoren

Die Sarkopenie gilt aktuell als der am besten charakterisierte prognostische Faktor im Bereich der Lebererkrankungen, da er für Patient*innen mit Leberzirrhose zugleich eine kritische systemische Folge der Grunderkrankung darstellt (72). Patient*innen mit

fortgeschrittener Lebererkrankung weisen schwerwiegende Defizite im Aminosäuren- und Proteinstoffwechsel auf, einhergehend mit einem Verlust von Muskelmasse und -Funktion (73). Mittlerweile wurde die Sarkopenie-Evaluation in aktuelle Leitlinien für chronische Lebererkrankungen inkorporiert (72).

Während die Assoziation der Leberzirrhose mit dysfunktionalem Proteinstoffwechsel und dem drastischen Verlust von Muskelmasse früh beschrieben und als behandlungsbedürftig erkannt wurde, soll im Folgenden ebenfalls eine Perspektive über die Body Composition-Veränderungen im Kontext der MASLD aufgezeigt werden. Hier ist eine metabolische Dysfunktion mit Lipidüberladung ein wichtiger Mechanismus eingeschränkter Leberfunktion und der chronischen Lebererkrankung (74). Eine progrediente Dysfunktionalität des weißen Fettgewebes, mit mangelnder Fettspeicherung und eingeschränkter metabolischer Flexibilität führt auch in Abwesenheit von viralen, alkoholischen oder toxischen Stimuli zur hepatischen und systemischen Inflammation und hepatischem Schaden, mit dem klinischen Krankheitsbild der MASLD (75).

Dem Muskelgewebe kommt im Rahmen des Glukosestoffwechsels eine zentrale Rolle als anteilig größter Verbraucher von Glukose zu, die beim metabolischen Syndrom vor allem durch eine Insulinresistenz mit gestörter Glukoseaufnahme gekennzeichnet ist (76). Patient*innen mit Typ 2 Diabetes haben neben einer verringerten Glukoseaufnahme zusätzlich selbst bei zunächst erhaltener absoluter Muskelmasse eine Umstrukturierung mit einer Dominanz glykolytischer Typ 2 Muskelfasern und einem Verlust oxidativer Typ 1 Fasern, welche physiologisch die höchste Glukoseverwertung zeigen (77). Folgerichtig konnte für sarkopene Individuen ein erhöhtes Risiko für MASLD und für eine Progression der Fettlebererkrankung im Vergleich zu Personen mit normaler Muskelmasse ermittelt werden (78). Interessanterweise bestand gleichzeitig in einer UK-Biobank-weiten Studie eine erniedrigte Sarkopenie-Prävalenz bei MASLD im Vergleich zu gesunden Kontrollen (79).

Über den Stellenwert einer pathologischen intramuskulären Fettakkumulation, der Myosteatose, ist bekannt, dass sie ebenfalls zu einer abnehmenden metabolischen und kinetischen Funktionalität der Muskulatur führt (80). Das Vorhandensein von Myosteatose im Kontext von Lebererkrankungen ist kürzlich als negativer prognostischer Faktor identifiziert worden, da sie mit einer höheren Rate an Komplikationen und einem verkürzten Gesamtüberleben nach Lebertransplantation assoziiert ist (81, 82). In der MASLD ist die Myosteatose hochprävalent und korreliert hochgradig mit erhöhter kardiovaskulärer und metabolischer Komorbidität (79).

1.5 Zielsetzung der Arbeit

Ziel dieser Arbeit ist es, prognostische Wirtsfaktoren im kurativ behandelten HCC und CCA zu charakterisieren. Hierfür wurden (1.), Keimbahnmutationen in Genen, die im TME involviert sind, und (2), die präoperative Körperzusammensetzung im Zusammenhang mit Krankheitsätiologie, Krankheitsfreiem- und Gesamtüberleben untersucht. Diese Faktoren können präoperativ aus routinemäßig erhobenen Parametern, wie Blut und Schnittbildgebung abgeleitet werden, und Beiträge zur klinischen Entscheidungsfindung leisten.

(1.) Wir hypothetisierten, dass funktionelle Genpolymorphismen, die entweder eine veränderte Genexpression oder ein verändertes Produkt auf Proteinebene verursachen, prognostisch relevant sein können, wenn sie in Genen auftreten, die für essenzielle Mediatoren im TME kodieren.

(2.) Wir untersuchten die Körperzusammensetzung von Patient*innen mit CCA und charakterisierten ihren Zusammenhang mit Krankheitsätiologie und Prognose.

Zusammenfassend untersucht diese kumulative Habilitationsschrift, ob neben den klinisch etablierten Tumor-zentrierten prognostischen und prädiktiven Faktoren wie Lymphknoteninvasion und Mutationsstatus, auch genetische und physische Wirtsaspekte die Prognose nach der Resektion primärer maligner Lebertumoren beeinflussen (Abbildung 5).

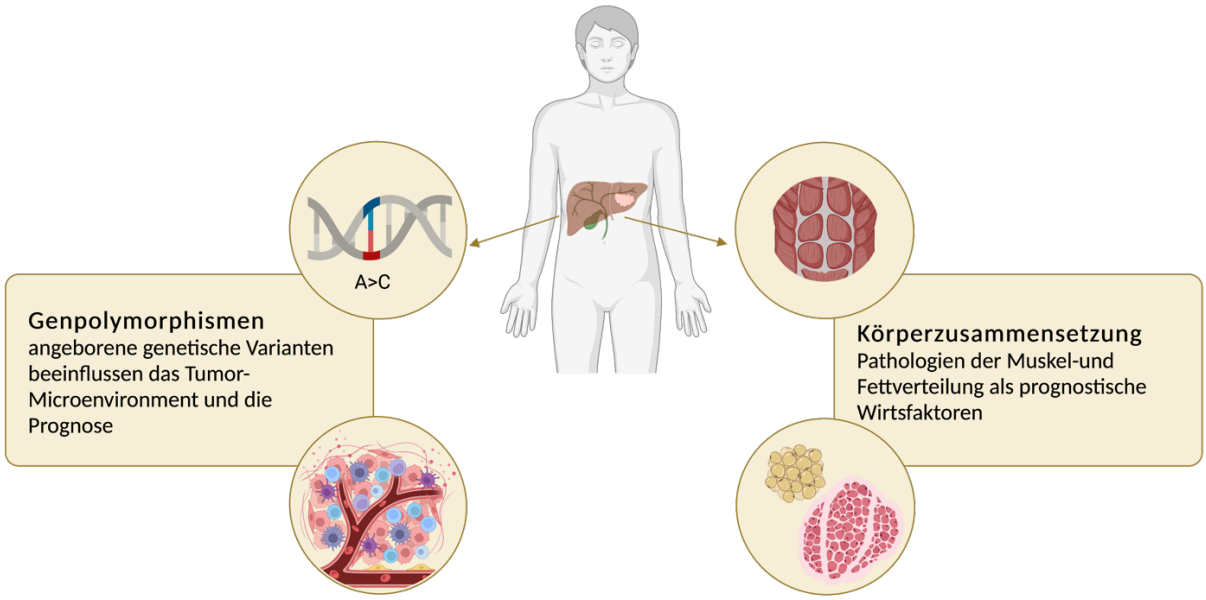


Abbildung 5: untersuchte Wirtsfaktoren als prognostische Determinanten in primären Lebertumoren
Genpolymorphismen und Body Composition als angeborene bzw. akquirierte prognostische Faktoren. Genpolymorphismen als Keimbahn-Varianten haben potenziell in jeder Körperzelle einen Einfluss und tragen so insbesondere zu Prozessen wie immunologischen Antworten und Neovaskularisation bei. Körperzusammensetzung als genetisch beeinflusster, aber überwiegend akquirierter Wirtsfaktor wurde in dieser Arbeit im Hinblick auf Muskelqualität und Quantität, sowie Fettquantität und -Verteilung analysiert. *Abbildung erstellt mittels Biorender.com.*

2. Ergebnisse eigener Arbeiten

2.1 Der IL-8 Rezeptor Genpolymorphismus rs2234671 ist im perihilären Cholangiokarzinom mir rekurrenzfreiem-, krebsspezifischen und Gesamtüberleben assoziiert

2.2 Genetische Varianten des Interleukin-1β (rs1143634) und Interleukin-8 (rs4073) haben nach Resektion des iCCA prognostischen Wert

2.3 Angiogenese-und Hypoxie-assoziierte Genpolymorphismen beeinflussen die Prognose nach Resektion des HCC

2.4 Body Composition als prognostischer Faktor im iCCA und pCCA

2.5 Die metabolisch assoziierte Fettlebererkrankung (MASLD) ist im intrahepatischen Cholangiokarzinom mit pathologischer Körperzusammensetzung assoziiert.

2.1 Der IL-8 Rezeptor Genpolymorphismus rs2234671 ist im perihilären Cholangiokarzinom mit rekurrenzfreiem-, krebsspezifischen und Gesamtüberleben assoziiert

***Lurje I**, Czigany Z, Bednarsch J, Gaisa NT, Dahl E, Knüchel R, Miller H, Ulmer TF, Strnad P, Trautwein C, Tacke F, Neumann UP, Lurje G.*
Genetic Variant of CXCR1 (rs2234671) Associates with Clinical Outcome in Perihilar Cholangiocarcinoma. Liver Cancer. 2022 Jan 25;11(2):162-173.

Abstract Zitat (83):

„Background: Perihilar cholangiocarcinoma (pCCA) is a rare primary liver malignancy. Even in patients amenable to surgery, outcomes are often dismal. Here, we aimed to identify prognostic markers for patient outcomes by analyzing functionally relevant single-nucleotide polymorphisms (SNPs) in genes with a role in tumor inflammation and angiogenesis. We analyzed 11 polymorphisms in the inflammation-angiogenesis axis (*VEGF, EGF, EGFR, IL-1b, IL-6, CXCL8 (IL-8), IL-10, CXCR1, HIF1A*, and *COX2* genes) for their prediction of tumor recurrence and survival in pCCA patients undergoing surgery in a curative intent.
Methods: Samples were obtained from 111 patients with pCCA undergoing liver resection in curative intent. DNA was extracted and analyzed using polymerase chain reaction-restriction fragment length polymorphism protocols and correlated with patients' outcomes.
Results: Out of the assessed variants, only the *CXCR1* (also: interleukin-8-receptor alpha - *IL-8RA*) +860C>G heterozygous polymorphism (rs2234671) was associated with decreased disease-free survival (DFS), cancer-specific survival (CSS), and overall survival (OS) (18/111 (16.2%), median DFS 14 months, log-rank p = 0.007; median CSS 31 months, log-rank p = 0.007; and median OS 6 months, log-rank p = 0.002), compared to the GG genotype (92/111 (82.9%), median DFS 55 months, median CSS 63 months, and median OS 33 months). In the multivariate analysis, +860C>G remained an independent prognostic factor for DFS (adjusted p = 0.008), CSS (adjusted p = 0.001), and OS (adjusted p = 0.001).
Conclusion: Genetic variant of *CXCR1* +860C>G may serve as a molecular marker for DFS, CSS, and OS in patients undergoing curative-intent surgery for pCCA, indicating that the analysis of SNPs in genes involved in immune-mediated angiogenesis may help to identify patient subgroups at high risk for dismal oncological and overall outcome.“

In dieser Arbeit untersuchten wir SNPs als hochprävalente Keimbahnvarianten auf ihren prognostischen Wert im pCCA. Genetische Varianten, die eine veränderte Genexpression oder ein verändertes Genprodukt im Bereich der Tumorimmunologie und Neovaskularisation bewirken waren von besonderem Interesse, da in diesen Bereichen

regulär eine Tumor-Wirt Wechselwirkung stattfindet. Ein Panel von 11 SNPs in 10 Genen wurde anhand ihrer relativen Häufigkeit, ihrer dokumentierten Schlüsselfunktion in Neoangiogenese- und inflammatorischen Pathways, sowie anhand der biologischen Relevanz des Polymorphismus ausgewählt. Die Genotypisierung wurde in einer Kohorte von über 100 Patient*innen mit pCCA, die sich einer Leberteilresektion in kurativer Intention an der Universitätsklinik RWTH Aachen unterzogen haben, durchgeführt. Hervorzuheben gilt hier, dass zur Erreichung eines möglichst homogenen Kollektivs, Patient*innen mit intrahepatischen oder distalen Tumoren aufgrund der verschiedenen Tumorbiologie und Resektionsstrategie, sowie Patient*innen mit Neuroendokrinen oder HCC-CCA Mischtumoren, sowie Patient*innen mit neoadjuvanter Behandlung ausgeschlossen wurden.

Das entscheidende Ergebnis der Studie war, dass der G+860C SNP im C-X-C Motif Chemokine Receptor (*CXCR)1*-Gen, hochsignifikant und statistisch unabhängig mit krankheitsfreiem, krebsspezifischem und Gesamtüberleben assoziiert war. Patient*innen mit homozygotem C/C (Wildtyp) hatten zwar eine ähnliche Gesamtrate an Rekurrenz wie jene mit einem G Allel, das mediane Überleben war bei ihnen aber etwa fünfmal so lang (33 Monate C/C vs. 6 Monate G/C).

CXCR1 ist der Rezeptor für IL-8, welches im TME des CCA eine zentrale Rolle als Mediator von Neoangiogenese und Rekrutierer und Polarisator immunsuppressiver MDSCs einnimmt. Der Rezeptor CXCR1 ist exprimiert auf Monozyten und Neutrophilen, wo er die Rekrutierung dieser Leukozyten und ihre Polarisation zu einem immunsuppressiven Phänotyp bewirkt (84). Des Weiteren ist IL-8 ein wichtiges Überlebenssignal für maligne Zellen, welches von ihnen selbst und von CAFs abgegeben wird (85). Der untersuchte *CXCR1* G+860C SNP führt bei gleicher quantitativen Genexpression hochwahrscheinlich zu einer Veränderung der Signaltransduktion. Er führt zu einer Missense-Variante, durch Austausch der Serin Aminosäure zu Threonin in der extrazellulären Rezeptordomäne in Position 276, deren benachbarte Aminosäure (277) eine Disulfidbrücke zu der Cystein-Seitenkette von Position 30 ausbildet (86, 87). Während die exakte Veränderung der Tertiärstruktur durch den Polymorphismus unbekannt ist, ist es naheliegend, dass dies der Grund für eine veränderte biologische Funktion ist (83, 88).

Zusammenfassend war diese Studie die erste Dokumentation eines prognostisch relevanten Genpolymorphismus im CCA. Unsere Daten stärken die Theorie, dass erstens, IL-8 und CXCR1-signalling wichtige Elemente des TME von pCCAs sind und zweitens, die Analyse von Genpolymorphismen iCCA-Patient*innen mit erhöhtem Risiko für schlechtes onkologisches und allgemeines Outcome Identifizieren kann.

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Genetic Variant of CXCR1 (rs2234671) Associates with Clinical Outcome in Perihilar Cholangiocarcinoma

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Keywords
Perihilar cholangiocarcinoma · Single-nucleotide polymorphism · IL-8 · CXCR1

Abstract

Background: Perihilar cholangiocarcinoma (pCCA) is a rare primary liver malignancy. Even in patients amenable to surgery, outcomes are often dismal. Here, we aimed to identify prognostic markers for patient outcomes by analyzing functionally relevant single-nucleotide polymorphisms (SNPs) in genes with a role in tumor inflammation and angiogenesis. We analyzed 11 polymorphisms in the inflammation-angiogenesis axis (*VEGF, EGF, EGFR, IL-1b, IL-6, CXCL8 (IL-8), IL-10, CXCR1, HIF1A*, and *COX2* genes) for their prediction of tumor recurrence and survival in pCCA patients undergoing surgery in a curative intent. ***Methods:*** Samples were obtained from 111 patients with pCCA undergoing liver resection in curative intent. DNA was extracted and analyzed using polymerase chain reaction-restriction fragment length polymorphism protocols and correlated with patients' outcomes. ***Results:*** Out of the assessed variants, only the *CXCR1* (also: interleukin-8-receptor alpha – *IL-8RA*) +860C>G heterozygous polymorphism (rs2234671) was associated with decreased disease-free survival (DFS), cancer-specific survival (CSS), and overall survival (OS) (18/111 (16.2%), median DFS 14 months, log-rank $p = 0.007$; median CSS 31 months, log-rank $p = 0.007$; and median OS 6 months, log-rank $p = 0.002$), compared to the GG genotype (92/111 (82.9%), median DFS 55 months, median CSS 63 months, and median OS 33 months). In the multivariate analysis, +860C>G remained an independent prognostic factor for DFS (adjusted $p = 0.008$), CSS (adjusted $p = 0.001$), and OS (adjusted $p = 0.001$). ***Conclusion:*** Genetic variant of *CXCR1* +860C>G may serve as a molecular marker for DFS, CSS, and OS in patients undergoing curative-intent surgery for pCCA, indicating that the analysis of SNPs in genes involved in immune-mediated angiogenesis may help to identify patient subgroups at high risk for dismal oncological and overall outcome.

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Introduction

Cholangiocarcinoma (CCA) is the second most common primary liver cancer. A rising mortality from CCA has been reported worldwide during the last decade [1], despite the improvement of surgical and palliative treatment [2, 3]. More than 50% of CCAs originate from the proximal extrahepatic bile ducts, are termed perihilar CCA (pCCA), and constitute the most common CCA entity in the Western world [4]. Many pCCA cases are sporadic, arising in the absence of the known risk factors such as chronic biliary inflammation, cholestasis, hepatobiliary parasitic infections, and liver cirrhosis [1]. Clinicopathological characteristics such as lymph node status and poor differentiation remain the best-studied, but imperfect prognostic, factors [5, 6]. Therefore, the identification of preoperatively available molecular markers of prognosis as an adjunct to traditional staging systems may facilitate selection of patients who may benefit from surgical and adjuvant treatment strategies.

Angiogenesis – the induction of tumor neovascularization – is a prerequisite for tumor growth and survival. Over 50 years ago, Judah Folkman pioneered the hypothesis that vascular endothelial growth factor (VEGF) is a central driver of angiogenesis in solid malignancies, and since then, the predominant role of VEGF for tumor angiogenesis has become apparent [7]. More recently, it has been shown that the early angiogenic response to VEGF is also dependent on the interplay with immune cells that confer paracrine proliferative effects on the endothelium. As such, secreted VEGF attracts circulating monocytes and stimulates angiogenesis by forming SDF-1 traps [8]. In this regard, several cell populations in the tumor microenvironment (TME), such as myeloid-derived suppressor cells, tumor-associated macrophages, and cancer-associated fibroblasts (CAFs), support tumor growth, angiogenesis, and immune escape [9]. It has been proposed that the latter may play a crucial role in the regulation of inflammation and early-onset angiogenesis and in turn may impact the process of tumor growth and disease progression [10–12]. In addition, IL-8 has been reported to play a major role in VEGF-independent tumor angiogenesis in gastric and colon cancer [13, 14]. In particular, an induction of IL-8 preserved the angiogenic phenotype in HIF1-α-deficient colon cancer cells [15]. The G-protein-coupled receptor for IL-8, CXCR1, is expressed on granulocytes, monocytes, mast cells, and some natural killer cells [16] and is often overexpressed in malignant cells and their TME, where it confers proangiogenic and immunosuppressive effects [17, 18]. We hypothesized that functional *VEGF, EGF, EGFR, IL-1b, IL-6, IL-8, IL-10, CXCR1* (*IL-8RA*), *HIF1a*, and *COX2* polymorphisms could be associated with differences in clinical outcome in a large Western cohort of pCCA treated with curative-intent surgery.

Materials and Methods

Study Population and Pathology

A total of 113 unrelated consecutive patients with localized pCCA without signs of systemic disease underwent curative-intent surgery at the University Hospital RWTH Aachen between October 2010 and September 2019. Patients undergoing associating liver partition and portal vein ligation for staged hepatectomy ($n = 2$) were excluded from the analysis because associating liver partition and portal vein ligation for staged hepatectomy is not considered the mainstay of treatment in CCA, but rather a rescue operation in a small subset of patients with insufficient liver regeneration, where it is associated with dismal prognosis [3, 19]. Clinicopathological and survival data were obtained from a prospectively managed institutional database and a senior hepatobiliary pathologist (NTG) reviewed the tumor histology to confirm appropriate patient selection. The Institutional Biobank (RWTH-cBMB) and the Department of Pathology provided patient material for genotyping (NTG, ED, and RKC). This study was conducted after approval by the Institutional Review Board of the RWTH Aachen University (EK 360/15 and EK 173/06) and in accordance with good clinical practice guidelines and the current Declaration of Helsinki.

Staging and Surgical Technique

All patients who were referred for surgical treatment of pCCA to our institution underwent a detailed clinical workup. None of the patients underwent neoadjuvant chemotherapy. Intrahepatic and distal CCA were not included in this analysis due to the markedly different tumor biology and surgical treatment. The preoperative workup included appropriate cross-sectional imaging to rule out the presence of distant metastases and CT or dynamic magnetic resonance imaging of the liver to visualize the invasion of major vessels in the liver hilum and an endoscopic retrograde cholangiopancreatography or magnetic resonance cholangiopancreatography to assess the extent of disease in the liver hilum. In selected cases, patients with suspected extrahepatic disease on conventional cross-sectional imaging underwent positron emission tomography-CT. Patients with extrahepatic disease were not included in this study. The surgical procedure for pCCA was carried out as previously described by Neuhaus et al. [20, 21]. Briefly, a "no-touch" hilar *en bloc* resection approach, as defined by extended liver resection with portal vein resection and reconstruction, was carried out in all cases [2, 6]. Lymphadenectomy of the pericholedochal, periportal, common hepatic, posterior pancreaticoduodenal, and the celiac lymph nodes was routinely performed. All surgical specimens underwent routine histopathological workup according to current national guidelines, WHO- and UICC classifications. Tumor type, histopathological grading, and staging, loco-regional lymph node metastasis, resection margins, and vessel invasion were evaluated by an experienced board-certified staff pathologist.

Table 1. Primer sequences, restriction enzymes, and reaction conditions

Gene (allele, SNP)	Forward primer sequence	Reverse primer sequence	Annealing temp. (°C)	Restriction enzyme
VEGF+936 (C>T, rs3025039)	AGA CTC CGG CGG AAG CAT	TGT ATG TGG GTG GGT GTG TC	60	NlaIII
EGF+61 (A>G, rs4444903)	CAT TTG CAA ACA GAG GCT CA	TGT GAC AGA GCA AGG CAA AG	60	AluI
EGFR-1562 (G>A, rs2227983)	TGC TGT GAC CCA CTC TGT CT	CCA GAA GGT TGC ACT TGT CC	59	BstNI
IL-1b + 3954 (C>T, rs1143634)	GTT GTC ATC CAG ACT TTG ACC	TTC AGT TCA TAT GGA CCA GA	58	TaqaI
IL-6-174 (G>C, rs1800795)	GCC TCA ATG ACG ACC TAA GC	TCA TGG GAA AAT CCC ACA TT	55	NlaIII
IL-8-251 (T>A, rs4073)	TGC CAT TAA AAG AAA ATC ATC CA	CAT TTA AAA TAC TGA AGC TCC ACA	56	MfeI
IL-10-592 (T>G, rs1800872)	GAG CAC TAC CTG ACT AGC ATA TAA G	GTG GGC TAA ATA TCC TCA AAG T	60	RsaI
CXCR1 +860 (Ex2), (C>G, rs2234671)	CTC ATG AGG ACC CAG GTG AT	GGT TGA GGC AGC TAT GGA GA	60	AluI
HIF1a-1772 (C>T, rs11549465)	CCC AAT GGA TGA TGA CTT CC	AGT GGT GGC ATT AGC AGT AGG	59	Tsp-45 I
COX2 +8473 (A>G, rs5275)	GTT TGA AAT TTT AAA GTA CTT TTG AT	TTT CAA ATT ATT GTT TCA TTG C	53	BclI
COX2 −765 (G>C, rs20417)	ATT CTG GCC ATC GCC GCT TC	CTC CTT GTT TCT TGG AAA GAG ACG	55	AciI

COX, cyclooxygenase; CXCR, chemokine receptor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HIFa, hypoxia-inducing factor alpha; IL, interleukin; SNP, single-nucleotide polymorphism; VEGF, vascular endothelial growth factor.

Single-Nucleotide Polymorphism Selection

The polymorphisms we tested were selected by a pathway approach with the goal of selecting genes known to modulate inflammation and tumor-associated angiogenesis (Table 1). We used the following criteria to select genes for study: (a) that the gene is a part of a pathway for which there is evidence to support its involvement in tumor angiogenesis; (b) that the gene has a well-documented genetic polymorphism with an expected biological relevance; and (c) that the frequency of the polymorphism is high enough to enable a statistically meaningful association with clinical outcome. A total of 11 single-nucleotide polymorphisms (SNPs) in 10 genes were selected, including *VEGF, EGF, EGFR, IL-1B, IL-6, CXCL8 (IL-8), IL-10, CXCR1* (*IL-8RA*), *HIF1A*, and *COX2* (Table 1).

Genotyping

Formalin-fixed paraffin-embedded nontumor tissues were collected and genomic DNA was extracted using the QIAamp DNA extraction kit (Qiagen, CA, Valencia, USA) according to the manufacturer's protocol. Photometric analysis of DNA quality and content was conducted (NanoDrop, Thermo Fisher, Waltham, MA, USA). Polymerase chain reaction-restriction fragment length polymorphism technique was used for genotyping, as previously described [22, 23]. Briefly, forward- and reverse primers flanking the SNP region were used for amplification and the amplicon was then digested with appropriate DNA restriction endonucleases (New England Biolabs, Ipswich, MA, USA) (Table 1). The reaction products were separated using agarose gel electrophoresis with a 4% agarose gel at 120 mV for 60 min and visualized (GelDoc, Bio-Rad Laboratories GmbH, Feldkirchen, Germany). For quality assurance purposes, a total of 10% positive and negative samples were randomly selected for each polymorphism and genotyped with genotype concordance ≥99%.

Endpoints and Statistical Analysis

Patient outcome was assessed with the endpoints disease-free survival (DFS), defined as the period between surgery and first recurrence; cancer-specific survival (CSS), defined as the period between surgery and death in those patients that recurred while patients with other causes of death were censored at the time of death; and overall survival (OS), defined as the period between liver resection and death without censoring for perioperative mortality. For DFS, patients were censored if they died without recurring, whereas patients lost to follow-up were censored at the time of last contact. Differences were tested using a two-tailed Fisher's exact test and a χ^2 test for categorical variables and the Mann-Whitney U test for continuous variables as well as the Kruskal-Wallis test for nonparametric samples with more than 2 groups. Differences in DFS and OS between genotypes were assessed with Kaplan-Meier survival curves and the log-rank test. For SNPs with a frequency of the homozygous minor allele of <10% in the study population, associations between genotypes and clinical outcome were analyzed in a dominant model. Otherwise, a codominant or additive model was employed. Associations of factors with DFS, CSS, and OS were analyzed with univariable and multivariable Cox proportional hazard models, with hazard ratios presented with 95% confidence intervals (CI). Significant variables in the univariable analyses were included in the multivariable analyses. The level of significance was set to $p < 0.05$. Analyses were performed with SPSS Statistics (v23, IBM Corp., Armonk, NY, USA).

Results

A total of 111 patients with localized pCCA were included in this study. The cohort comprised 75 (68%) men and 38 (32%) women with a median age of 66 years at the time of surgery. Six (6/111, 5%) of pCCAs were classified as Bismuth I, 8 (7%) as Bismuth II, 30 as Bismuth IIIa (27%), 32 as Bismuth IIIb (29%), and 35 as Bismuth IV (32%). Thirty-six patients had preoperative cholangitis (36/111, 32%). Due to cholestasis, 88 (80%) patients re-

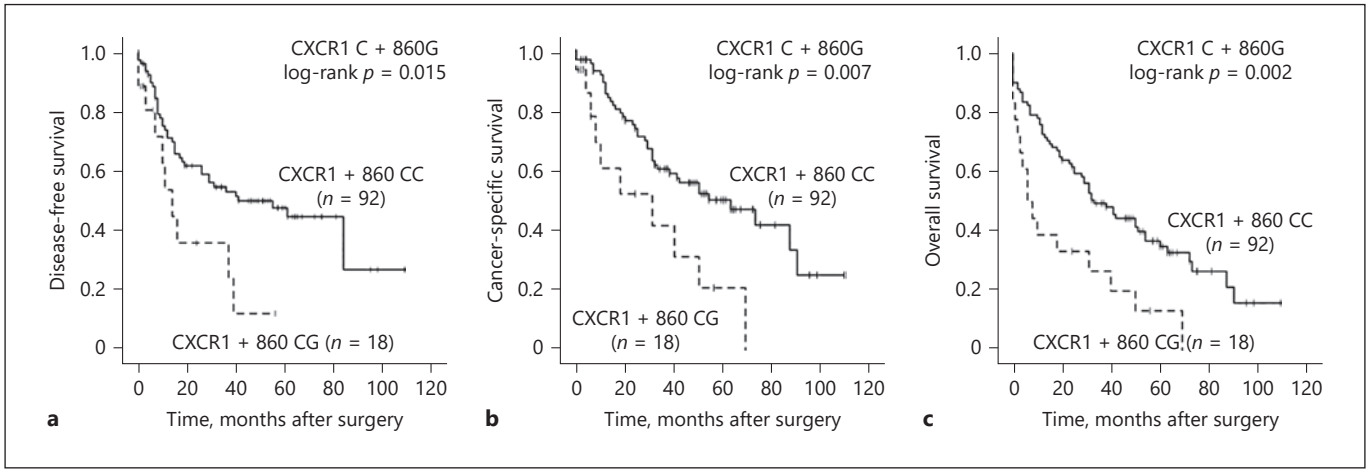

Fig. 1. DFS (**a**), CSS (**b**), and OS of patients (**c**) by CXCR1 polymorphism.

ceived preoperative endoscopic biliary drainage and/or percutaneous biliary drainage (26/111, 23%). Portal vein embolization was performed in 47 (42%) patients. No patient received neoadjuvant chemotherapy. All patients received systematic lymphadenectomy (111/111, 100%) and most patients (103/111, 93%) underwent vascular resection with reconstruction. One patient (1/111, 1%) received an anatomical resection, 9 (8%) and 13 (12%) underwent right and left hepatectomy, respectively, while 20 (18%) and 30 (27%) underwent extended right and left hepatectomy, respectively. Twenty-three (23/111, 21%) patients were treated with right trisectionectomy, 6 patients (5%) with left trisectionectomy, and 9 patients (8%) with hepatoduodenectomy (extended patient data in online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000521613). Median follow-up was 32 months. DFS was 37 months, and 51 patients (46%) recurred in the observation time. Median CSS was 50 months, with 50 patients (45%) recurring prior to death. Median OS was 31 months, with 78 patients (70%) deceased during the observation period.

CXCR1 G+860C and Outcome Parameter

Of the 11 tested polymorphisms, only *CXCR1* G+860C correlated significantly with outcome parameters. Genotyping for *CXCR1* G+860C was successful in 99.0% of cases (110/111). Ninety-two patients (83%, 92/111) of were homozygous for the *CXCR1* +860 C allele, 16% (18/111) heterozygous (C/G), and no patient was homozygous for the *CXCR1* +860 G allele. When analyzing the association of the *CXCR1* +860 polymorphism with postoperative complications, patients with the C/G genotype had a significantly higher rate of postoperative 90-day mortality (6/18, 33.3%) than patients with the wild-type alleles (12/92, 13.0%, $p = 0.37$). No significant difference regarding 90-day Clavien-Dindo ≥ CD3b complications, intensive care, and hospital stay, and 90-day comprehensive complication index (CCI) was noted (online suppl. Table 2). The G+860C genotype was equally distributed between Bismuth stages (online suppl. Table 3, $p = 0.828$).

The *CXCR1* +860 polymorphism was significantly associated with all three outcome parameters. Patients homozygous for the *CXCR1* +860 C allele (wild type) had a median DFS of 55 months (95% CI: 21.6–88.4), while patients with the G/C genotype had a median DFS of 14 months (95% CI: 7.7–20.3, log-rank $p = 0.015$, Fig. 1a). Forty-five percentage (41/92) of patients homozygous for the C allele and 55% (10/18) of heterozygous patients (C/G) recurred during the observation time. The *CXCR1* +860 polymorphism showed a significant association with CSS. Patients with the C/C homozygous genotype had a median CSS of 63 months (95% CI: 35.3–90.7), while patients with a C/G genotype had a median CSS of 31 months (95% CI: 0.0–62.8, log-rank $p = 0.007$, Fig. 1b). Forty-three percentage (40/92) of patients homozygous for the *CXCR1* +860C allele and 55% (10/18) of patients with a heterozygous (C/G) genotype died from their oncological disease. Median OS for patients homozygous for the *CXCR1* +860C allele was 33 months (95% CI: 21.7–44.3) and for patients with the *CXCR1* +860C/G genotype 6 months (95% CI: 0.5–11.5, log-rank $p = 0.002$, Fig. 1c). Sixty-six percentage (61/92) of patients with the CC genotype and 89% (16/18) of patients with the GC genotype deceased during the observation period (Table 2).

Table 2. Frequencies of SNPs and their association with OS, CSS, and DFS

Gene, SNP	*N**, (%)	DFS			CSS			OS		
		median DFS, *m* (95% CI)	relative risk (95% CI)	*p* value†	median CSS, *m* (95% CI)	relative risk (95% CI)	*p* value†	median OS, m (95% CI)	relative risk (95% CI)	*p* value†
VEGF+936 (C>T)[1]				0.207			0.352			0.655
CC	80 (72.1)	40 (15.3–64.7)	–		50 (20.2–79.8)	–		31 (17.8–44.2)	–	
CT	29 (26.1)	31 (3.6–58.4)	–		38 (0.0–76.4)	–		29 (6.4–51.6)	–	
TT	1 (0.9)								–	
EGF+61 (A>G)[1]				0.968			0.816			0.934
AA	55 (49.5)	39 (3.6–74.4)	–		54 (30.7–77.3)	–		31 (15.1–46.9)	–	
AG	54 (48.6)	36 (17.6–54.4)	–		50 (22.3–77.7)	–		31 (22.7–39.3)	–	
GG	1 (0.9)							–	–	
EGFR-1562 (G>A)				0.535			0.336			0.602
GG	38 (34.2)	26 (0.0–53.8)	–		50 (23.5–76.5)	–		24 (7.4–40.6)	–	
GA	51 (45.9)	84 (35.5–132.5)	–		87 (3.3–170.7)	–		31 (23.1–38.9)	–	
AA	15 (13.5)	36 (8.1–63.9)	–		42 (14.9–69.1)	–		32 (4.4–59.6)	–	
IL-1b + 3954 (C>T)[1]				0.160			0.090			0.230
CC	65 (58.6)	61 (20.9–101.1)	–		63 (30.8–95.2)	–		31 (13.2–48.8)	–	
CT	38 (34.2)	29 (10.8–47.2)	–		40 (26.3–53.7)	–		32 (18.6–45.4)	–	
TT	5 (4.5)								–	
IL-6-174 (G>C)				0.881			0.817			0.791
GG	40 (36.0)	40 (6.0–73.9)	–		63 (42.0–84.0)	–		32 (13.9–50.1)	–	
GC	60 (54.0)	29 (7.3–50.7)	–		38 (16.5–59.5)	–		29 (21.8–36.2)	–	
CC	11 (10.0)	41 (0.3–81.7)	–		50 (16.1–83.9)	–		41 (0.0–85.0)	–	
IL-8-251 (T>A)				0.891			0.931			0.651
TT	35 (31.5)	40 (11.5–68.5)	–		54 (28.7–79.3)	–		41 (19.2–62.8)	–	
TA	46 (41.4)	36 (9.3–62.7)	–		50 (14.0–86.0)	–		32 (15.1–48.9)	–	
AA	28 (25.2)	61 (0.0–122.7)	–		38 (6.7–69.3)	–		19 (3.8–34.2)	–	
IL-10-592 (T>G) [1]				0.444			0.473			0.374
TT	8 (7.2)	84 (39.6–161.6)	–		87 (13.8–160.2)	–		25 (11.1–38.9)	–	
TG	36 (32.4)								–	
GG	64 (57.7)	31 (9.9–52.1)	–		50 (31.6–68.4)	–		31 (18.1–43.9)	–	
CXCR1 +860 (G>C)				**0.015**			**0.007**			**0.002**
CC	92 (82.9)	55 (21.6–88.4)	1		63 (35.3–90.7)	1		33 (21.7–44.3)	1	
CG	18 (16.2)	14 (7.7–20.3)	2.315 (1.145–4.682)		31 (0.0–62.8)	2.520 (1.252–5.095)		6 (0.5–11.5)	2.322 (1.327–4.063)	
GG	0 (0.0)							–	–	
HIF1a-1772 (C>T)[1]				0.932			0.634			0.993
CC	75 (67.6)	37 (20.3–53.7)	–		50 (34.6–65.4)	–		32 (17.0–47.0)	–	
CT	27 (24.3)	36 (0.0–80.3)	–		73 (10.3–135.7)	–		31 (11.1–51.0)	–	
TT	8 (7.2)							–	–	
COX2 +8473 (A>G)				0.747			0.611			0.951
AA	52 (46.8)	37 (22.3–51.7)	–		54 (28.2–79.8)	–		38.0 (25.7–50.3)	–	
AG	46 (41.4)	26 (5.8–46.2)	–		40 (11.5–68.5)	–		24.0 (9.9–38.1)	–	
GG	12 (10.8)	55 (0.0–130.8)	–		73 (10.2–135.8)	–		19.0 (0.0–45.2)	–	
COX2 –765 (G>C)				0.640			0.310			0.185
GG	2 (1.8)	36 (0.5–71.5)	–		41 (20.9–61.1)	–		33 (21.561–44.439)	–	
GC	35 (31.5)		–			–			–	
CC	73 (65.8)	37 (9.6–64.5)	–		54 (26.3–81.7)	–		20 (6.7–33.3)	–	

† Based on log-rank test. * Only samples with successful genotyping included.

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Table 3. Patient characteristics in association with DFS, CSS, and OS

	N (%)	Med DFS (95% CI)	RR	*p* value†	Med CSS (95% CI)	RR	*p* value†	Med OS (95% CI)	RR	*p* value†
Sex				0.435			0.418			0.397
Male	75 (67.6)	40 (13.6–66.4)	–		50 (20.9–79.1)	–		32 (19.0–45.0)	–	
Female	36 (32.4)	29 (0.0–67.1)	–		50 (20.8–79.2)	–		25 (8.8–41.2)	–	
Age, years				0.858			0.874			0.750
≤65	47 (42.3)	40 (14.0–66.1)	–		42 (10.4–73.6)	–		29 (7.1–50.9)	–	
>65	64 (57.7)	31 (5.0–57.1)	–		50 (26.0–74.0)	–		31 (22.7–39.3)	–	
BMI, kg/m^2				0.264			0.551			0.481
≤25	54 (48.6)	61 (31.8–89.2)	–		63 (37.3–88.7)	–		32 (17.8–46.2)	–	
>25	57 (51.4)	29 (8.8–49.2)	–		40 (18.8–61.2)	–		29 (15.6–42.4)	–	
ASA				0.868			0.850			0.817
I/II	51 (45.9)	41 (6.1–75.9)	–		50 (29.5–70.5)	–		40 (19.0–61.0)	–	
III/IV	56 (50.5)	37 (2.5–71.5)	–		50 (16.9–83.1)	–		31 (21.5–40.5)	–	
Cholangitis				0.173			0.261			0.196
No	71 (64.0)	61 (28.7–93.3)	–		42 (21.2–62.8)	–		32 (20.2–43.8)	–	
Yes	36 (32.4)	26 (1.2–50.8)	–		63 (31.1–95.0)	–		31 (16.2–45.8)	–	
PVE				0.714			0.481			0.564
No	64 (57.7)	39 (10.2–67.8)	–		41 (11.0–71.0)	–		31 (6.2–41.8)	–	
Yes	47 (42.3)	41 (0.0–83.6)	–		50 (28.4–71.6)	–		28 (13.0–43.1)	–	
EBD				0.108			**0.045**			0.179
No	23 (20.7)	n.a.	–		42 (26.0–58.0)	2.483 (0.984–6.266)		51 (n.a.)	–	
Yes	88 (79.3)	36 (14.3–57.7)	–		n.a	1		31 (23.3–38.7)	–	
PBD				0.441			0.358			0.620
No	84 (75.7)	41 (8.3–73.7)	–		42 (19.9–64.1)			31 (17.0–53.4)	–	
Yes	26 (23.4)	31 (0.0–63.6)	–		54 (29.0–79.0)	–		29 (4.6–53.4)	–	
Bismuth classification				0.130			0.389			0.706
I	6 (5.4)	19 (0.0–53.3)	–		41 (20.7–61.3)	–		28 (12.6–43.4)	–	
II	8 (7.2)	8 (0.0–17.8)	–		20 (12.8–27.2)	–		18 (0.0–40.2)	–	
IIIa	30 (27.0)	84 (3.1–164.9)	–		87 (22.2–151.8)	–		19 (0.0–56.6)	–	
IIIb	32 (28.8)	n.a	–		n.a	–		32 (2.2–61.8)	–	
IV	35 (31.5)	31 (9.2–52.8)	–		42 (24.1–59.9)	–		32 (18.9–44.2)	–	
Albumin, g/L				0.602			0.628			0.529
≤42	71 (64.0)	29 (4.5–53.5)	–		42 (17.1–66.9)	–		25 (13.1–36.9)	–	
>42	40 (36.0)	55 (30.8–79.2)	–		69 (31.9–106.1)	–		40 (26.1–53.9)	–	
AST, U/L				0.195			0.400			0.924
≤40	43 (38.7)	84 (29.9–138.1)	–		69 (40.6–97.4)	–		32 (13.1–50.9)	–	
>40	68 (61.3)	31 (5.4–56.6)	–		40 (12.7–67.3)	–		24 (19.4–38.6)	–	
ALT, U/L				0.812			0.447			0.291
≤40	20 (18.0)	29 (0.0–62.9)	–		41 (22.0–60.0)	–		25 (13.3–36.7)	–	
>40	91 (82.0)	41 (12.6–69.4)	–		54 (30.1–77.9)	–		32 (18.3–45.7)	–	
GGT, U/L				0.662			0.613			0.646
≤100	8 (7.2)	61 (0.0–129.3)	–		63 (16.6–109.4)	–		32 (0.0–71.7)	–	
>100	103 (92.8)	37 (21.5–52.5)	–		50 (34.4–65.6)	–		31 (23.2–38.8)	–	

Table 3 (continued)

	N (%)	Med DFS (95% CI)	RR	*p* value†	Med CSS (95% CI)	RR	*p* value†	Med OS (95% CI)	RR	*p* value†
Bilirubin, mg/dL				0.271			0.193			0.223
≤1	52 (46.8)	74 (23.584–124.416)	–		69 (37.818–100.182)	–		33 (13.119–52.881)	–	
>1	59 (53.2)	31 (4.431–57.569)	–		40 (17.405–62.595)	–		23 (7.115–38.885)	–	
Alkaline phosphatase, U/L				0.200			0.182			0.067
≤100	5 (4.5)	n.a	–		n.a	–		n.a	–	
>100	106 (95.5)	37 (21.404–52.596)	–		50 (33.923–66.077)	–		29 (21.100–36.900)	–	
Platelet count, 1/nL				0.745			0.304			0.708
≤200	15 (13.5)	74 (0.000–160.025)	–		90 (30.040–149.960)	–		6 (0.000–30.616)	–	
>200	96 (86.5)	39 (14.004–63.996)	–		50 (31.929–68.071)	–		31 (20.920–41.080)	–	
INR				0.091			0.110			0.104
≤1	43 (38.7)	61 (32.608–89.392)	–		73 (44.417–101.583)	–		50 (28.362–71.638)	–	
>1	68 (61.3)	29 (5.552–52.448)	–		38 (24.837–51.163)	–		25 (12.283–37.717)	–	
Hemoglobin, g/dL				**0.000**			**0.000**			**0.017**
≤12	42 (37.8)	14 (5.108–22.892)	2.914 (1.627–5.220)		25 (14.805–35.195)	2.636 (1.504–4.620)		18 (5.312–30.688)	1.704 (1.085–2.676)	
>12	69 (62.2)	74 (50.260–97.740)	1		73 (54.474–91.526)	1		40 (20.171–59.829)	1	
CRP, mg/L				0.262			0.531			0.333
≤10	46 (41.4)	61 (n.a.)	–		63 (n.a.)	–		32 (21.107–42.893)	–	
>10	65 (58.6)	36 (21.251–50.749)	–		50 (34.418–65.582)	–		29 (7.987–50.013)	–	
Operative time, min				0.796			0.546			0.134
≤360	31 (27.9)	74 (9.218–138.782)	–		69 (24.894–113.106)	–		51 (8.924–93.076)		
>360	80 (72.1)	39 (11.974–66.026)	–		50 (38.498–61.502)	–		29 (18.023–39.977)	–	
Blood transfusions				**0.001**			**0.004**			**0.002**
No	59 (53.2)	74 (54.245–93.755)	1		73 (49.484–96.516)	1		54 (21.245–86.755)	1	
Yes	52 (46.8)	14 (8.228–19.772)	2.569 (1.427–4.626)		31 (21.322–40.678)	2.247 (1.278–3.952)		15 (3.516–26.484)	1.972 (1.256–3.095)	
FFP				**0.001**			**0.003**			**0.002**
No	39 (35.1)	n.a	1		n.a	1		69 (23.408–114.592)	1	
Yes	72 (64.9)	17 (1.145–32.855)	2.981 (1.478–6.012)		33 (21.374–44.626)	2.655 (1.356–5.198)		19 (5.357–32.643)	2.227 (1.324–3.744)	
R status				0.511			0.171			0.239
R0	84 (75.7)	40 (2.360–77.640)	–		69 (40.707–97.293)	–		32 (21.210–42.790)	–	
R1/Rx	26 (23.4)	39 (29.421–48.579)	–		40 (20.435–59.565)	–		23 (2.804–43.196)	–	
MVI				0.087			0.069			0.223
No	79 (71.2)	74 (25.194–122.806)	–		73 (40.075–105.925)	–		40 (25.791–54.209)	–	
Yes	25 (22.5)	17 (11.481–22.519)	–		31 (17.402–44.598)	–		23 (1.784–44.216)	–	
Perineural invasion				0.360			0.168			0.128
Pn0	15 (13.5)	NA	–		69 (n.a.)	–		50 (19.143–80.857)	–	
Pn1	75 (67.6)	39 (24.534–53.466)	–		41 (19.717–62.283)	–		25 (13.968–36.032)	–	
LVI				0.053			0.335			0.055
No	79 (71.2)	61 (31.141–90.859)	–		63 (40.313–85.687)	–		41 (25.007–56.993)	–	
Yes	24 (21.6)	15 (5.342–24.658)	–		20 (0.000–41.446)	–		12 (2.398–21.601)	–	
Tumor grading				**0.016**			**0.001**			**0.000**
G1/G2	80 (72.1)	55 (18.162–91.838)	1		54 (27.268–80.732)	1		41 (24.641–57.359)	1	
G3/G4	25 (22.5)	8 (0.000–33.636)	2.334 (1.138–4.787)		13 (10.215–15.785)	2.992 (1.556–5.755)		7 (3.736–10.264)	3.076 (1.844–5.129)	

Table 3 (continued)

	N (%)	Med DFS (95% CI)	RR	*p* value†	Med CSS (95% CI)	RR	*p* value†	Med OS (95% CI)	RR	*p* value†
Tumor stage UICC				**0.048**			**0.049**			**0.009**
I/II	47 (42.3)	84 (16.803–151.197)	1		73 (40.769–105.231)	1		50 (34.524–65.476)	1	
III/IV	63 (56.8)	37 (5.989–68.011)	1.806 (0.991–3.291)		41 (19.750–62.250)	1.768 (0.992–3.151)		19 (6.897–31.103)	1.837 (1.151–2.933)	
pT category				–			0.138			**0.004**
pT1-2	72 (64.9)	39 (5.343–72.657)	–		54 (23.636–84.364)	–		41 (24.058–57.942)	1	
pT3-4	38 (34.2)	40 (4.101–75.899)			41 (4.541–77.459)	–		12 (5.959–18.041)	1.902 (1.205–3.003)	
N category				**0.005**			**0.017**			**0.007**
pN0	64 (57.7)	74 (38.081–109.919)	1		73 (38.649–107.351)	1		50 (32.572–67.428)	1	
pN1	45 (40.5)	15 (0.000–44.057)	2.260 (1.250–4.086)		31 (20.762–41.238)	1.990 (1.115–3.555)		18 (4.985–31.015)	1.853 (1.166–2.944)	
ICU time, days				0.056			**0.015**			**0.000**
≤1	51 (45.9)	n.a	–		69 (40.749–97.251)	1		50 (23.886–76.114)	1	
>1	60 (54.1)	26 (9.049–42.951)	–		31 (15.179–46.821)	1.985 (1.126–3.502)		18 (7.879–28.121)	2.263 (1.421–3.603)	
Hospitalization, days				0.237						0.207
≤14	44 (39.6)	55 (n.a.)	–		73 (35.544–110.456)	–	0.495	50 (26.956–73.044)	–	
>14	67 (60.4)	36 (9.781–62.219)	–		42 (16.628–67.372)	–		29 (18.194–39.806)	–	
CCI				**0.026**			**0.002**			**0.000**
≤40	57 (51.4)	84 (22.299–145.701)	1		73 (47.371–98.629)	1		54 (32.986–75.014)	1	
>40	53 (47.7)	26 (6.309–45.691)	1.909 (1.064–3.424)		31 (19.339–42.661)	2.388 (1.351–4.223)		12 (1.696–22.304)	2.722 (1.714–4.324)	
Adjuvant therapy				**0.007**			**0.036**			0.341
No	66 (59.5)	74 (51.306–96.694)	1		73 (46.834–99.166)	1		31 (1.869–60.131)	–	
Yes	45 (40.5)	19 (0.000–38.239)	2.220 (1.217–4.047)		38 (26.310–49.690)	1.843 (1.028–3.307)		31 (25.832–36.168)	–	
Tumor recurrence										**0.000**
No	–	–	–		–	–		72 (43.218–100.782)	1	
Yes	–	–	–		–	–		24 (14.004–33.996)	2.556 (1.593–4.101)	

EBD, endoscopic biliary drainage; PBD, percutaneous biliary drainage; PVE, portal vein embolization. † Based on log-rank test.

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Table 4. Multivariable Cox regression analysis of OS and DFS

	DFS			CSS			OS		
	n (%)*	relative risk (95% CI)†	*p* value	*n* (%)**	relative risk (95% CI)†	*p* value	*n* (%)***	relative risk (95% CI)†	*p* value
CXCR1 +860, *n* (%)			0.008			0.001			0.001
CC§	84 (84)	1		84 (84)	1		77 (83.7)	1	
GC	16 (16)	3.679 (1.399–9.672)		16 (16)	4.957 (1.922–12.781)		15 (16.3)	3.761 (1.727–8.190)	
Hb, *n* (%)			0.003			0.012			–
≤12	38 (38)	2.805 (1.407–5.592)		38 (38)	2.392 (1.216–4.705)		–	–	
>12	62 (62)	1		62 (62)	1		–	–	
Adjuvant therapy, *n* (%)			0.015			0.032			–
No	63 (63)	1		63 (63)	1		–	–	
Yes	37 (37)	2.359 (1.180–4.715)		37 (37)	2.072 (1.067–4.024)		–	–	
L, *n* (%)			–			–			0.008
L0	–	–		–	–		75 (81.5)	1	
L1	–	–		–	–		17 (18.5)	2.743 (1.301–5.783)	
CCI, *n* (%)			–			–			0.017
≤40	–	–		–	–		47 (51.1)	1	
>40	–	–		–	–		45	2.311 (1.158–4.613)	

Only significant variables and outcomes are shown. Significant parameters in univariate analysis were included in the respective multivariate analysis for DFS, CSS, and OS. For DFS, hemoglobin (≤12/>12 g/dL), intraoperative blood and FFP transfusions, tumor grading (G1-2/G3-4), UICC (I-II/III-IV), N category (pN0/pN1), CCI (≤40/>40), adjuvant therapy, and the CXCR1 +860C>G SNP were included. For CSS, EBD, hemoglobin (≤12/>12 g/dL), intraoperative blood and FFP transfusions, tumor grading (G1-2/G3-4), UICC (I-II/III-IV), N category (pN0/pN1), ICU time (≤1/>1 day), CCI (≤40/>40), adjuvant therapy, and the CXCR1 +860C>G SNP were included. For OS, preoperative cholangitis, hemoglobin (≤12/>12 g/dL), intraoperative blood and FFP transfusions, lymphovascular invasion, tumor grading (G1-2/G3-4), UICC (I-II/III-IV), tumor stage (pT1-2/pT3-4), N category (pN0/pN1), ICU time (≤1/>1 day), CCI (≤40/>40), tumor recurrence, and the CXCR1 +860C>G SNP were included. * 100 patients with complete data were included in the model. ** 100 patients with complete data were included in the model. *** 92 patients with complete data were included in the model. § Determined as the favorable allele.

In the multivariable analysis incorporating significant clinicopathological parameters from the univariable analysis (Table 3), the *CXCR1* rs2234671 polymorphism remained significantly associated with DFS, CSS, and OS. As such, the *CXCR1* +860 genotype (G/C relative risk [RR] 3.679 [95% CI: 1.399–9.672] $p = 0.008$), preoperative hemoglobin (≤12 RR 2.805 [95% CI: 1.407–5.592] $p = 0.003$), and adjuvant therapy (RR 2.359 [95% CI: 1.180–4.715] $p = 0.015$) were significantly associated with DFS. *CXCR1* +860 (G/C RR 4.957 [95% CI: 1.922–12.781] $p = 0.001$), preoperative hemoglobin (≤12 RR 2.392 [95% CI 1.216–4.705] $p = 0.012$), and adjuvant therapy (RR 2.072 [95% CI: 1.067–4.024] $p = 0.032$) were also significantly associated with CSS. *CXCR1* +860 (G/C RR 3.761 [95% CI: 1.727–8.190] $p = 0.001$), lymph node status (L1 RR 2.743 [95% CI: 1.301–5.783] $p = 0.008$), and CCI (>40 RR 2.311 [95% CI: 1.158–4.613] $p = 0.017$) were significantly associated with OS (Table 4).

Discussion

We were able to demonstrate that a germline polymorphism of *CXCR1* independently predicts tumor recurrence and survival in a large homogenous patient group with pCCA. To the best of our knowledge, this is the first study to show that the *CXCR1* +860C>G polymorphism may be an important prognostic marker for pCCA tumor relapse and overall survival, independent of lymph node status, hemoglobin, and perioperative complications.

pCCA is a rare but highly aggressive primary hepatic malignancy. While the last years have witnessed considerable advances in characterizing the genetic and transcriptomic landscape of CCA, the tumor biology of CCA remains understudied compared to other gastrointestinal malignancies [24]. Suggestions to expand traditional TNM staging with clinical adjuncts have been made [25], but relatively few biomarker-based staging systems were implemented so far [26, 27]. The role of tumor neovascularization in cancer, especially the prominent role of VEGF, is well established [13, 28]. Tumor cells and immune cells from the TME, such as infiltrating CD14 (+) CD16 (+) monocytes and CAFs, abundantly secrete VEGF and promote angiogenesis and metastasis [29, 30]. However, clinical studies investigating the efficacy of VEGF inhibition for CCA have reported overall discouraging results [31, 32], suggesting that alternative mechanisms may support CCA neoangiogenesis. Besides directly stimulating neovascularization by secreting soluble angiogenic factors, malignant cells actively interact with the TME to enhance neovascularization. In this regard, the inflammatory TME amplifies VEGF-dependent angiogenesis and promotes tumor progression through VEGF-independent pathways [8]. In turn, endothelial cells can attract leucocytes to facilitate angiogenesis, tumor growth, and metastasis. As such, the release of IL-8 from endothelial cells recruits and activates granulocytes and monocytes and triggers their transformation into immunosuppressive myeloid-derived suppressor cells [17].

A prominent stromal reaction is inherent to pCCA, with a characteristic hypoxic microenvironment and a reduced vascularity in comparison to HCC [1]. A dense network of CAFs and endothelial cells surrounds the tumor and intensely interacts with the malignant cells as well as with resident and migratory immune cells [30]. In vitro studies have identified CAFs as a source of soluble mediators with direct proinvasive effects on cocultured CCA cells, with IL-8 as one of the main secreted mediators [11]. As such, excessive IL-8 secretion by senescent CAFs drives invasion and metastasis in other cancer entities with a prominent desmoplastic reaction, such as pancreatic cancer [14, 33, 34]. Recent CCA single-cell sequencing data showed that the majority of CAFs in CCA exhibit a microvasculature signature with an upregulation of genes responsible for hypoxia response and are localized in proximity to microvascular regions, underlining their mediator role between malignant cells and neoangiogenesis. In vitro and in vivo data showed that the presence of vascular CAFs promotes CCA tumor growth [35]. While genetic variants of IL-8 and its receptor CXCR1 (also interleukin-8-receptor alpha—*IL-8RA*) were linked with clinical outcome in gastric and colon cancer [13, 14], the induction of IL-8 signaling preserved the angiogenic phenotype in HIF1-α-deficient colon cancer cells, suggesting a critical role of IL-8 in tumor-associated angiogenesis, independent of VEGF [15]. These findings support our hypothesis that altered IL-8 signaling may impact immunosuppressive and proangiogenic signals in the TME.

The *CXCR1* rs2234671 860C>G polymorphism causes a missense variant with a Ser > Thr substitution at position 276, located in the extracellular domain of the receptor [36, 37]. While the polymorphism is not located in an expression quantitative trait locus, it seems likely that the polymorphism causes altered receptor signaling [38]. As such, the exchanged amino acid directly adjoins to the amino acid in position 277, which forms a disulfide bond to a cysteine side chain in position 30 [39], therefore potentially altering the tertiary protein structure of the receptor.

The wild-type *CXCR1* +860 alleles (CC) have been linked to improved response to bevacizumab (anti-VEGF) and increased DFS in colorectal, pancreatic, lung, renal, breast, and gastric cancer [38], underlining the impact of the *CXCR1* +860 SNP on VEGF-dependent angiogenesis. Previously, the *CXCR1* rs2234671 polymorphism has been associated with an increased susceptibility to breast cancer [40]. To the best of our knowledge, the prognostic role of the C>G polymorphism has not yet been investigated in CCA. Interestingly, patients with gallstones and carriers of the *CXCR1* +860C>G polymorphism had a 26-fold risk for the development of gallbladder cancer, compared to those without gallstones [41]. Furthermore, high IL-8 protein expression in surgical pCCA cases has been associated with microvascular density and dismal survival [42].

When analyzing the genotype of the *CXCR1* receptor polymorphism (rs2234671), we observed that carriers of the G-allele had inferior DFS, CSS, and OS in both univariable and multivariable analyses. The results of the present study must be interpreted with caution due to its retrospective nature. The findings from this study will need to be replicated in other studies on pCCA and validated in independent trials. Despite originating from a single-center cohort, a strength of this report is the rigorous definition of a homogenous patient collective with only treatment-naïve, perihilar disease, managed with a universal surgical approach of hilar *en bloc* resection with lymphadenectomy. Due to the relatively low incidence of pCCA, many single-center studies are conducted in cohorts with heterogeneous CCA localization and pretreatments, limiting the comparability of results [43].

Taken together and consistent with our hypothesis, a genetic variant of an immunoregulatory gene involved in tumor inflammation and angiogenesis independently predicted clinical outcome in patients undergoing curative-intent surgery for pCCA. Our findings suggest that the assessment of the patients' angiogenic potential based on *CXCR1* genotypes may predict postoperative disease progression and survival. Potentially, this may aid the identification of novel therapeutic targets. Biomarker-embedded clinical trials and validation in an independent cohort of patients are required to confirm our findings.

Statement of Ethics

This research complies with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study protocol was reviewed and approved by the Institutional Review Board of the RWTH Aachen University (EK 360/15 and EK 173/06). Written informed consent was not required due to the retrospective nature of the study (Institutional Review Board of the RWTH Aachen University, EK 360/15, EK 173/06).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The study was designed by the initiating study team (I.L., Z.C., G.L., U.P.N., and J.B.). Data collection and analysis were performed by I.L., Z.C., J.B., H.M., N.T.G., U.P.N., and G.L. Laboratory experiments were conducted by I.L. and H.K. The manuscript was drafted by I.L., Z.C., and G.L. Further authors (F.T., P.S., C.T., E.D., N.T.G., T.F.U., R.K., and U.P.N.) have substantially contributed to the final version of the manuscript. All authors have read and approved the final version of the manuscript.

Data Availability Statement

All relevant data were reported within the article. Further supporting data will be provided upon written request addressed to the corresponding author.

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2.2 Genetische Varianten des Interleukin-1β (rs1143634) und Interleukin-8 (rs4073) haben nach Resektion des intrahepatischen Cholangiokarzinoms prognostischen Wert

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Genetic polymorphisms in interleukin-1β (rs1143634) and interleukin-8 (rs4073) are associated with survival after resection of intrahepatic cholangiocarcinoma.

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Abstract Zitat (89):

“Intrahepatic cholangiocarcinoma (iCCA) is a rare, understudied primary hepatic malignancy with dismal outcomes. Aiming to identify prognostically relevant single-nucleotide polymorphisms, we analyzed 11 genetic variants with a role in tumor-promoting inflammation (VEGF, EGF, EGFR, IL-1B, IL-6, CXCL8 (IL-8), IL-10, CXCR1, HIF1A and PTGS2 (COX-2) genes) and their association with disease-free (DFS) and overall survival (OS) in patients undergoing curative-intent surgery for iCCA.

Genomic DNA was isolated from 112 patients (64 female, 48 male) with iCCA. Germline polymorphisms were analyzed with polymerase chain reaction-restriction fragment length polymorphism protocols. The IL-1B +3954 C/C (73/112, hazard ratio (HR)=1.735, p=0.012) and the IL-8 -251 T/A or A/A (53/112 and 16/112, HR=2.001 and 1.1777, p=0.026) genotypes were associated with shorter OS in univariable and multivariable analysis. The IL-1B +3954 polymorphism was also associated with shorter DFS (HR=1.983, p=0.012), but this effect was not sustained in the multivariable model. A genetic risk model of 0, 1 and 2 unfavorable alleles was established and confirmed in multivariable analysis.

This study supports the prognostic role of the IL-1B C+3954T and the IL-8 T-251A variant as outcome markers in iCCA patients, identifying patient subgroups at higher risk for dismal clinical outcomes.”

Tumor-Angiogenese und lokale Immunsuppression sind zentrale Elemente des TME intrahepatischer Gallenwegstumore. Die zentrale Fragestellung dieser Arbeit war, ob Genpolymorphismen, die die Expression oder Funktion von Proteinen beeinflussen, die zentrale Mediatoren im TME sind, nach Resektion des iCCAs prognostisch relevant sind. Spezielles Interesse galt hierbei den Mediatoren, die die Attraktion und die immun-suppressiven Funktionen myeloider Zellen, sowie die Tumor-Angiogenese im TME beeinflussen. In einem Pathway-basierten Ansatz wurden SNPs mit dokumentierter funktioneller Relevanz, ausreichender Allelhäufigkeit und biologisch zentraler Rolle des

Produkts im TME ausgewählt und in einer großen chirurgischen Kohorte von Patient*innen mit iCCA genotypisiert. Neben klinisch-pathologischen und laborchemischen Faktoren wurde krankheitsfreies und Gesamtüberleben erhoben.

Ein wichtiges Ergebnis dieser Studie war, dass Patient*innen mit dem *IL-1B* 3954 C/C Genotyp ein statistisch unabhängiges, signifikant kürzeres Gesamtüberleben als Patient*innen mit einem oder zwei T Allelen. Gleichzeitig hatten Patient*innen mit dem IL-8-251 T/T Genotyp ein signifikant längeres Gesamtüberleben. Die Kombination dieser Parameter in einen genetischen Risikoscore erlaubte die Zuordnung des Kollektivs in drei Gruppen, mit einem Medianen Langzeitüberleben von 36 Monaten ohne Hochrisiko-Allel, 25 Monaten mit einem, und 13 Monaten mit 2 Hochrisiko-Allelen. Weitere signifikante klinische Faktoren in der multivariablen Analyse für Allgemeinüberleben waren eine nicht-R0 Resektion, mikrovaskuläre und lymphovaskuläre Invasion, sowie Union Internationale Contre le Cancer (UICC) Stadium III/IV. Bis auf das UICC-Stadium sind diese Faktoren präoperativ zumeist nicht verfügbar und können so, im Gegensatz zu dem prognostischen SNP-Score, nicht zur operativen Risikostratifikation für einen Eingriff beitragen, der selbst in erfahrenen Zentren mit hochgradiger Morbidität und Mortalität assoziiert ist.

Zusammenfassend zeigte in diesem Patient*innenkollektiv die Analyse von Genpolymorphismen, deren Produkte im TME involviert sind, eine prognostische Relevanz genetischer Varianten des IL-1b und IL-8, welche zentrale immunsuppressive und pro-angiogenese Funktionen im TME haben. IL-1β und IL-8 sind Zytokine, die vornehmlich die Rekrutierung von Makrophagen- und Neutrophilenpopulationen mediieren, welche im TME als MDSCs immunsuppressive Funktionen entfalten (90, 91). Die hier analysierten Polymorphismen haben einen zuvor dokumentierten Effekt einer erhöhten Zytokinsekretion von IL-1β und IL-8 (92, 93). Die gezeigte prognostische Assoziation mit postoperativem onkologischem Outcome und Gesamtüberleben kann nicht nur Potenzial für Patientenstratifikation, sondern auch als Anhaltpunkt für weitere translationale, mechanistische oder Outcome-Studien dienen.

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Genetic polymorphisms in interleukin-1β (rs1143634) and interleukin-8 (rs4073) are associated with survival after resection of intrahepatic cholangiocarcinoma

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Intrahepatic cholangiocarcinoma (iCCA) is a rare, understudied primary hepatic malignancy with dismal outcomes. Aiming to identify prognostically relevant single-nucleotide polymorphisms, we analyzed 11 genetic variants with a role in tumor-promoting inflammation (*VEGF*, *EGF*, *EGFR*, *IL-1B*, *IL-6*, *CXCL8 (IL-8)*, *IL-10*, *CXCR1*, *HIF1A* and *PTGS2 (COX-2)* genes) and their association with disease-free (DFS) and overall survival (OS) in patients undergoing curative-intent surgery for iCCA. Genomic DNA was isolated from 112 patients (64 female, 48 male) with iCCA. Germline polymorphisms were analyzed with polymerase chain reaction-restriction fragment length polymorphism protocols. The *IL-1B* +3954 C/C (73/112, hazard ratio (HR) = 1.735, *p* = 0.012) and the *IL-8* -251 T/A or A/A (53/112 and 16/112, HR = 2.001 and 1.1777, p = 0.026) genotypes were associated with shorter OS in univariable and multivariable analysis. The *IL-1B* +3954 polymorphism was also associated with shorter DFS (HR = 1.983, p = 0.012), but this effect was not sustained in the multivariable model. A genetic risk model of 0, 1 and 2 unfavorable alleles was established and confirmed in multivariable analysis. This study supports the prognostic role of the *IL-1B* C+3954T and the *IL-8* T-251A variant as outcome markers in iCCA patients, identifying patient subgroups at higher risk for dismal clinical outcomes.

Cholangiocarcinoma (CCA) is one of the most aggressive gastrointestinal cancers with a rising worldwide incidence over the last decade[1]. Despite the improvement of surgical techniques and palliative regimens, therapeutic options remain limited and outcomes are dismal[2]. From the anatomical and surgical perspective, CCA can be classified into intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA) disease. While iCCAs arise above the second-order bile ducts, pCCAs originate above the cystic duct and below the second-order bile ducts and dCCAs below the cystic duct, and, as a consequence, surgical approaches differ significantly between entities[3,4].

Intrahepatic CCA (iCCA) represents approximately 10–20% of CCA and is an understudied tumor entity[3]. Predisposing factors include chronic biliary inflammation such as primary sclerosing cholangitis, as well as cholelithiasis and liver cirrhosis, but more general risk factors such as type 2 diabetes and smoking have been described as well[5]. To date, surgery represents the only curative treatment for iCCA, with dismal survival rates of 20%-35% after 5 years[6]. Clinico-pathological characteristics such as lymphovascular invasion and poor differentiation

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remain the best-studied prognostic factors, which, however, have a limited value for the preoperative identification of patients at risk for poor postoperative outcomes[6,7]. Therefore, finding prognostic markers as an adjunct to traditional staging systems may facilitate the selection of patients who require additional or a more aggressive adjuvant treatment approaches and a closer oncological follow-up[8].

The tumor microenvironment (TME) of iCCA is abundant in mediator responses that drive tumor growth and invasion while abrogating anti-tumor immune responses including antigen presentation and infiltration of activated cytotoxic T cells. Typically, a prominent desmoplastic reaction with a proliferation of cancer-associated fibroblasts (CAF) and an infiltration of immunosuppressive myeloid and lymphoid populations are present[9]. Neoangiogenesis, an essential prerequisite for tumor growth, is driven by vascular endothelial growth factor (VEGF) and supported by monocytes[10,11]. Furthermore, infiltrating immune cells convey tolerogenic effects that abrogate efficient antigen cross-presentation and cytotoxic T cell antitumor activity[12,13].

We hypothesized that functional gene polymorphisms encoding for proteins that are critically involved in the tumor microenvironment may have prognostic value in iCCA by altering the systemic and local concentration of mediators relevant for the TME. We hypothesized that an altered expression of proteins involved in the attraction of suppressive myeloid populations such as tumor-associated neutrophils (TANs) and tumor-associated macrophages (TAM)—like interleukin (IL)-1β or Hypoxia-inducible factor (HIF)-1α—may impact prognosis in these patients[14,15]. Further selected single nucleotide polymorphism (SNP) candidates were in genes encoding for mediators in VEGF-dependent and independent angiogenesis (VEGF, IL-8)[16,17]. Thus, we analysed 11 polymorphisms in ten genes with a role in tumor inflammation and tumor-related immunosuppression to identify patient subgroups with dismal oncological and overall outcome after surgical resection of cholangiocarcinoma.

Patients and methods

Study population. In this retrospective single-center study, data of $N = 112$ consecutive patients with localized iCCA undergoing curative-intent surgery at the University Hospital RWTH Aachen were analysed. Clinicopathological and survival data for this study was obtained from a prospectively managed institutional database spanning 2010–2019. A part of the included cohort had previously been analyzed to determine the efficacy of the surgical ALPPS technique for iCCA[18], the prognostic role of pathological factors[7] and small nerve fibers[19]. Patients with mixed hepatocellular carcinoma (HCC)-CCA histology or neuroendocrine tumor differentiation were not included in the analysis, nor were pCCA and dCCAs, due to different tumor biology, prognostic factors, and surgical treatment. Patients with extrahepatic or metastatic disease were excluded, as well. An overview of the selection criteria is provided in Supplementary Fig. 1. A senior hepatobiliary pathologist (NTG) reviewed the tumor histology. Patient material for genotyping was provided by the institutional biobank (RWTH-cBMB) and the Department of Pathology (NTG, ED, RKC). This study was approved by the institutional review board of the RWTH Aachen University (EK 360/15, EK 173/06) and conducted in accordance with good clinical practice guidelines and the current Declaration of Helsinki. For this study informed consent has been waived by Institutional review board of the RWTH Aachen University, EK 360/15, EK 173/06 due to the anonymity and retrospective nature of the study. An ex-ante sample size calculation was not performed due to the hypothesis-generating, exploratory study design.

Staging and surgical technique. Preoperative work-up included appropriate cross-sectional imaging to rule out distant metastases and CT or magnetic resonance imaging (MRI) of the liver to visualize hilar vessel invasion and, if necessary, endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance cholangiopancreatography (MRCP) to assess hilar disease extent. Patients with suspected metastatic disease on conventional imaging underwent positron emission tomography (PET)-CT. In cases of insufficient estimated future liver remnant on liver volumetry, portal vein embolization (PVE) and, if necessary, ALPPS, were employed to allow right-sided hepatectomy. Indication for surgical resection was based on the recommendation of senior hepatobiliary surgical staff and approved by the local multidisciplinary tumor board. Depending on tumor extent, the resection volume ranged from atypical/non-anatomical to extended resections[7].

An experienced board-certified staff pathologist performed the routine histopathological work-up and reported tumor type, histopathological grading and staging, loco-regional lymph node metastasis, resection margins and vessel invasion.

SNP selection. The polymorphisms were selected in a pathway-centered approach, with the aim of selecting genes involved in tumor-associated inflammation and neovascularization, as well as tumor immune suppression (Supplementary Table 1). The following prerequisites were set: (a) that the gene is a part of a pathway involved in tumor-associated inflammation and tumor immunosuppression, (b) that the respective polymorphism is well-documented and confers a biological effect, and (c) that the frequency of the polymorphism is sufficient to enable a statistically meaningful association with clinical outcomes. In line with previous studies, this was estimated to be the case if at least 15% of the general population carry the minor allele of the genetic variant[20]. A total of 11 SNPs in ten genes were selected, including *VEGF, Epidermal Growth Factor* (*EGF*), *EGF-Receptor* (*EGF-R*), *IL-1B, IL-6, C-X-C motif chemokine ligand* (*CXCL*)*8* (*IL-8*), *IL-10, CXC chemokine receptor* (*CXCR*)*1, HIF1A and Prostaglandin-Endoperoxide Synthase* (*PTGS2, COX2*) (Table 1).

Genotyping. Formalin-fixed paraffin-embedded non-tumor tissues were collected and the QIAamp DNA extraction kit (Qiagen, CA, Valencia, USA) was used to extract genomic DNA according to the manufacturer's protocol. DNA quality and content was analysed photometrically (NanoDrop, Thermo Fisher, MA, USA). The polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique was employed for genotyping, as previously reported[21,22]. The SNP region was amplified in 35 PCR cycles with forward- and

	n = 112	Median DFS (95%*CI*)	*HR*	*p*†	Median OS (95%CI)	*HR*	*p*†
Sex							
Male	48 (42.9)	11 (3.799–18.201)		.984	19 (14.026–23.974)		.332
Female	64 (57.1)	12 (8.055–15.945)			22 (8.991–35.009)		
Age, years							
≤ 65	50 (46.3)	10 (4.826–15.174)		.121	20 (7.675–32.325)		.189
> 65	58 (53.7)	16 (5.245–26.755)			21 (10.767–31.233)		
BMI, kg/m^2							
≤ 25	54 (50.0)	12 (8.834–15.166)		.456	25 (14.655–35.345)		.438
> 25	54 (50.0)	12 (5.069–18.931)			18 (10.975–25.025)		
PVE							
No	101 (90.2)	12 (8.371–15.629)		.293	19 (5.054–32.946)		.556
Yes	11 (9.8)	27 (2.564–51.436)			21 (12.068–29.932)		
EBD							
No	91 (84.3)	30 (7.940–52.060)		.147	22 (13.540–30.460)		.567
Yes	17 (15.7)	10 (7.281–12.719)			13 (7.697–18.303)		
PBD							
No	107 (99.1)	n.a		.922	21 (15.483–26.517)	1	**.000**
Yes	1 (0.9)	0 (n.a.)			0 (n.a.)	26.796 (2.998–239.470)	
Albumin, g/l							
≤ 42	35 (32.7)	8 (3.261–12.739)		.145	13 (5.233–20.767)		.093
> 42	72 (67.3)	13 (6.737–19.263)			28 (17.830–38.170)		
AST, U/l							
≤ 40	63 (58.9)	13 (5.926–20.074)		.646	20 (15.442–24.558)		.863
> 40	44 (41.1)	10 (7.171–12.829)			25 (13.677–36.323)		
ALT, U/l							
≤ 40	56 (52.3)	16 (8.345–23.655)		.473	21 (9.721–32.279)		.763
> 40	51 (47.7)	8 (3.753–12.247)			20 (9.671–30.329)		
GGT, U/l							
≤ 100	45 (42.1)	13 (3.341–22.659)		.225	25 (15.125–34.875)		.080
> 100	62 (57.9)	11 (6.586–15.414)			19 (12.674–25.326)		
Bilirubin, mg/dl							
≤ 1	85 (79.4)	12 (6.830–17.170)		.909	20 (15.746–24.254)		.986
> 1	22 (20.6)	8 (1.598–14.402)			22 (4.523–39.477)		
Alkaline phosphatase, U/l							
≤ 100	37 (34.6)	18 (8.585–27.415)		.076	28 (n.a.)	1	**.018**
> 100	70 (65.4)	10 (7.035–12.965)			18 (9.172–26.828)	1.854 (1.096–3.137)	
Platelet count, 1/nl							
≤ 200	27 (25.2)	8 (1.038–14.962)		.465	18 (3.400–32.600)		.051
> 200	80 (74.8)	12 (4.556–19.444)			25 (13.197–36.803)		
INR							
≤ 1	57 (53.3)	13 (5.176–20.824)		.200	25 (15.593–34.407)		.125
> 1	50 (46.7)	11 (7.227–14.773)			14 (7.408–20.592)		
Hemoglobin, g/dl							
≤ 12	29 (27.1)	11 (1.866–20.134)		.078	6 (0.000–13.911)	1	**.000**
> 12	78 (72.9)	13 (5.490–20.510)			25 (4.034–45.966)	0.426 (0.262-0.690)	
CRP, mg/l							
≤ 10	56 (52.3)	13 (6.215–19.785)		.133	28 (4.327–51.673)	1	**.008**
> 10	51 (47.7)	10 (5.677–14.323)			14 (3.779–24.221)	1.845 (1.155–2.946)	
Knife-to-skin time, min							
≤ 300	63 (58.3)	13 (6.240–19.760)		.168	22 (14.682–29.318)		.307
> 300	45 (41.7)	8 (2.638–13.362)			20 (5.522–34.478)		
Blood transfusions							
No	71 (65.7)	15 (7.864–22.136)	1	**.025**	25 (14.793–35.207)	1	**.008**
Yes	37 (34.3)	8 (1.237–14.763)	1.756 (1.054–2.926)		10 (0.000–20.479)	1.866 (1.163–2.993)	
FFP							
No	64 (59.3)	12 (6.499–17.501)		.344	25 (16.927–33.073)		.100
Continued							

	n = 112	Median DFS (95%*CI*)	*HR*	*p*†	Median OS (95%CI)	*HR*	*p*†
Yes	44 (40.7)	12 (7.183–16.817)			14 (4.115–23.885)		
R1 status							
R0	80 (74.1)	12 (5.259–18.741)		.068	29 (10.817–47.183)	2.136 (1.261–3.618)	**.003**
R1/Rx	23 (21.3)	8 (6.200–9.800)			9 (3.131–14.869)		
MVI							
No	63 (63.0)	13 (0.000–28.857)	1	**.006**	25 (12.755–37.245)	1	**.000**
Yes	37 (37.0)	9 (6.106–11.894)	2.076 (1.250–3.447)		20 (14.607–25.393)	1.339 (0.818–2.192)	
LVI							
No	71 (72.4)	12 (4.656–19.344)	1	**.015**	40 (16.205–63.795)	1	**.000**
Yes	27 (27.6)	4 (0.000–10.174)	2.022 (1.115–3.668)		4 (2.735–5.265)	4.274 (2.536–7.204)	
Tumor grading							
G1/G2	61 (56.5)	12 (6.592–17.408)		.417	28 (15.116–40.884)		.120
G3/G4	31 (28.7)	10 (5.852–14.148)			12 (2.726–21.274)		
Tumor stage UICC							
I/II	53 (55.7)	18 (9.317–26.683)	1	**.000**	50 (28.949–71.051)	1	**.000**
III/IV	42 (44.2)	7 (3.635–10.365)	2.509 (1.462–4.305)		9 (4.469–13.531)	3.463 (2.085–5.753)	
pT category							
pT1–2	98 (90.7)	12 (7.233–16.767)		.237	22 (13.909–30.091)	1	**.009**
pT3–4	9 (8.3)	9 (1.791–16.209)			9 (6.228–11.772)	2.571 (1.218–5.428)	
N category							
pN0	55 (57.9)	18 (9.534–26.466)	2.017 (1.172–3.469)	**.008**	50 (34.936)	1	**.000**
pN1	40 (42.1)	6 (1.545–10.455)			7 (1.687–12.313)	3.129 (1.885–5.196)	
Length of ICU stay, days							
≤ 1	70 (64.8)	13 (8.394–17.606)		.289	29 (12.239–45.761)	1	**.007**
> 1	38 (35.2)	8 (4.894–11.106)			7 (0.000–15.359)	1.877 (1.169–3.012)	
Length of hospitalization, days							
≤ 14	60 (55.6)	12 (6.058–17.942)		.279	29 (4.196–53.804)	1	**.003**
> 14	48 (44.4)	8 (3.550–12.450)			13 (1.965–24.035)	1.970 (1.238–3.134)	
CCI							
≤ 40	72 (64.3)	12 (6.413–17.587)		.167	28 (6.693–49.307)	1	**.006**
> 40	35 (31.3)	8 (4.656–11.344)			13 (.000–27.602)	1.937 (1.195–3.140)	
Adjuvant therapy							
No	51 (45.5)	39 (n.a.)	1	**.000**	22 (12.882–31.118)		.425
Yes	56 (50.0)	7 (5.742–8.258)	3.320 (1.893–5.820)		20 (11.527–28.473)		
Tumor recurrence							
No	42 (37.5)	n.a		n.a	25 (0.000–86.963)		.134
Yes	65 (58.0)	n.a			20 (16.995–23.005)		

Table 1. Patient characteristics in association with disease-free and overall survival in iCCA. *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *BMI* body mass index, *CCA* cholangiocarcinoma, *CCI* comprehensive complication index, *CI* confidence interval, *CRP* C-reactive protein, *DFS* disease-free survival, *EBD* endoscopic biliary drainage, *FFP* fresh frozen plasma, *GGT* gamma-glutamyl transferase, *HR* hazard ratio, *ICU* intensive care unit, *INR* international normalized ratio, *LVI* lymphovascular invasion, *MVI* microvascular invasion, *OS* overall survival, *PBD* percutaneous biliary drainage, *PVE* portal venous embolization, *UICC* union internationale contre le cancer. †Based on log-rank test. Significant values are in bold.

reverse-primers, which were designed with the National Library of Medicine gene database and then controlled for alternative binding sites with the NCBI/National Center for Biotechnology Information primer blast function. The amplicon was digested with appropriate DNA restriction endonucleases specific for the SNP regions (New England Biolabs, MA, USA) (Supplementary Table 2). Then, the reaction products were separated on a 4% agarose gel at 120 mV for 60 min and visualized (GelDoc, Bio-Rad Laboratories GmbH, Feldkirchen, Germany) together with a 50 base pair DNA ladder. Based on the visualized fragment length and count, it was determined whether the region targeted by the restriction nucleases was digested. Appropriate positive (homocygous genotype of the smaller digested fragment) and negative controls (mastermix plus restriction enzyme, without DNA) were included on the gels. For quality control, 10% of positive and negative samples were randomly selected and re-genotyped with a genotype concordance ≥ 98%.

Endpoints and statistical analysis. Disease-free survival (DFS) was defined as the period between surgery and first recurrence and patients were censored if they died without recurring. Overall survival (OS) was defined as the period between surgery and death without censoring for perioperative mortality. Individuals lost to follow-up were censored at the time of last patient contact. Differences in categorical variables were evaluated using two-tailed Fisher's exact test and chi-squared test, in continuous variables with the *Mann*–Whitney *U* test. Kruskal–Wallis test was used to compare non-parametric variables with more than two groups. Continuous clinical variables were dichotomized at the median for the categorical presentation in the survival analysis. Differences in DFS and OS between genotypes were assessed with Kaplan–Meier analysis and the log-rank test for group comparison. For SNPs with a homozygous minor allele frequency < 10% in the study population, a dominant model was employed to test associations between genotypes and clinical outcome. Otherwise, a codominant or additive model was used. Uni- and multivariable Cox proportional hazard models were employed to analyze the association of factors with DFS and OS. Hazard ratios (HR) were presented with 95% confidence intervals (CI). Due to the large number of examined variables, only variables significant in the univariable analyses were included in the multivariable analyses, with an exclusion of parameters with potential collinearity. The level of significance was set to $p < 0.05$. Analyses were performed with SPSS Statistics (v23, IBM Corp., Armonk, NY, USA).

Statement of ethics. This research complies with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Study approval statement. This study protocol was reviewed and approved by institutional review board of the RWTH Aachen University (EK 360/15, EK 173/06). For this study informed consent has been waived by Institutional review board of the RWTH Aachen University, EK 360/15, EK 173/06 due to the anonymity and retrospective nature of the study.

Results

Clinical and histopathological characteristics and clinical outcome. Of the 112 patients undergoing curative-intent surgery for localized iCCA, 48 (43%) patients were male and 64 (57%) were female. Median age in this cohort at the time of surgical resection was 65 (range: 31–87) years. A total of 57 (51%) of patients received adjuvant chemotherapy, predominantly (27%, 30/112) with Gemcitabine/Cisplatin regimens and 11 (10%) patients underwent adjuvant radiotherapy (Table 1, Supplementary Table 3). During the follow-up period, 64 (57%) patients recurred and 74 (66%) died. Median follow-up was 25 months, with a median DFS of 12 months and median OS of 25 months. Blood transfusions, microvascular and lymphovascular invasion, lymph node positivity, UICC stage III/IV and adjuvant treatment were significantly associated with DFS and OS, while, preoperative alkaline phosphatase > 100 U/l, preoperative Hemoglobin < 12 g/dl, preoperative C-reactive protein > 10 g/dl, resection status Rx or R1, Comprehensive complication index (CCI) > 40, prolonged hospitalization > 14 days, T category T3 or T4 and intensive care unit stay > 1 day were associated with inferior OS but not DFS. There were no further associations between clinical, demographic or histopathological characteristics and DFS or OS (Table 1).

DFS and OS in CCA associated with IL-1B C+3954 T SNP. Genotyping for *IL-1B* C+3954 T (rs1143634) was successful in 112/112 patients (100%), with 65% (73/112) homozygous for the C-allele (C/C), 28% (31/112) heterozygous (C/T) and 7% (8/112) homozygous for the T-allele (T/T), corresponding to allele frequencies of C = 0.790 and T = 0.210, and therefore with great similarity to the reference allele frequencies in European populations, which are C = 0.763 and T = 0.237[23]. The C/T and T/T genotypes were pooled in an additive model ("any T allele") due to the low incidence of the homozygous T/T genotype. Median DFS for patients with the *IL-1B* +3954 C/C genotype was 9 months (95% CI 5.9–12.1 months, HR = 1.983), while for patients with any T allele (C/T or T/T) it was 24 months (estimates not reached for 95% CI, log-rank p = 0.012, Table 2). Patients homozygous for the *IL-1B* +3954 C-allele (C/C) had a median OS of 19 months (95% CI 13.0–19.0 months, HR = 1.735), while patients with a *IL-1B* +3954 C/T or T/T genotype had a median OS of 44 months (95% CI 3.9–84.0 months, log-rank p = 0.034) (Fig. 1). The clinical variables significantly associated with DFS or OS (Table 1) were equally distributed across the C/C and C/T / T/T groups (Supplementary Table 4).

OS in CCA associated with IL-8 T-251A SNP. The genotyping for *IL-8* T-251A (rs4073) was successful in 95% (106/112) of cases, in the remaining 6 cases the quantity of the extracted genomic DNA was insufficient for analysis. Eighteen percent (18%, 20/112) of patients were homozygous for the *IL-8* -251 A-allele (A/A), 47% (53/112) heterozygous *IL-8* -251 T/A and 30% (33/112) homozygous for the T-allele (T/T). Thus, the allele frequencies in our cohort (A = 0.439, T = 0.561) were consistent with the allele frequencies reported in European reference populations (A = 0.454, T = 0.546)[24]. Clinico-pathological characteristics were equally distributed across genotypes (Supplementary Table 5). While *IL-8* T-251A were not significant for DFS, a significant association with survival was observed: Patients with a A/A genotype had a median OS of 32 months (95% CI 6.3–57.7 months), patients with an *IL-8* -251 T/A genotype had a median OS of 13 months (95% CI 2.7–23.3 months, HR 2.001), whereas patients homozygous for the T-allele (T/T) had a median OS of 40 months (95% CI 14.8–65.2 months, HR 1.177, log-rank $p = 0.026$) (Fig. 1).

Multivariable analysis and combined subgroup analysis. We did not observe statistically significant associations between other tested genes involved in the tumor immune environment and DFS or OS (Table 2).

Gene, SNP		n, (%)	Disease-free survival (DFS)			Overall survival (OS)		
			Median DFS, m (95% *CI*)	Hazard ratio (95% *CI*)	$p^{\dagger}$	Median OS, m (95% *CI*)	Hazard ratio (95% *CI*)	$p^{\dagger}$
VEGF + 936 (C > T, rs3025039)	CC	76 (67.9)	13 (5.3–20.7)	1 (reference)	.157	21 (10.0–32.0)	1 (reference)	.674
	CT	35 (31.3)	9 (4.0–14.0)	1.425 (0.855–2.375)		20 (12.4–27.6)	1.110 (0.678–1.816)	
	TT	1 (0.9)						
EGF + 61 (A > G, rs4444903)	AA	48 (42.9)	16 (8.2–23.8)	1 (reference)	.669	21 (5.5–36.5)	1 (reference)	.753
	AG	64 (57.1)	10 (5.5–14.5)	1.111 (0.676–1.826)		22 (13.8–30.2)	1.077 (0.674–1.721)	
	GG	0 (0.0)						
EGFR-1562 (G > A, rs2227983)	GG	38 (33.9)	10 (5.8–14.2)	1 (reference)	.847	22 (7.6–36.4)	1 (reference)	.262
	GA	45 (40.2)	16 (8.2–23.8)	1.013 (0.565–1.815)		28 (6.8–49.2)	1.185 (0.645–2.178)	
	AA	27 (24.1)	8 (.9–15.1)	1.190 (0.605–2.343)		13 (7.1–18.9)	0.737 (0.426–1.277)	
IL-1B +3954 (C > T, rs1143634)	TT	8 (7.1)	24 (n.a.)	1 (reference)	**.012**	44 (3.9–84.0)	1 (reference)	**.034**
	CT	31 (27.7)						
	CC	73 (65.2)	9 (5.9–12.1)	1.983 (1.139–3.453)		19 (13.0–25.0)	1.735 (1.043–2.886)	
IL-6-174 (G > C, rs1800795)	GG	41 (36.6)	12 (0.0–28.6)	1 (reference)	.413	29 (0.0–69.2)	1 (reference)	.237
	GC	53 (47.3)	12 (6.3–17.7)	1.112 (0.644–1.918)		18 (10.3–25.7)	1.537 (0.914–2.587)	
	CC	16 (14.3)	8 (2.9–13.1)	1.590 (0.781–3.239)		22 (13.1–30.9)	1.465 (0.714–3.004)	
IL-8-251 (T > A, rs4073)	TT	33 (29.5)	12 (3.3–20.7)	1 (reference)	.679	40 (14.8–65.2)	1 (reference)	**.026**
	TA	53 (47.3)	12 (7.7–16.3)	1.203 (0.674–2.149)		13 (2.7–23.3)	2.001 (1.144–3.498)	
	AA	20 (17.9)	8 (0.2–15.8)	1.339 (0.661–2.714)		32 (6.3–57.7)	1.177 (0.566–2.445)	
IL-10-592 (T > G, rs1800872)	TT	5 (4.5)	12 (6.9–17.1)	1 (reference)	.380	19 (11.9–26.1)	1 (reference)	.928
	TG	53 (47.3)						
	GG	54 (48.2)	10 (5.6–14.4)	1.239 (0.758–2.023)		22 (15.9–28.1)	0.979 (0.616–1.557)	
CXCR1 + 860(Ex2) (C > G, rs2234671)	CC	102 (91.1)	12 (8.6–15.4)	1 (reference)	.638	20 (16.3–23.7)	1 (reference)	.662
	CG	10 (8.9)	7 (1.9–12.1)	0.789 (0.286–2.175)		25 (0.0–82.8)	0.817 (0.353–1.892)	
	GG	0 (0.0)						
HIF1α-1772 (C > T, rs11549465)	CC	88 (78.6)	11 (8.0–14.0)	1 (reference)	.461	19 (11.3–26.6)	1 (reference)	.676
	CT	23 (20.5)	18 (8.1–27.9)	0.804 (0.443–1.459)		28 (5.0–51.0)	0.889 (0.507–1.558)	
	TT	0 (0.0)						
PTGS2 (COX2) + 8473 (A > G, rs5275)	AA	49 (43.8)	12 (9.2–14.8)	1 (reference)	.669	20 (8.9–31.0)	1 (reference)	.630
	AG	48 (42.9)	12 (6.3–17.7)	0.988 (0.588–1.661)		25 (11.9–38.1)	0.891 (0.547–1.451)	
	GG	12 (10.0)	18 (2.1–33.9)	0.632 (0.283–1.650)		29 (0.0–62.9)	0.694 (0.307–1.567)	
PTGS2 (COX2)-765 (G > C, rs20417)	GG	81 (72.3)	11 (6.9–15.1)	1 (reference)	.993	22 (12.9–31.1)	1 (reference)	.360
	GC	25 (22.3)	13 (3.8–22.3)	0.998 (0.578–1.722)		29 (2.6–55.4)	0.783 (0.459–1.334)	
	CC	4 (3.5)						

Table 2. Genetic polymorphisms in association with disease-free and overall survival. Based on Cox proportional hazards model, for DFS including: Blood transfusions, microvascular invasion, lymphovascular invasion, lymph node positivity, UICC stage. For OS including: Alkaline phosphatase, hemoglobin, C-reactive protein, blood transfusions, microvascular invasion, lymphovascular invasion, lymph node positivity, UICC stage, comprehensive complication index and hospitalization. Also see Supplementary Table 2 and 3. *n.a.* estimates not reached, *CI* confidence interval, *CXCR* chemokine receptor, *EGF* epidermal growth factor, *EGFR* epidermal growth factor receptor, *HIF-1α* hypoxia-inducing factor alpha, *IL* interleukin, *PTGS* prostaglandin-endoperoxide synthase 2, *SNP* single-nucleotide polymorphism, *VEGF* vascular endothelial growth factor, *iCCA* intrahepatic cholangiocarcinoma. [†]Based on log-rank test. [$]Significances given for the T/A *vs.* T/T groups and for the "2 unfavorable" *vs.* "0 unfavorable" groups. Significant values are in bold.

Multivariable analysis of the significant SNPs adjusted for the significant clinico-pathological variables from univariable outcome analysis was performed. DFS did not independently correlate with any SNP. However, multivariable analysis confirmed an independent prognostic effect of the *IL-1B* +3954 (p = 0.013) and the *IL-8* -251 (p = 0.026) polymorphism for OS (Table 3, Supplementary Table 6).

Aiming to establish a novel genetic risk-score based on *IL-1β* +3954 and *IL-8* -251, we further stratified the cohort into patients without unfavorable alleles (*IL-1B* +3954 T/T or T/C genotype and *IL-8* -251 T/T genotype, n = 14), with 1 unfavorable allele (*IL-1B* +3954 C/C genotype or *IL-8* -251 T/A or A/A genotype, n = 39) and with 2 unfavorable alleles (*IL-Bβ* +3954 C/C genotype and *IL-8* -251 T/A or A/A genotype, n = 49). While this stratification did not reach a significant association with DFS (p = 0.056, Fig. 2A), it was significantly associated with OS (no unfavorable allele, 36 months median OS, 1 unfavorable allele, 25 months median OS, 2 unfavorable alleles, 13 months median OS, p = 0.005, Fig. 2B). Multivariable analysis with significant clinico-pathological characteristics from univariable analysis confirmed the independent prognostic effect of this allele grouping (p = 0.007, Table 3, Supplementary Table 7).

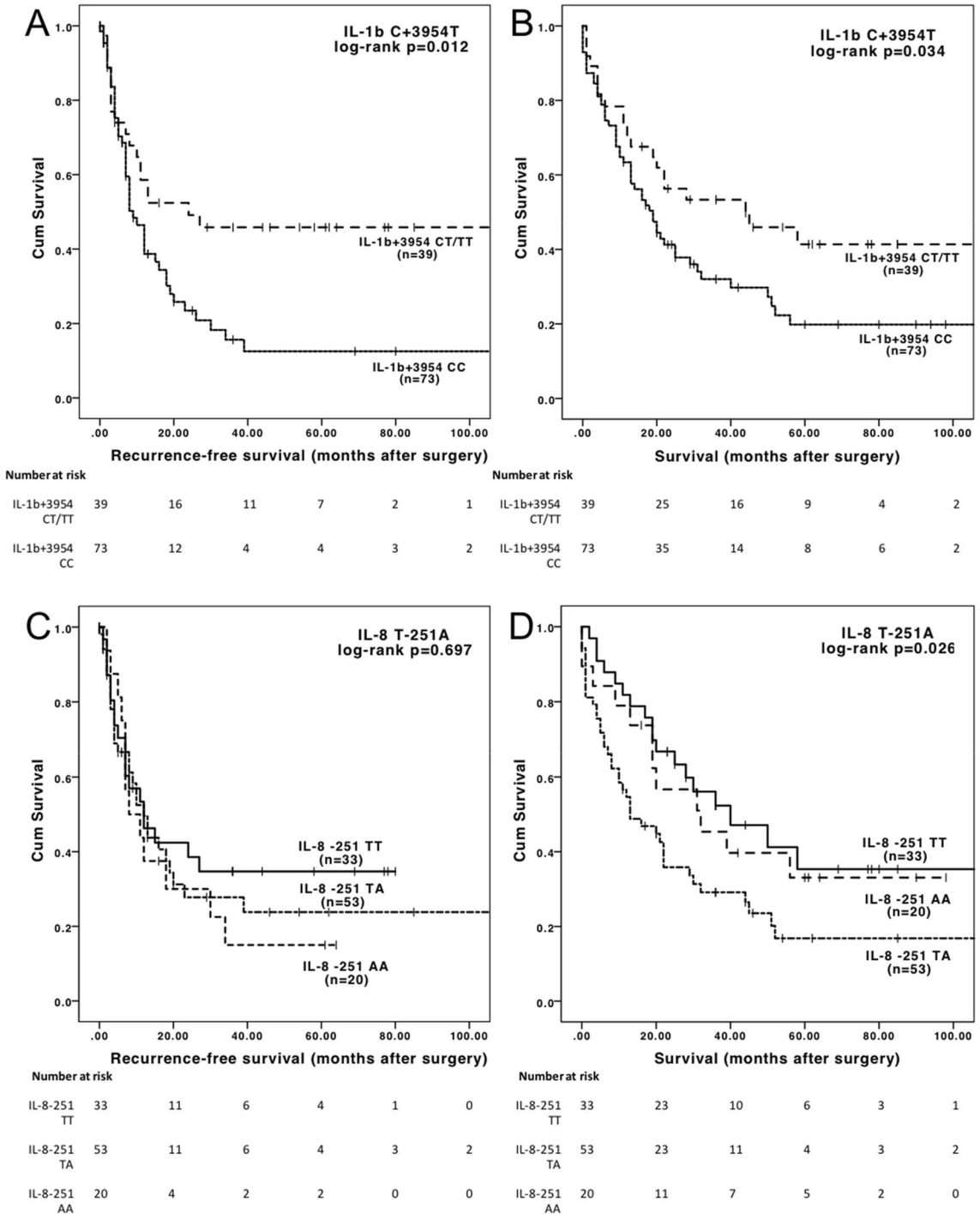

Figure 1. (**A**) Recurrence-free and (**B**) overall survival of patients by *IL-1B* C+3954 T polymorphism. (**C**) Recurrence-free and (**D**) overall survival of patients by *IL-8* T-251A polymorphism. Indicated p values are pooled, the post-hoc pairwise comparisons are as follows for (**D**) *IL-8*-251 TT versus *IL-8*-251 TA log-rank p = 0.013; *IL-8*-251 TT versus *IL-8*-251 AA log-rank $p = 0.677$; *IL-8*-251 TA versus *IL-8*-251 AA log-rank $p = 0.119$.

Conclusion

Intrahepatic CCA is a relatively rare, but highly aggressive gastrointestinal malignancy that frequently recurs even after major liver resections[7]. In this study, we analyzed polymorphisms in genes driving tumor-associated immunosuppression and neovascularization to determine their prognostic value in a large and homogenous Western cohort of iCCA patients. As such, we found that patients with the *IL-1B* +3954 C/C genotype had shorter DFS and OS, while patients with an *IL-8* -251 T/A or A/A genotype had shorter OS. Both polymorphisms were

	Disease-free survival (DFS)		Overall survival (OS)	
	Hazard ratio (95% *CI*)[§]	*p*	Hazard ratio (95% *CI*)[#]	*p*
IL-1B +3954 C > T *(rs1143634)*		.526		**.013**
C/T or T/T (favorable)	1		1	
C/C (unfavorable)	1.233 (.645–2.360)		2.444 (1.204–4.962)	
IL-8 -251 T > A *(rs4073)*		n.a.[&]		**.026**
T/T (favorable)	n.a.[&]		1	
T/A (unfavorable)	n.a.[&]		2.318 (1.158–4.640)	
A/A (unfavorable)	n.a.[&]		1.967 (.363–2.577)	
Combined		n.a.[&]		
0 unfavorable	n.a.[&]		1	**.007**
1 unfavorable	n.a.[&]		0.880 (0.268–2.895)	
2 unfavorable	n.a.[&]		2.395 (0.747–7.895)	

Table 3. Multivariable Cox regression analysis of *IL-1B* and *IL-8* polymorphisms disease-free and overall survival in iCCA. Based on Cox proportional hazards model, for DFS including: blood transfusions, microvascular invasion, lymphovascular invasion, lymph node positivity, UICC stage. For OS including: alkaline phosphatase, hemoglobin, C-reactive protein, blood transfusions, microvascular invasion, lymphovascular invasion, lymph node positivity, UICC stage, comprehensive complication index and hospitalization. Also see Supplementary Table 2 and 3. *CI* confidence interval, *iCCA* intrahepatic cholangiocarcinoma, *IL* interleukin. [§]83 patients with complete data were included in the model. [#]84 patients with complete data were included in the model. [&]Not significant in univariable analysis (log-rank test), therefore variable was not included in multivariable analysis. Significant values are in bold.

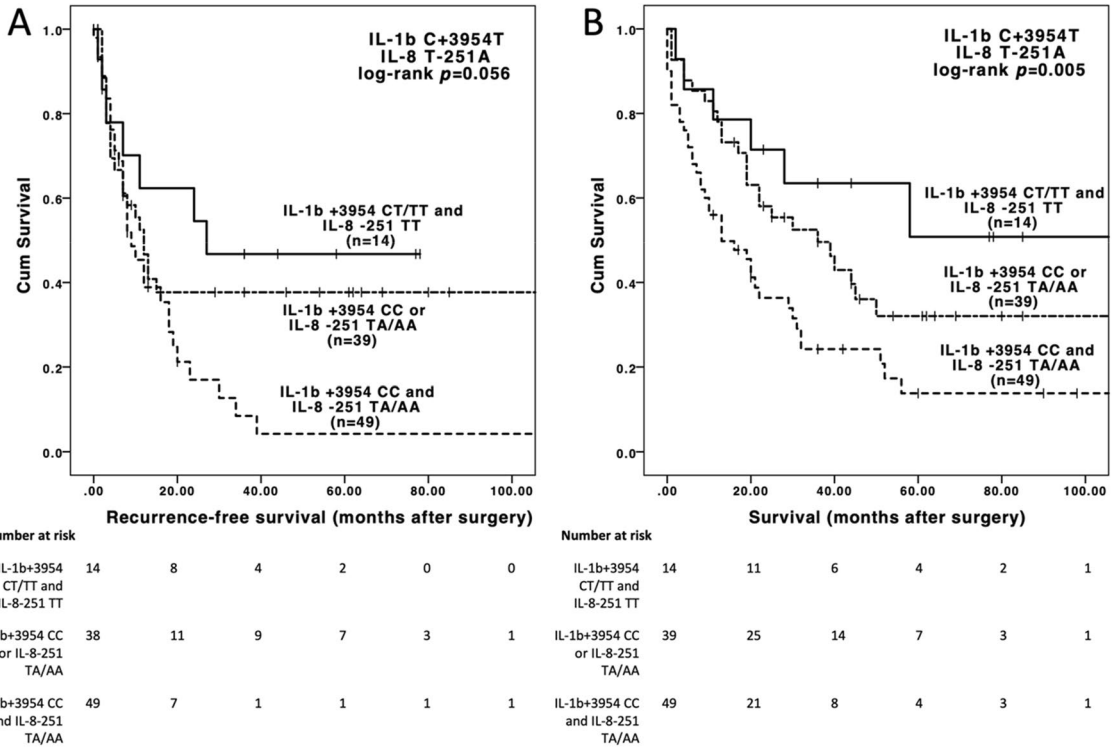

Figure 2. (**A**) Recurrence-free and (**B**) overall survival of patients by 0, 1 or 2 unfavorable *IL-1B* and *IL-8* alleles, *IL-1B* +3954 CC and *IL-8*-251 TA/AA genotypes were considered unfavorable. Indicated p values are pooled, the post hoc pairwise comparisons are as follows: (a) 0 unvafourable versus 1 unfavorable allele log-rank $p = 0.517$; 0 unvafourable versus 2 unfavorable alleles log-rank $p = 0.013$; 1 unvafourable versus 2 unfavorable alleles log-rank $p = 0.083$. (b) 0 unvafourable versus 1 unfavorable allele log-rank $p = 0.263$; 0 unvafourable versus 2 unfavorable alleles log-rank $p = 0.01$; 1 unvafourable versus 2 unfavorable alleles log-rank $p = 0.12$.

confirmed as independent prognostic factors for OS in multivariable analysis. Combining these allowed for patient stratification into survival groups by the number of unfavorable alleles.

IL-1β signals through binding to the receptor IL-1R1, which is widely expressed on various leucocyte populations and frequently across epithelial tissues[25]. Physiological effects include the expansion of hematopoietic progenitors, regulation of emergency hematopoiesis and prolonged survival of neutrophils and monocytes-macrophages[26]. The oncological relevance of IL-1β signaling was recently demonstrated in IL1β-deficient mice, which showed inhibited tumor growth in various tumor entities and retained antitumor immunity[27]. IL-1β signaling drives carcinogenesis by several mechanisms, including sustained inflammation with preferential macrophage and neutrophil recruitment, angiogenesis and immunosuppression[28].

Recently, a TME-based prognostic classification of iCCA identified a distinct M2-polarized macrophage-dominated subtype (I3), which was associated with inferior survival compared to subtypes devoid of any immune infiltration (I1) and lymphoid- and myeloid-enriched tumors (I2)[29]. Interestingly, the potential of targeting IL-1β-mediated cancer immune evasion has been translated into clinical trials in other gastrointestinal malignancies[30].

In our cohort, patients with the *IL-1B* +3954 (rs1143634) had a median OS of 19 months as opposed to 44 months with a C/T or T/T genotype. Functional data on the *IL-1B* rs1143634 SNP is limited to non-oncological studies, with evidence that in the systemic circulation, the SNP translates to higher IL-1β production by monocytes without any qualitative changes of the protein, both *in vitro*[31] and at sites of infection[32]. Due to a lack of functional data from hepatic or tumor immunology, the exact effects on the CCA TME remain to be determined.

We furthermore observed an independent association of *IL-8* T-251A SNP with OS. IL-8 signaling has been previously identified as a central regulator of VEGF-independent and HIF1α-independent angiogenesis in gastrointestinal malignancies[33], signaling through the CXCR1/CXCR2 receptors[34]. Typical origins of IL-8 in the iCCA TME are suggested to be endothelial cells and CAFs, along with infiltrating myeloid cells[35,36]. CXCR1 is physiologically found on granulocytes, monocytes, mast cells and natural killer cells, but also on cancer cells and the TME, where the signaling mediates immunosuppressive responses[34].

Previously, the *IL-8* -251 T > A polymorphism has been linked to shortened DFS in stage III colon cancer and to shortened DFS and OS in localized gastric cancer[20,37]. The SNP is localized in the *IL-8* promoter region and effects a higher expression of IL-8 with higher serum levels compared to wildtype individuals[38]. Furthermore, the IL-8 -251 A allele has been associated to increased IL-8 mucosal tissue levels, inflammation, metaplasia and carcinogenesis in individuals with Helicobacter pylori infection[39]. In this study, no clear association of the A/A genotype with DFS or OS was demonstrated. Only a small subgroup of our cohort (20 patients, 17.9%) harbored the *IL-8* -251 A/A genotype, potentially increasing the risk of type 2 error, and, at the same time, the risk of type 1 error for significant findings for the TA subgroup.

Our group was the first to recently describe a relevant prognostic value of gene polymorphisms in patients with CCA[40]. As such, the *CXCR1 (rs2234671)* SNP, (IL-8 receptor) was associated with decreased DFS and OS in surgical pCCA patients. This polymorphism is presumed to enhance intracellular CXCR1-signaling, leading to stronger IL-8 effects[41]. In keeping with the prognostic effects of *IL-8* variations observed in this study, this underlines the importance of the IL-8 pathway in the TME of CCA. In keeping with emerging evidence on biological differences of the two tumor localizations[3], this difference in the relevance of prognostic polymorphisms supports the concept of pluralistic roles in the TME.

As with most clinical outcome studies, this analysis has some inherent limitations. First, this is a retrospective, single-center analysis that requires prospective external validation. Second, while this is a very homogenous cohort in terms of patient selection and surgical approach, the present study completed recruitment in 2019, the same year when the BILCAP study provided universal level I evidence for adjuvant capecitabine treatment[42]. However, sufficiently powered biomarker studies with long-term outcomes in this rare tumor entity may require several more years to complete patient recruitment under the BILCAP selection criteria. Third, while the exclusion of patients with extrahepatic spread and inoperable disease afforded an extremely homogenous patient cohort, our findings may not be representative of all patients with iCCA. Fourth, while this cohort is relatively large for a single-center study, the relatively low total number of events leads to a risk of statistical overfitting, thus warranting further external validation. Fifth, we examined only ten genes in a pathway-driven approach, with the potential to expand the current analysis to larger gene panels. Our preliminary findings should thus be regarded as hypothesis-generating until confirmed in independent cohorts.

The potential of the present study, compared to other prognostic factors for hepatobiliary malignancies, is the fact that genetic variants can be accessed from any genetic material, including blood leucocytes, thus constituting a potential preoperative biomarker. As patients with iCCA often require extensive and high-risk resections, the present study may contribute to preoperative oncological and outcome considerations.

This study in a large and homogenously treated iCCA cohort reveals a potential prognostic value of the *IL-1B* +3954 and the *IL-8* -251 polymorphism for OS after curative-intent surgery for iCCA, consistent with our hypothesis that genetic variants of tumor-mediated immune suppression and angiogenesis may have additional clinical value for prognostic patient stratification. Potentially, our findings may translate into the identification of novel therapeutic targets for this understudied tumor entity. Biomarker-embedded clinical trials and a validation in independent patient cohorts are required to confirm our findings.

Data availability

The datasets generated and/or analysed during the current study are available in the ClinVar repository (ClinVar assession number: SCV004011734—SCV004011744, summary report: https://submit.ncbi.nlm.nih.gov/api/2.0/files/9wosnzv0/sub13574875__100__submitter_report_b.txt/?format=attachment). Further relevant data were reported within the article. Further supporting data will be provided upon written request addressed to the corresponding author.

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Author contributions

The study was designed by the initiating study team (I.L., Z.C., G.L., U.P.N.). Data collection and analysis were performed by I.L., Z.C., N.T.G., U.P.N., and G.L. Laboratory experiments were conducted by I.L. The manuscript was drafted by I.L., Z.C. and G.L. Further authors (F.T., P.S., C.T., E.D., N.T.G., R.K. and U.P.N.) have substantially contributed to the final version of the manuscript. All authors have read and approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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2.3 Angioneogenese-und Hypoxie-assoziierte Genpolymorphismen beeinflussen die Prognose nach Resektion des Hepatozellulären Karzinoms

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Abstract Zitat (94):
„Tumor angiogenesis plays a pivotal role in hepatocellular carcinoma (HCC) biology. Identifying molecular prognostic markers is critical to further improve treatment selection in these patients. The present study analyzed a subset of 10 germline polymorphisms involved in tumor angiogenesis pathways and their impact on prognosis in HCC patients undergoing partial hepatectomy in a curative intent. Formalin-fixed paraffin-embedded (FFPE) tissues were obtained from 127 HCC patients at a German primary care hospital. Genomic DNA was extracted, and genotyping was carried out using polymerase chain reaction (PCR)-restriction fragment length polymorphism-based protocols. Polymorphisms in interleukin-8 (IL-8) (rs4073; p = 0.047, log-rank test) and vascular endothelial growth factor (VEGF C + 936T) (rs3025039; p = 0.045, log-rank test) were significantly associated with disease-free survival (DFS). After adjusting for covariates in the multivariable model, IL-8 T-251A (rs4073) (adjusted p = 0.010) and a combination of "high-expression" variants of rs4073 and rs3025039 (adjusted p = 0.034) remained significantly associated with DFS. High-expression variants of IL-8 T-251A may serve as an independent molecular marker of prognosis in patients undergoing surgical resection for HCC. Assessment of the patients' individual genetic risks may help to identify patient subgroups at high risk for recurrence following curative-intent surgery.“

Die HCC-Rekurrenz nach erfolgter Resektion stellt mit einer Inzidenz von 40-70% innerhalb der ersten 5 postoperativen Jahre ein zentrales Problem der onkologischen Behandlung dar (95). In einem Pathway-basierten Ansatz analysierten wir funktionelle Polymorphismen der Angiogenese- und Hypoxie (*CXCL8, VEGF, EGFR, EGF-A, TP53, CXCR1, IL-1B, HIF1A, IL6, IL10*) von Patient*innen mit HCC, die sich einer Leberteilresektion in kurativer Intention unterzogen haben und analysierten ihre Assoziation zu onkologischer Prognose. Nach Extraktion genomischer DNA aus Formalin-fixiertem Patientenmaterial, wurden die entsprechenden Gene amplifiziert und mittels Restriktionsverdau genotypisiert.

Besonderheiten der analysierten Kohorte liegen in der, für chirurgische Verhältnisse, hohen Rate an Patient*innen mit fortgeschrittener Grunderkrankung der Leber:

etwa 50% der Patient*innen hatten eine Leberzirrhose, und etwa 40% sogar ein BCLC Tumorstadium B/C. Entsprechend vorherigen Publikationen war auch in diesem Patient*innkollektiv die Rekurrenzrate innerhalb des knapp 3-jährigen Follow-up-Zeitraums bei etwa 50%, also eine zentrale Limitation der chirurgischen Therapie in kurativer Intention.

Die Analyse von Keimbahn-Polymorphismen ergab eine signifikante Assoziation des *IL-8* T-251A Polymorphismus und der *VEGF* C+936T Polymorphismus mit krankheitsfreiem Überleben. Die Kombination der beiden prognostischen Allele erlaubte die Gruppierung in Patient*innen ohne unvorteilhafte Allele (medianes rekurrenzfreies Überleben, 33 Monate), mit einem unvorteilhaften Allel (medianes rekurrenzfreies Überleben, 20 Monate) und mit zwei unvorteilhaften Allelen (medianes rekurrenzfreies Überleben, 7 Monate).

Die hier gezeigten Ergebnisse bestätigen nicht nur die prognostische Relevanz von SNPs in Tumor-Wirt Wechselwirkungen, sondern zeigen klar eine genetische Hochrisiko-Konstellation auf, bei der Patient*innen eine signifikant frühere HCC-Rekurrenz aufweisen. Angesichts der Tatsache, dass das Rekurrenzmuster des HCCs nach Resektion fast immer ein Rezidiv in hepatischer Lokalisation impliziert (33), und bei rechtzeitiger Diagnose weiter in kurativer Intention loko-regional oder chirurgisch behandelbar ist (95), birgt die Identifikation dieser Hochrisikogruppe das Potenzial, mittels erhöhter Follow-up Frequenz rechtzeitig Rezidive zu erkennen oder diese Patient*innen potenziellen zukünftigen adjuvanten Therapien zuzuführen.

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Impact of Angiogenesis- and Hypoxia-Associated Polymorphisms on Tumor Recurrence in Patients with Hepatocellular Carcinoma Undergoing Surgical Resection

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Simple Summary: Hepatocellular carcinoma remains a leading cause of cancer-related death and the most common primary hepatic malignancy in the Western hemisphere. Previous research found that angiogenesis-related cytokines and elevated levels of interleukin 8 and vascular endothelial growth factor (VEGF) shorten the expected time of survival. Moreover, factors of tumor angiogenesis- and hypoxia-driven signaling pathways are already associated with worse outcome in disease-free survival in several tumor entities. Our study investigates the prognosis of hepatocellular carcinoma patients based on a selection of ten different single-nucleotide polymorphisms from angiogenesis, carcinogenesis, and hypoxia pathways. Our study with 127 patients found supporting evidence that polymorphisms in angiogenesis-associated pathways corelate with disease-free survival and clinical outcome in patients with hepatocellular carcinoma.

Abstract: Tumor angiogenesis plays a pivotal role in hepatocellular carcinoma (HCC) biology. Identifying molecular prognostic markers is critical to further improve treatment selection in these patients. The present study analyzed a subset of 10 germline polymorphisms involved in tumor angiogenesis pathways and their impact on prognosis in HCC patients undergoing partial hepatectomy in a curative intent. Formalin-fixed paraffin-embedded (FFPE) tissues were obtained from 127 HCC patients at a German primary care hospital. Genomic DNA was extracted, and genotyping was carried out using polymerase chain reaction (PCR)–restriction fragment length polymorphism-based protocols. Polymorphisms in interleukin-8 (IL-8) (rs4073; $p = 0.047$, log-rank test) and vascular endothelial growth factor (VEGF C + 936T) (rs3025039; $p = 0.045$, log-rank test) were significantly associated with disease-free survival (DFS). After adjusting for covariates in the multivariable model,

IL-8 T-251A (rs4073) (adjusted $p = 0.010$) and a combination of "high-expression" variants of rs4073 and rs3025039 (adjusted $p = 0.034$) remained significantly associated with DFS. High-expression variants of IL-8 T-251A may serve as an independent molecular marker of prognosis in patients undergoing surgical resection for HCC. Assessment of the patients' individual genetic risks may help to identify patient subgroups at high risk for recurrence following curative-intent surgery.

Keywords: hepatocellular carcinoma; single-nucleotide polymorphism; IL8

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy, and its mortality ranks fourth among solid tumors, behind carcinomas of the lung, colon, and the stomach [1,2]. As most HCCs develop in the background of chronic liver disease, liver transplantation is considered the optimal curative therapy because it treats both the tumor and the underlying liver disease [3–6]. In the context of donor allograft shortage, surgical resection has emerged as a viable treatment strategy even beyond early Barcelona Clinic Liver Cancer (BCLC) stages [3,7]. Despite continuous advances in patient selection, surgical technique, and medical treatments [5,6], the rate of local tumor recurrence exceeds 50% 4 years after partial hepatectomy [8,9]. Therefore, the implementation of prognostic molecular markers as an adjunct to traditional staging systems may not only be helpful in identifying patients prone to recurrence but can also aid patient-specific treatment selection.

In 1971, Judah Folkman introduced the hypothesis that angiogenesis, the formation of new blood vessels from endothelial precursors, is a prerequisite for the growth and progression of solid malignancies. Gaining access to the host vascular system and the generation of a tumor blood supply are rate-limiting steps in tumor growth and progression [10]. Among proangiogenic factors, vascular endothelial growth factor (VEGF), a sub-family of growth factors which is induced under hypoxic conditions through hypoxia-inducible factor (HIF)-1α promotor binding, plays a pivotal role in tumor angiogenesis [11,12]. The relevance of VEGF-dependent pathways for hepatocarcinogenesis was recently emphasized by the data from a phase 3 clinical trial, establishing the combination therapy of the VEGF inhibitor bevacizumab and the immune checkpoint inhibitor atezolizumab as the first-line systemic treatment in advanced HCC [13].

Additionally, tumors can sustain angiogenesis in a VEGF-independent manner [14]. As such, interleukin (IL)-8 (CXCL8) signaling preserves the angiogenic phenotype in HIF1-α-deficient colon cancer cells, indicating a critical role of IL-8 in tumor-associated angiogenesis, independent of VEGF [15]. Furthermore, elevated serum levels of IL-8 and single-nucleotide polymorphisms (SNPs) of VEGF and IL-8 are associated with shorter disease-free survival (DFS) and overall survival (OS) in HCC and other gastrointestinal malignancies [16–20].

Based on these data, we here employed a pathway-focused approach to investigate whether functional single-nucleotide polymorphisms of genes involved in angiogenesis, tumorigenesis, and hypoxia (Table 1) are associated with differences in clinical outcome in HCC patients undergoing partial hepatectomy in a curative intent.

Table 1. Genes, their function, and the minor allele frequency.

No.	Gene	Allele	SNP [1]	Allele Function	Minor Allele Freq.	Description	Ref.
1	IL-8 − 251	A > T	rs4073	A-allele: higher IL-8 plasma levels	45%	The dominant allele AT and AA is associated with a poor prognosis and may increase CXCL 6, which stimulates endothelial production	[21–25]
2	VEGF + 936	C > T	rs3025039	T-allele: lower VEGF plasma levels	20%	De novo vascularization, endothelial proliferation Increased risk of NASH C-allele associated with higher VEGF production	[21,26–28]
3	EGFR-497	G > A	rs2227983	A-allele: lower EGFR ligand binding, growth stimulation	29%	Identified in gastrointestinal and colorectal tumors	[29,30]
4	EGFA 61G	A > G	rs4444903	A-allele: lower EGF serum levels	45%	Increased susceptibility to hepatitis C	[30–32]
5	p53	C > G	rs1042522	Tumor suppressor, induces cell cycle arrest	35%	Associates with LiFraumeni syndrome, is detected as inducer of different types of cancer	[33]
6	CXCR	G > C	rs2234671	C-allele: higher ligand binding	10%	Interleukin-8 acts through CXCR receptor	[34]
7	IL-1b	C > T	rs1143634	Increased tumor growth and proliferation	20%	Stomach cell cancer, gall bladder cancer, breast cancer, and liver cell cancer	[35]
8	HIF1 A588T	C > T	rs11549465	T-allele with cancer associated	8%	Associated with tumor size and location in colorectal carcinoma, tumor tissue is over-stimulated with HIF1	[36,37]
9	IL-10	T > G	rs1800872	Higher Il-10, more immunosuppressive effect	30–40%	Increases risk of liver cell cancer, negatively modulates NFKappaB and TNF alpha production (inhibits gene expression)	[38]
10	IL-6	G > C	rs1800795	Proinflammatory cytokine, response to cancer	20%	Increased risk of cervical and renal cancer Stimulates endothelial production	[39,40]

Abbreviations used: CI, confidence interval; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; IL-1/6/8/10, interleukin-1/6/8/10; CXC2, chemokine receptor; HIFa, hypoxia-inducing factor alpha; NASH, non-alcoholic fatty liver disease; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-alpha, tumor-necrosis factor alpha; p53, protooncogene 53, [1] SNP, single nucleotide polymorphism Database NCBI.

2. Results

2.1. Patients Characteristics

Between 2010 and 2017, 127 patients with localized hepatocellular carcinoma were included in this study. Thirty-seven (37/127, 29.1%) patients were female and 90/127 (70.9%) were male; the median age was 67 years (15–89). The median body mass index (BMI) was 27 kg/m^2 (16.8–41). Sixty-four (64/127, 50.4%) patients had histologically confirmed cirrhosis, 76/127 (59.8%) had a single tumor, and 51/127 (39.3%) had more than one HCC lesion. Average diameter of tumor nodules on final pathology was 68 mm (6–228 mm). Median alpha-fetoprotein (AFP) at time of operation was 147.9 µL/L. Seventy-seven (77/127, 60.6%) patients belonged to BCLC category 0/A and fifty (50/123, 39.4%) patients to category B/C. Clinico-pathological characteristics are presented in Tables 2 and 3.

Table 2. Demographic and clinico-pathological characteristics and disease-free survival.

Variable	Cutoff	Total No. Patients (%)	Event in Group (%)	Median DFS (Months) (95% CI)	Relative Risk (95% CI)	*p* Value [1]
Sex	Female	37 (29.1%)	19 (51.4%)	38 (17.1–58.9)	0.763 (0.443–1.314)	0.329
	Male	90 (70.9%)	43 (47.8%)	26 (16.1–35.3)	1 (reference)	
Age (years)	<65	48 (37.8%)	26 (44.2%)	26 (12.7–39.3)	1.222 (0.736–2.032)	0.438
	>65	79 (62.2%)	36 (45.6%)	32 (17.0–47.0)	1 (reference)	
BMI	<25	51 (40.2%)	22 (43.1%)	35 (15.4–54.6)	0.790 (0.469–1.330)	0.375
	>25	76 (59.8%)	40 (52.6%)	26 (05.3–15.7)	1 (reference)	
Diameter (mm)	<50	49 (38.6%)	24 (49.0%)	39 (26.9–51.1)	0.695 (0.415–1.165)	0.168
	>50	78 (61.4%)	38 (48.7%)	21 (12.5–29.5)	1 (reference)	
T-category	T1/2	95 (74.8%)	51 (53.7%)	39 (12.1–65.9)	**0.407 (0.244–0.679)**	**<0.0001**
	T3/4	32 (25.2%)	12 (37.5%)	12 (05.1–18.9)	1 (reference)	
N-category	0	29 (22.8%)	13 (55.2%)	26 (05.4–47.0)	0.976 (0.543- 1.756)	0.936
	N	98 (74.7%)	41 (44.8%)	30 (12.9–47.1)	1 (reference)	
HCC tumor nodes	=1	51 (39.3%)	35 (68.6%)	12 (07.6–16.4)	**0.543 (0.342–0.862)**	**0.0001**
	>1	76 (59.8%)	27 (35.5%)	67 (43.3–90.7)	1 (reference)	
Steatosis	No	79 (61.9%)	39 (49.3%)	30 (18.2–41.8)	1.093 (0.652–1.833)	0.736
	Yes	48 (38.1%)	23 (47.9%)	25 (0.00–61.7)	1 (reference)	
Cirrhosis	No	63 (49.6%)	26 (41.3%)	58 (14.5–101.5)	**0.519 (0.308–0.874)**	**0.012**
	Yes	64 (50.4%)	36 (48.8%)	24 (15.7–32.3)	1 (reference)	
AFP (µL/L)	<100	50 (39.4%)	27 (54.0%)	32 (22.4–41.6)	0.732 (0.455–1.180)	0.200
	>100	77 (60.6%)	35 (55.5%)	24 (09.5–38.5)	1 (reference)	
BCLC Stage	0/A	77 (57.5%)	33 (42.8%)	65 (33.6–96.4)	**0.291 (0.171–0.496)**	**<0.0001**
	B/C	50 (39.4%)	33 (66.0%)	11 (06.7–15.2)	1 (reference)	

Values are given as median with 95% CI or numbers and (percentages). Results of the regression analysis are given as relative risk with 95% confidence interval. Bold is used to highlight significant results. [1] Based on log-rank test. BCLC—Barcelona Clinic Liver Cancer.

Table 3. Demographic and clinico-pathological characteristics and overall survival.

Variable	Cutoff	Total No. Patients (%)	Event in Group (%)	Median OS (Months) (95% CI)	Relative Risk (95% CI)	*p* Value [2]
Sex	Female	37 (29.1%)	15 (40.5%)	78 (56.0–89.3)	**0.410 (0.231–0.723)**	**0.002**
	Male	90 (70.9%)	59 (65.6%)	24 (16.0–32.0)	1 (reference)	
Age (years)	<65	48 (37.8%)	27 (46.2%)	38 (00.0–77.8)	0.840 (0.523–1.351)	0.473
	>65	79 (62.2%)	47 (59.5%)	32 (18.7–45.3)	1 (reference)	
BMI	<25	51 (40.2%)	28 (54.9%)	31 (12.3–49.7)	0.9 (0.562–1.441)	0.660
	>25	76 (59.8%)	46 (60.5%)	35 (22.2–47.8)	1 (reference)	
Diameter (mm)	<50	49 (38.6%)	25 (51.0%)	58 (36.2–79.8)	**0.601 (0.370–0.976)**	**0.035**
	>50	78 (61.4%)	49 (62.8%)	23 (15.6–30.4)	1 (reference)	
T-category	T1/2	95 (74.8%)	47 (51.6%)	47 (26.8–67.2)	**0.261 (0.060–1.131)**	**0.002**
	T3/4	32 (25.2%)	24 (55.0%)	15 (06.4–23.6)	1 (reference)	
N-category	0	29 (22.8%)	14 (48.3%)	65 (26.5–103.5)	0.591 (0.324–1.077)	0.082
	N	98 (74.7%)	46 (59.0%)	32 (18.0–46.0)	1 (reference)	
HCC tumor nodes	=1	51 (39.3%)	38 (50.0%)	42 (22.9–61.1)	**0.543 (0.342–0.862)**	**0.010**
	>1	76 (59.8%)	35 (70.0%)	20 (12.0–28.0)	1 (reference)	
Steatosis	No	79 (61.9%)	51 (65.4%)	30 (18.2–29.2)	1.590 (0.963–2.623)	0.070
	Yes	48 (38.1%)	22 (45.8%)	58 (29.2–86.8)	1 (reference)	

Table 3. *Cont.*

Variable	Cutoff	Total No. Patients (%)	Event in Group (%)	Median OS (Months) (95% CI)	Relative Risk (95% CI)	*p* Value [2]
Cirrhosis	No	63 (49.6%)	30 (47.6%)	55 (25.9–84.1)	0.515 (0.322–0.825)	0.005
	Yes	64 (50.4%)	44 (68.7%)	24 (11.9–36.1)	1 (reference)	
AFP (µL/L)	<100	50 (39.4%)	27 (54.0%)	42 (18.8–65.2)	1.025 (0.618–1.699)	0.924
	>100	77 (60.6%)	47 (61.0%)	29 (04.2–20.8)	1 (reference)	
BCLC Stage	0/A	77 (57.5%)	33 (42.8%)	47 (27.7–66.2)	**0.493 (0.309–0.789)**	**0.003**
	B/C	50 (39.4%)	33 (66.0%)	17 (09.1–24.9)	1 (reference)	

Values are given as median with 95% CI, numbers and (percentages). Results of the regression analyses are given as relative risk with 95% confidence interval. Bold is used to highlight significant results. [2] Based on log-rank test.

The median OS was 30.5 months (range 0–122 months), and the median DFS was 23 months (range 0–117 months). During the observation period, 74 (58.2%) patients died and 62 (48.8%) experienced tumor recurrence (Table 2). T-category 3 or 4 ($p < 0.001$), more than one tumor node ($p < 0.001$), BCLC stage B/C ($p < 0.001$), and the presence of hepatic cirrhosis ($p = 0.012$) were significantly associated with a shorter disease-free survival. T-category higher than 3 ($p = 0.002$), more than one tumor nodule ($p = 0.010$), a tumor diameter over 50 mm (p value = 0.035), male sex ($p = 0.002$), and the presence of hepatic cirrhosis ($p = 0.005$) were associated with shorter OS.

2.2. Analysis of IL-8 T-251A and Clinical Outcome

Genotyping of IL-8 T-251A was successful in 125 of 127 patients and was used for the subsequent analysis. Of these, sixty-two (62/125, 49.6%) patients recurred, while 63/125 (50.4%) patients did not show a recurrence within the observation period. An A/A genotype was identified in 14 (11.3%) patients, who showed a median DFS of median 20 (0–40.1) months compared to 65 patients with the T/T and 46 with the A/T allele with a median survival of 32 months (CI 18.34–5.8). IL-8 T-251A had a minor allele frequency of 11.3% and was analyzed in a codominant model. The unfavorable A/A allele was interpreted against A/T and T/T as favorable alleles (Table 4, Figure 1a). The *p*-value of the log-rank test was 0.047 (Table 4, Figure 1a). IL-8 T-251A was included in the multivariable analysis. IL-8 251 SNPs showed no significant association with OS.

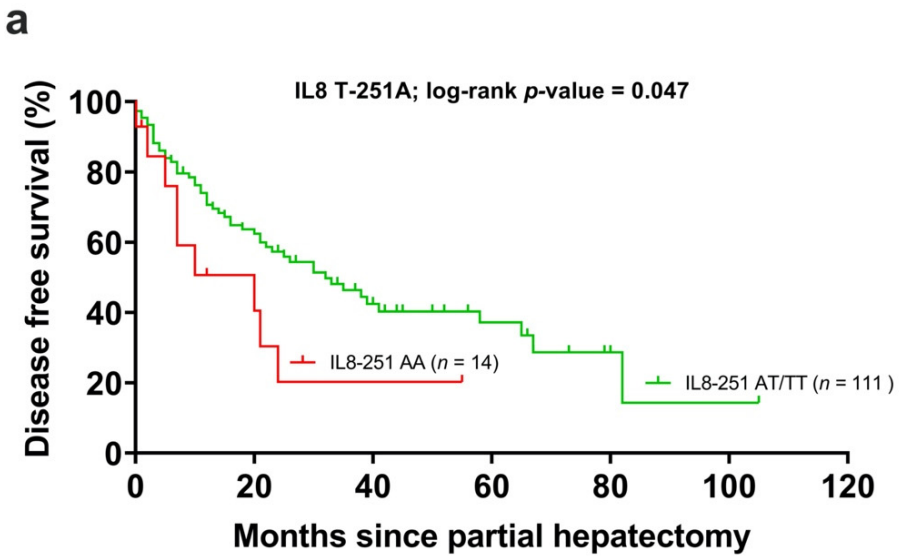

Figure 1. *Cont.*

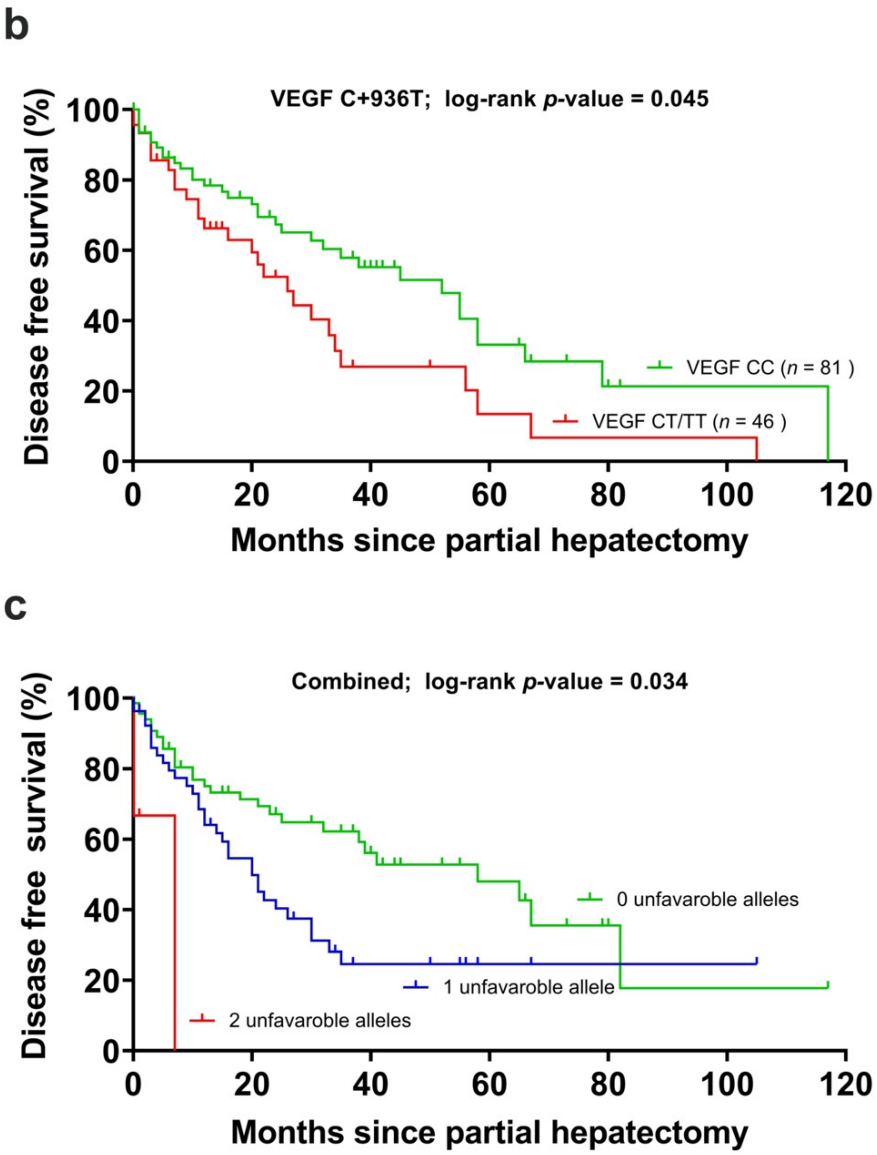

Figure 1. (**a**) Disease-free survival in the carriers of the interleukin-8 T-251A polymorphism, (**b**) the vascular endothelial growth factor C + 936T (VEGF) polymorphism and (**c**) the unfavorable alleles grouped together. Censored cases indicate the time of last follow-up for those patients who had neither recurred nor died at the time of the analysis of data.

Table 4. Polymorphisms of genes analyzed and disease-free survival (DFS) as well as overall survival in patients with hepatocellular carcinoma.

SNP		*n*	DFS			OS		
			Median DFS (Months) (95% CI)	**RR (95% CI)**	***p* Value**	**Median OS (Months) (95% CI)**	**RR (95% CI)**	***p* Value**
IL-8					**0.047**			0.366
	AA	14	20 (8.7–40.1)	**2.040 (1.000–4.179)**		22 (8.7–35.3)	0.710 (0.337–1.479)	
	AT [1]	65	32 (18.3–45.8)	1 (reference)		32 (10.5–53.5)	1 (reference)	
	TT [1]	46						
VEGF					**0.045**			0.350
	CT [1]	41	20 (12.0–30.0)	**1.668 (1.007–2.764)**		27 (18.1–36.0)	1.038 (0.646–1.667)	
	TT [1]	5						
	CC	81	41 (12.5–69.5)	1 (reference)		38 (27.0–50.0)	1 (reference)	
IL-1					0.120			0.229
	CC	54	51 (39.8–61.8)	0.583 (0.321–1.060)		38 (20.5–55.5)	0.657 (0.392–1.1)	
	TT	21	20 (12.1–27.8)	1.060 (0.545–2.063)		38 (0.0–90.0)	0.671 (0.335–1.342)	
	CT	43	25 (17.0–33.0)	1 (reference)		23 (8.0–38.0)	1 (reference)	

Table 4. *Cont.*

SNP		*n*	DFS			OS		
			Median DFS (Months) (95% CI)	**RR (95% CI)**	***p* Value**	**Median OS (Months) (95% CI)**	**RR (95% CI)**	***p* Value**
IL-6					0.301			0.415
	GG	54	25 (11.8–38.2)	1.552 (0.873–2.759)		32 (16.9–47.1)	0.856 (0.522–1.404)	
	CC	23	32 (1.4–62.7)	1.458 (0.719–2.955)		65 (12.2–117.7)	0.632 (0.318–1.258)	
	GC	50	38 (2.7–73.3)	1 (reference)		30 (14.8–45.2)	1 (reference)	
IL-10					0.580			0.456
	TT	7	21 (6.4–35.6)	1.172 (0.343–4.009)		33 (17.3–47.8)	0.914 (0.271–3.079)	
	GG	74	30 (16.0–44.0)	1.356 (0.763–2.410)		30 (21.0–39.0)	1.359 (0.802–2.301)	
	GT	41	30 (13.0–47.0)	1 (reference)		41 (19.7–46.3)	1 (reference)	
EGFA					0.145			0.073
	AA [1]	63	24 (0.9–47.1)	1.463 (0.877–2.441)		29 (15.8–42.2)	1.519 (0.959–2.406)	
	GG [1]	10						
	AG	50	30 (13.2–46.8)	1 (reference)		38 (18.7–57.3)	1 (reference)	
EGFR					0.393			0.523
	GG	50	18 (7.8–28.2)	1.272 (0.721–2.243)		23 (14.0–32.0)	1.177 (0.703–1.972)	
	AA	27	38 (0.0–76.3)	0.807 (0.385–1.690)		38 (0–81.9)	0.819 (0.420–1.597)	
	AG	45	33 (23.1–42.9)	1 (reference)		47 (22.4–71.6)	1 (reference)	
CXCR					0.693			0.847
	GG	29	30 (13.4–46.6)	1.012 (0.527–1.941)		42 (4.8–79.2)	0.836 (0.455–1.538)	
	CC	11	21 (15.5–26.5)	1.414 (0.632–3.162)		30 (22.5–37.5)	0.960 (0.436–2.115)	
	GC	87	35 (17.4–52.6)	1 (reference)		31 (15.8–46.2)	1(reference)	
p53					0.897			0.673
	GG	43	38 (3.1–72.9)	0.653 (0.343–2.000)		38 (25.0–51.0)	0.944 (0.410–2.171)	
	CC	36	24 (7.8–40.2)	0.961 (0.528–1.800)		30 (12.0–48.1)	1.254 (0.687–2.3)	
	GC	16	25 (7.8–40.2)	1 (reference)		35 (15.4–55.0)	1 (reference)	
HIF					0.229			0.779
	CC	7	30 (16.7–43.4)	0.555 (0.135–2.279)		40 (0.0–90.4)	1.214 (0.439–3.355)	
	TT	18	56 (36.1–75.2)	0.518 (0.222–1.209)		23 (6.9–39.1)	1.223 (0.656–2.280)	
	CT	102	26 (18.7–41.3)	1 (reference)		35 (24.1–45.9)	1 (reference)	

[1] Polymorphisms were grouped together in a codominant model. Results of the regression analyses are given as relative risk with 95% confidence interval. Bold is used to highlight significant results.

2.3. Analysis of VEGF C + 936T and Clinical Outcome

The genotyping of VEGF C + 936T was successful in all 127 patients. During the observation period, 61/127 (48%) patients showed recurrence. Of these, 41 with allele C/C after 41 months (CI 5–77), 22 with C/T allele after 21 months (CI 8–4) and 5 with allele T/T after 6 months (CI 2–10), respectively. The *p*-value of the log-rank test was 0.023 (Table 4, Figure 1b). Due to the small group of patients with a T/T allele, we performed a dominant analysis (C/T and T/T grouped together) with a *p*-value of 0.045. VEGF was included in the multivariable analysis. OS showed no significant association with the VEGF SNP and was, therefore, not further analyzed.

2.4. Combined Analysis of VEGF C + 936T, IL-8 T-251A and Clinical Outcome

When VEGF C + 936T (adjusted *p*-value = 0.332) and IL-8-251 T > A (adjusted *p*-value = 0.010) were stratified by cirrhosis, T-category, more than one tumor node, and polymorphisms in IL8 remained significantly associated with DFS. VEGF C + 936T did not remain associated with DFS. Patients with zero unfavorable alleles (IL-8-251 AT/TT and VEGF +936 CC allele) had lower risk of tumor recurrence than patients with one unfavorable allele (RR 1.853 (1.045–3.284)) or two unfavorable genes (RR 4.910 (1.047–23.031), adjusted *p*-value 0.034) (Table 5, Figure 1c). The median disease-free survival was 20 months in the group with one unfavorable allele and was 7 months with two unfavorable alleles compared to 33 months in the favorable group.

Table 5. Multivariable analyses of IL-8, VEGF, and DFS.

SNP	Multivariable Analysis			
	n	**Adjusted RR**	**Adjusted *p* Value**	**Median DFS (Months) (95% CI)**
IL-8 AA = unfavorable	14	**2.817 (1.278–6.168)**	**0.010**	33 (19.6–44.4)
IL-8 AT/TT = favorable	111	1 (reference)		20 (0.0–40.1)
VEGF TT/CT = unfavorable	46	1.351 (0.736–2.480)	0.332	20 (12.0–28.0)
VEGF CC = favorable	79	1 (reference)		39 (10.2–67.8)
SNP	**Combined Analysis**			
	n	**Adjusted RR**	**Adjusted *p* Value**	**Median DFS (Months) (95% CI)**
0 unfavorable	70	1 (reference)	**0.034**	58 (32.4–83.6)
1 unfavorable	54	1.853 (1.045–3.284)		20 (13.8–26.2)
2 unfavorable	3	4.910 (1.047–23.031)		7 (0.0–10.0)

Adjusted: based on cirrhosis, T-status, and HCC tumor nodes, which were significant in DFS. Results of the regression analyses are given as relative risk with 95% confidence interval. Bold is used to highlight significant results.

2.5. Analysis of Other Tested Germline Polymorphisms

In total, 10 genes were tested; however, none of the further genes showed any relevant association with DFS or OS. The detailed data are presented in Table 4.

3. Discussion

In this study, we aimed to clarify the impact of angiogenesis- and hypoxia-associated germline polymorphisms on tumor recurrence in HCC patients who underwent partial hepatectomy. Here, we demonstrate that a proangiogenic and functional germline polymorphism of the IL-8 gene significantly correlates with DFS in HCC patients undergoing curative-intent surgery.

Despite recent advancements in the surgical treatment of HCC, the high rate of tumor recurrence results in an overall poor clinical prognosis [3]. Although great efforts have been made to optimize patient selection based on clinical and molecular parameters, data on how germline polymorphisms influence clinical outcomes after surgical resection for HCC are scarce [41]. The prominent role of tumor angiogenesis in HCC biology is reflected in the prominent arterial neo-vascularization of HCC as one of the hallmarks of malignant hepatocyte transformation [42]. Targeted agents such as sorafenib, a small-molecule multityrosine kinase inhibitor, have been employed in the treatment of advanced HCC [17,43,44], and more recently, the administration of atezolizumab, a checkpoint inhibitor, in combination with an anti-VEGF antibody (bevacizumab) showed promising results [13].

VEGF is one of the main regulators of tumor angiogenesis and is regulated by a large variety of transcription factors such as HIF-1, nuclear factor κB (NF-κB), and signal transducer and activator of transcription 3 (STAT3) [14]. Activation of the VEGF signaling pathway leads to endothelial cell proliferation, migration, and formation of new vessels [45,46]. While the level of circulating VEGF protein is increased in HCC patients and has been linked to poor oncological outcomes [45], the expression levels of VEGF protein also correlate directly with tumor size, metastasis, and poor prognosis in various malignancies [47]. Functional DNA sequence variations within the VEGF gene lead to altered serum levels and activity and, as such, also affect clinical outcomes in different types of malignant disease [48,49]. The VEGF C + 936T SNP has been associated with an increased susceptibility to breast, lung, and colon cancer as well as with a shorter disease-free survival in colon cancer [20,50]. In our study, VEGF C + 936T and the presence of the T/T or C/T alleles was associated with shorter DFS in our univariable analysis but did not reach significance in the multivariable analysis. The DFS was 20 months in individuals with T/T or C/T alleles, in contrast to C/C homozygote patients who had a median DFS of 30 months.

IL-8, a member of the CXC chemokine family, is a potent and VEGF-independent mediator of tumor angiogenesis. In tumor biology, IL-8 conveys direct effects on tumor cells, mediating the

transition to a migratory, proliferative, or mesenchymal phenotype [51]. Furthermore, IL-8 modifies the tumor microenvironment, encouraging the recruitment of pro-tumorigenic immune cells, such as tumor-associated macrophages with an "M2-like" phenotype, ultimately leading to a positive feedback loop with increased IL-8 production, as well as recruitment of cancer-associated neutrophils, which, in turn, contribute to angiogenesis and invasion through the secretion of matrix metalloproteinase (MMP)-9 [52,53]. Furthermore, IL-8 conveys direct proangiogenic, chemotactic effects on endothelial cells via its receptors CXCR1 and -2 [51]. Most studies on IL-8 in HCC focus on the prognostic role of IL-8 expression and on the inhibition or activation of IL-8 in HCC models and cell lines [22,23,38,54,55]. As such, the findings of different groups have linked increased serum levels of IL-8 with tumor invasion, recurrence, and metastases [22,55]. Only scarce evidence is available on the prognostic role of IL-8 germline SNPs in HCC patients undergoing curative-intent surgery [56–58]. In 2000, Hull et al. identified the functional role of a common polymorphism IL-8 T-251A bp upstream of the IL-8 transcriptional start site, with over 50% of the United Kingdom population being heterozygous. Their experimental data suggested the association of the IL-8 T-251A A allele with increased IL-8 production [59]. Furthermore, high-expression variants of IL-8 T-251A (A/A) are linked to tumor recurrence and poor clinical outcome in various gastrointestinal malignancies, including colon and gastric cancer [18,20]. In patients with HCC, the homozygous IL-8-A/A allele was recently associated with a favorable clinical effect on transcatheter arterial chemoembolization (TACE) efficacy and lower levels of serum AFP [60]. Angiogenesis Liver CancEr 2 (ALICE-2), a retrospective multicenter study of 210 patients with advanced HCC, recently identified a panel of H1F-1α, VEGF-A, VEGF-C, VEGFR-1, and VEGFR-3 variants to be associated with better response to sorafenib treatment [29]. The present study demonstrated that patients with high-expression variants of IL-8 T-251A (A/A) developed significantly earlier tumor recurrence after partial hepatectomy with curative intent. On average, individuals with the homozygous minor allele A/A were recurrence-free for 20 months, compared to 32-month RFS in patients with at least one "protective" T allele (p-value: 0.034 in multivariable analysis).

The findings of this study should be interpreted in light of the potential limitations; nonetheless, this type of pilot study is an ideal forum for testing a novel hypothesis and generating data that need to be confirmed in a prospective study. First, our findings are based on a relatively small but homogeneous number of Central European HCC patients; and secondly, we examined only 10 genes within the angiogenesis pathway. While it is recognized that the observed associations and patterns require confirmation with an independent dataset, we have taken care to select candidate genes with a documented role in tumor angiogenesis that were also found to be associated with prognosis in previous studies [61].

4. Materials and Methods

4.1. Patients

Between 2010 and 2017, unrelated patients with localized HCC and no signs of systemic disease were treated with first-line surgical resection at the University Hospital of the Rheinisch-Westfälische Technische Hochschule (RWTH) Aachen (UH-RWTH) and were retrospectively included in this study. Clinico-pathological and follow-up data were obtained from a prospectively managed institutional database and analyzed retrospectively [62]. Clinical staging was performed according to the criteria of the International Union Against Cancer (UICC), BCLC, and Milan criteria. An experienced pathologist (N.T.G.) reviewed the histological specimens of the cohort (n = 127) to confirm the diagnosis. Patient samples were provided by the Institute of Pathology (UH-RWTH) and the institutional Biobank (RWTH-cBMB). The study was conducted in accordance with the requirements of the Institutional Review Board of the RWTH Aachen University (EK 360/15), the current version of the Declaration of Helsinki, and the guidelines for good clinical practice.

4.2. Selection of Candidate Polymorphisms

SNPs for molecular testing were selected based on our previous studies [20] and with a focus on tumor angiogenesis based on VEGF-dependent and -independent IL-8 pathways and the hypoxia-driven pathway. Besides well-documented function of the selected genes in the above-mentioned pathways, proof of a biological relevance of the respective SNPs was a necessary prerequisite. Additionally, the frequency of the polymorphism had to be high enough to allow a meaningful statistical analysis [20] (Tables 1 and 6).

Table 6. Forward and reverse primer sequences and restriction enzymes used.

No.	Gene	Allele	Primer Forward	Primer Reverse	Annealing Temp.	Enzyme
1	IL-8 251	A > T	TTG TTC TAA CAC CTG CCA CTC T	GGC AAA CCT GAG TCA TCA CA	60 °C	Mfe I
2	VEGF +936	C > T	AGA CTC CGG CGG AAG CAT	TGT ATG TGG GTG GGT GTG TC	60 °C	Nla III
3	EGFR -497	G > A	TGC TGT GAC CCA CTC TGT CT	CCA GAA GGT TGC ACT TGT CC	60 °C	Bstni
4	EGFA 61G	A > G	CAT TTG CAA ACA GAG GCT CA	TGT GAC AGA GCA AGG CAA AG	60 °C	Alu Ia
5	p53	C > G	ATC TAC AGT CCC CCT TGC CG	GCA ACT GAC CGT GCA AGT CA	60 °C	Bstni
6	CXCR1	G > C	CTC ATG AGG ACC CAG GTG AT	GGT TGA GGC AGC TAT GGA GA	60 °C	Alu I
7	IL-1b	C > T	GTT GTC ATC CAG ACT TTG ACC	TTC AGT TCA TAT GGA CCA GA	60 °C	Taq1
8	HIF1 A588T	C > T	CCC AAT GGA TGA TGA CTT CC	AGT GGT GGC ATT AGC AGT AGG	60 °C	Tsp-45 I
9	IL-10	T > G	GAGCACTACCTGACTAGCATATAAG	GTGGGCTAAATATCCTCAAAGT	60 °C	RSAI
10	IL-6	G > C	GCC TCA ATG ACG AC	TCA TGG GAA AAT CC	60 °C	NiaIII

Abbreviations used: VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; IL-1/6/8/10, interleukin-1/6/8/10; CXC2, chemokine receptor; HIFa, hypoxia-inducing factor alpha; p53, protooncogene 53.

4.3. Genotyping

From 127 formalin-fixed paraffin-embedded (FFPE) HCC samples, genomic DNA was extracted using the QIAmp DNA Isolation Kit (Qiagen, CA, USA) according to the manufacturer's protocol. DNA quality was determined photometrically (NanoDrop, Thermo Fisher, MA, USA). Samples were analyzed with the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique. After PCR amplification of probes using forward and reverse primers, the products were digested with restriction enzymes (New England Biolab, MA, USA). Reaction products were separated on a DNA Stain G (SERVA, Heidelberg, Germany) stained 4% agarose gel at 120 mV for 60 min and visualized under UV light using a GelDoc system (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). See Table 6 for forward and reverse primers, annealing temperatures, and restriction enzymes.

4.4. Study Endpoints and Statistical Analysis

The primary endpoint of this study was DFS, calculated from the date of surgery to the date of the first recurrence. Patients who did not recur were censored at the time of death or at last follow-up. The secondary endpoint was OS, defined as the period from surgery to the date of death from any cause or the last contact if the patient was alive.

Categorical data are presented as numbers and percentages and were compared using the chi-squared test, Fisher's exact test, or linear-by-linear association according to scale and number count. Data derived from continuous variables are presented as mean and standard deviation and comparisons between different time points were made using the Mann–Whitney U test. The association between each SNP and DFS and OS was examined using Kaplan–Meier curves and log-rank tests. For polymorphisms with a genotype frequency (homozygous minor allele) of <10%, the associations between genotypes and clinical outcome were analyzed in a dominant model. Otherwise, they were analyzed in a codominant or additive model.

Survival curves were generated using the Kaplan–Meier method and compared with the log-rank test. Univariable and multivariable associations of factors with patient survival and tumor recurrence were assessed using Cox proportional hazard models. Hazard ratios are presented with 95% confidence intervals (CI). Variables yielding significance in the univariable analysis were included in the multivariable analysis. The level of significance was set to $p < 0.05$. Analyses were performed using SPSS Statistics 23 (IBM Corp., Armonk, NY, USA).

5. Conclusions

Notwithstanding the aforementioned limitations, we have identified polymorphisms in IL-8 to be associated with tumor recurrence in HCC patients undergoing curative-intent surgery. Thus, the analysis of angiogenesis-related germline gene polymorphisms may facilitate more sophisticated patient selection by identifying patients at high risk for HCC recurrence. One might speculate that this subgroup of patients could potentially benefit from adjuvant anti-VEGF (e.g., bevacizumab) therapeutics. Despite these encouraging findings from our pilot study, independent, larger, controlled prospective biomarker-embedded clinical trials are warranted to validate our observations.

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2.4 Body Composition als prognostischer Faktor im iCCA und pCCA

***Lurje I**, Czigany Z, Eischet S, Bednarsch J, Ulmer TF, Isfort P, Strnad P, Trautwein C, Tacke F, Neumann UP, Lurje G.*
The prognostic impact of preoperative body composition in perihilar and intrahepatic cholangiocarcinoma.
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Abstract Zitat (96):
“Cholangiocarcinoma (CCA) is a rare but highly aggressive malignancy of the biliary system. Although it is amenable to surgical resection in early disease, outcomes are frequently dismal. Here, we investigated the prevalence of body composition (BC) alterations and their prognostic role for surgical patients with intrahepatic (iCCA) and perihilar (pCCA) disease. Patients undergoing curative-intent surgery for iCCA or pCCA between 2010 and 2019 at University Hospital Aachen were included. Axial computed tomography images were retrospectively assessed with a segmentation tool (3D Slicer) at the level of the third lumbar vertebra to determine lumbar skeletal muscle (SM) index, mean SM radiation attenuation, and visceral fat area. The related BC pathologies sarcopenia, myosteatosis, visceral obesity, and sarcopenic obesity were determined using previously described cutoffs. A total of 189 patients (86 with iCCA, 103 with pCCA) were included. Alterations of BC were highly prevalent in iCCA and pCCA, respectively: sarcopenia, 33% (28/86) and 39% (40/103); myosteatosis, 66% (57/86) and 66% (68/103); visceral obesity, 56% (48/86) and 67% (69/103); sarcopenic obesity, 11% (9/86) and 17% (17/103). Sarcopenia and myosteatosis did not have a significant prognostic role for disease-free survival (DFS) and overall survival (OS). Patients with iCCA with sarcopenic obesity (n = 9) had significantly shorter OS than patients without sarcopenic obesity (n = 7; log-rank p = 0.002; median OS, 11 months and 31 months; 1-year mortality, 55.6% [5/9] and 22% [17/77]; 5-year mortality, 88.9% [8/9] and 61% [47/77], respectively). In multivariable analysis, only tumor-related risk factors remained prognostic for DFS and OS. Sarcopenic obesity may affect clinical outcomes after curative-intent surgery for iCCA, indicating that imaging-based analysis of BC may hold prognostic value for long-term survival and could aid preoperative patient selection.”

Patient*innen mit CCA haben nicht nur eine gravierende onkologische Prognose, sondern auch eine deutlich eingeschränkte Lebensqualität mit Fatigue, Gebrechlichkeit, unintentioneller Gewichtsabnahme bis zur Kachexie, sowie Gelbsucht mit Cholangitiden (97). Wir stellten die Hypothese auf, dass die Analyse von Körperzusammensetzung in

Patient*innen mit CCA nicht nur Einblicke in ihren Allgemeinzustand geben kann, sondern auch prognostisch relevant sein kann.

In dieser Studie analysierten wir die Body Composition von Patient*innen die sich einer Tumorresektion eines iCCAs oder pCCAs in kurativer Intention unterzogen haben. Als Teil der routinemäßigen präoperativen Planung unterzog sich der Großteil des Kollektivs einer *in-house* abdominellen CT-Schnittbildgebung, welche wir mittels semi-automatischer Segmentierung auf der Höhe des 3. Lendenwirbels auf die Menge von Muskelmasse sowie viszeralen und subkutanen Fetts untersuchten. Des Weiteren wurde die Dichte der Muskulatur, als Reflektion des intramuskulären Verfettungsgrades (Myosteatose), untersucht. Neben klinisch-pathologischen Kriterien wurden postoperatives Outcome, sowie die rezidivfreie Zeitspanne und das Gesamtüberleben erhoben.

Ein erheblicher Anteil der Patient*innen mit sowohl iCCA als auch pCCA zeigten Pathologien in der Körperzusammensetzung: etwa die Hälfte der Patient*innen was übergewichtig oder adipös, etwa ein Drittel was Sarkopen, und ca. zwei Drittel hatten eine viszerale (intraabdominelle) Obesität und/oder eine Myosteatose. Während keine dieser hochprävalenten Body-Composition-Pathologien mit onkologischer- oder Gesamtprognose assoziiert waren, zeigten Patient*innen mit iCCA mit einer kombinierten Pathologie des Muskel-und Fettgewebes, nämlich der Sarkopenen Obesität, eine signifikante Verkürzung des Gesamtüberlebens, mit einem medianen Gesamtüberleben von 11 Monaten, verglichen mit 31 Monaten in Patient*innen ohne Sarkopene Obesität. Aufgrund der extrem kleinen Eventzahl konnte die Unabhängigkeit dieser Beobachtungen nur begrenzt untersucht werden.

Hieraus ergibt sich, dass eine kleine Subgruppe von iCCA-Patient*innen (ca. 10%), die übergewichtig oder adipös sind und gleichzeitig eine geringe Muskelmasse (Sarkopenie) aufweisen, eine hochvulnerable Gruppe für ein limitiertes Gesamtüberleben nach chirurgischer Resektion darstellen.

Die Daten unserer Studie unterstreichen, dass nicht nur die onkologische Grunderkrankung einen Einfluss auf Fitness und Körperzusammensetzung hat, sondern dass zwei hochprävalente Risikofaktoren – die weltweite Übergewichtsepidemie einerseits, und eine alternde Bevölkerung mit erniedrigter Muskelmasse andererseits – das Outcome von Patient*innen mit iCCA beeinflussen. Patient*innen mit Sarkopener Obesität bedürfen nicht nur eines optimierten peri- und postoperativen Managements, sondern stellen eine wichtige potenzielle Zielgruppe für Prä- und Rehabilitationsprogramme in zukünftigen Studien dar.

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ORIGINAL ARTICLE

The prognostic impact of preoperative body composition in perihilar and intrahepatic cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CCA) is a rare but highly aggressive malignancy of the biliary system. Although it is amenable to surgical resection in early disease, outcomes are frequently dismal. Here, we investigated the prevalence of body composition (BC) alterations and their prognostic role for surgical patients with intrahepatic (iCCA) and perihilar (pCCA) disease. Patients undergoing curative-intent surgery for iCCA or pCCA between 2010 and 2019 at University Hospital Aachen were included. Axial computed tomography images were retrospectively assessed with a segmentation tool (3D Slicer) at the level of the third lumbar vertebra to determine lumbar skeletal muscle (SM) index, mean SM radiation attenuation, and visceral fat area. The related BC pathologies sarcopenia, myosteatosis, visceral obesity, and sarcopenic obesity were determined using previously described cutoffs. A total of 189 patients (86 with iCCA, 103 with pCCA) were included. Alterations of BC were highly prevalent in iCCA and pCCA, respectively: sarcopenia, 33% (28/86) and 39% (40/103); myosteatosis, 66% (57/86) and 66% (68/103); visceral obesity, 56% (48/86) and 67% (69/103); sarcopenic obesity, 11% (9/86) and 17% (17/103). Sarcopenia and myosteatosis did not have a significant prognostic role for disease-free survival (DFS) and overall survival (OS). Patients with iCCA with sarcopenic obesity (n = 9) had significantly shorter OS than patients without sarcopenic obesity (n = 7; log-rank $p = 0.002$; median OS, 11 months and 31 months; 1-year mortality, 55.6% [5/9] and 22% [17/77]; 5-year mortality, 88.9% [8/9] and 61% [47/77], respectively). In multivariable analysis, only tumor-related risk factors remained prognostic for DFS and OS. Sarcopenic obesity may affect clinical outcomes after curative-intent surgery

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for iCCA, indicating that imaging-based analysis of BC may hold prognostic value for long-term survival and could aid preoperative patient selection.

INTRODUCTION

Cholangiocarcinoma (CCA) is a highly aggressive epithelial malignancy of the bile ducts that is estimated to account for 3% of all gastroenterological tumors.[1] Surgical resection represents the cornerstone of treatment, but only approximately 30% of CCAs are amenable to curative resection due to intrahepatic and extrahepatic tumor spread.[2,3]

The most common anatomical subclassification of CCA is the division into intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA) disease. The most common subset, pCCA, comprises 50%–60% of CCAs and arises above the cystic duct and below the second-order bile ducts. iCCAs originate above the second-order bile ducts and account for 10%–20% of CCAs, while dCCAs make up 20%–30% of all CCAs.[1] The most common risk factors for CCA are primary sclerosing cholangitis, cirrhosis, bile duct cysts (including Caroli's disease), hepatic cholelithiasis and cholelithiasis, as well as certain parasitic infections.[4]

Patients with CCA have a dismal oncological prognosis, and their disease is frequently accompanied by worsening of the general medical condition characterized by jaundice, cholangitis, unintentional weight loss, cachexia, and frailty.[5] While cachexia—the severe involuntary loss of lean body mass due to systemic inflammation and metabolic deregulation—is a well-characterized hallmark of advanced disease and confers unfavorable outcomes across numerous cancer entities,[6] the worldwide obesity epidemic has led to an increasing proportion of patients with masked wasting symptoms at presentation.[7] In this regard, expanding the analysis of body composition (BC) beyond classical metrics, like body mass index (BMI), has the potential to reveal wasting and alterations of lean tissues and is of prognostic value in oncological disease and liver disease.[8,9] As such, the quantification of muscle mass to detect sarcopenia has gained wide recognition as a prognostic parameter in solid tumors[10] and in the progression of end-stage liver disease.[8,11] More recently, myosteatosis, a qualitative characteristic of muscle composition, also emerged as a prognostic parameter in patients undergoing liver transplantation.[12,13] To date, little is known about the incidence of BC alterations in patients with surgical iCCA and pCCA and about the prognostic value of these as covariates.

We hypothesized that sarcopenia, myosteatosis, visceral obesity, and sarcopenic obesity may impact the disease course of CCA. In this study, we aimed to investigate the prognostic value of computed tomography (CT)-based diagnosis of BC pathologies in patients undergoing curative resection for iCCA and pCCA.

MATERIALS AND METHODS

Patients

Between May 2010 and December 2019, all consecutive patients undergoing curative-intent surgery for iCCA and pCCA at the University Hospital RWTH Aachen, Aachen, Germany, were considered for inclusion. Exclusion criteria were defined as (i) CT scans older than 3 months and/or those not including images from the third lumbar vertebra (L3) level or only other imaging modalities, like magnetic resonance imaging (MRI), available[12]; (ii) patients with dCCA, ampullary carcinoma, pancreatic adenocarcinoma, and (iii) neuroendocrine tumors. Clinicopathological and survival data were collected from a prospective institutional database. Preoperatively, all patients underwent a detailed workup to exclude systemic disease and to determine the extent of hepatic and hilar disease. This encompassed CT or gadolinium-enhanced MRI and endoscopic retrograde cholangiopancreatography or magnetic resonance cholangiopancreatography, as described.[14] The institutional surgical approach included a hilar *en bloc* resection for pCCA, as described.[15–17] The subsequent histopathological examination was standardized according to current versions of national guidelines, World Health Organization, and Union Internationale Contre le Cancer (UICC) classifications.

This study was conducted in accordance with the current version of the Declaration of Helsinki and good clinical practice guidelines (International Conference on Harmonization, Good Clinical Practice). Approval was granted by the institutional review board (EK 341/21). Informed consent was waived by the institutional review board (EK 341/21) due to the retrospective study design and analysis of available clinical data.

Segmentation and BC analysis

All CT scans were performed on a state-of-the-art multislice CT scanner. The technical parameters of CT imaging have been described.[12] An axial CT image at the L3 vertebra level from the most recent CT image was retrieved from the Picture Archiving and

Communication System for semiautomatic segmentation of skeletal muscle and adipose tissue on the 3D Slicer software platform and BC module (https://www.slicer.org/, version 4.1). Skeletal muscle was identified and quantified at attenuation values of −29 to 150 Hounsfield units (HU), with the muscle area on level L3 including psoas major, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis. Skeletal muscle index (SMI) was calculated by normalizing muscle area in square centimeters to patient stature in square meters. Skeletal muscle radiation attenuation (SM-RA), indicative of muscle density and myosteatosis, was quantified in HUs. Visceral fat area (VFA) was based on attenuation values −150 to −50 HU, and subcutaneous adipose tissue was based on attenuation values −190 to −30 HU. All measurements were performed by the same investigator who was blinded for the clinical outcome of these patients.

BMI was defined as weight in kilograms/height^2 in square meters, with values ≥25 kg/m^2 indicative of overweight/obesity. The definition of BC pathologies followed cancer-specific cutoffs described and validated in large patient cohorts as prognostic factors in gastrointestinal malignancies[7,18] to avoid an overfitting to our statistically small data set without an independent validation cohort. The cutoff for sarcopenia was SMI < 41 cm^2/m^2 in women and <43 cm^2/m^2 in men with BMI < 25 kg/m^2, and <53 cm^2/m^{2}in women and men with BMI ≥ 25 kg/m^2. Myosteatosis was assigned at levels of <41 HU for patients with a BMI < 25 kg/m^2 and <33 HU for patients with a BMI ≥ 25 kg/m^2.[7] VFA ≥ 100 cm^2 was used as a cutoff for visceral obesity, while sarcopenic obesity was diagnosed in patients with BMI ≥ 25 kg/m^2 and SMI ≤ 38.5 cm^2/m^2 in women and ≤52.4 cm^2/m^2 in men, as reported for cancer patients by Tan et al.[18] (Figure 1).

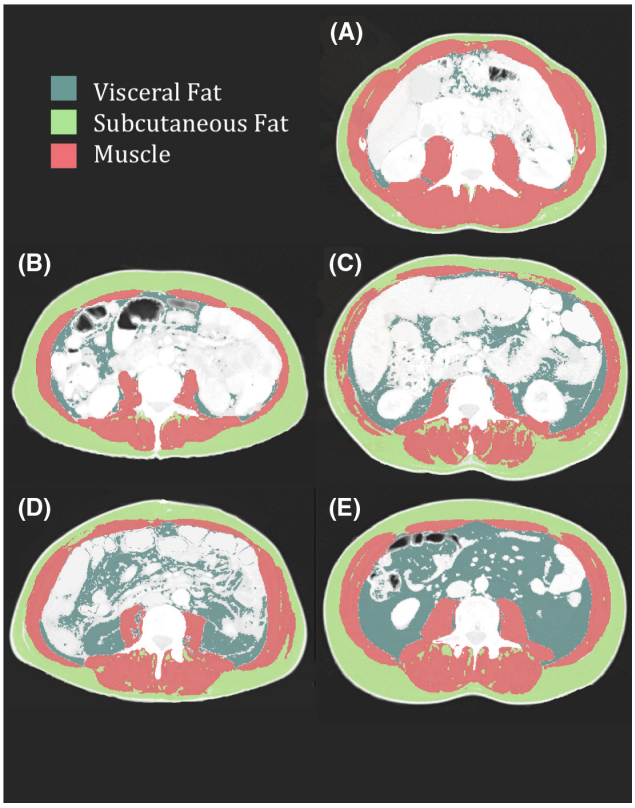

FIGURE 1 Representative axial computed tomography images of patients undergoing curative liver resection for intrahepatic cholangiocarcinoma after segmentation at the level of the third lumbar vertebra. The following attenuation values were used to define the respective areas: skeletal muscle area (red), 29–150 HU; subcutaneous fat area (light green), −190 to −30 HU; visceral fat area (dark green), −150 to −50 HU. Examples are given in (A–E). (A) No body composition pathology with normal muscle mass (SMI, 58.5 cm^2/m^2) and a low amount of intramuscular (SM-RA, 56.9 HU) and visceral (VFA, 12 cm^2) adipose tissue, and a normal BMI of 23 kg/m^2. (B) Sarcopenia, with a quantitatively reduced muscle mass (SMI, 35.6 cm^2/m^2). (C) Myosteatosis with a normal amount of muscle mass but an increased amount of intramuscular fat in dark green (SM-RA, 44.4 HU). (D) Visceral obesity, characterized by a large amount of visceral fat in dark green (VFA, 185 cm^2). (E) Sarcopenic obesity as the combination of low muscle mass and BMI (SMI, 46.0 cm^2/m^2; BMI, 26.5 kg/m^2). BMI, body mass index; HU, Hounsfield units; SMI, skeletal muscle index; SM-RA, skeletal muscle radiation attenuation; VFA, visceral fat area.

Study endpoints

Associations between pathological markers of tumor aggressiveness (lymph node invasion, perineural, lymphovascular and vascular invasion, multilocularity, tumor size) with the incidence of BC pathologies were assessed. The incidence of perioperative complications in patients with BC alterations was tested. We classified 90-day postoperative complications according to the Clavien-Dindo (CD) classification,[19] and the comprehensive complication index (CCI) was calculated as described.[20] Posthepatectomy liver failure (PHLF) was evaluated as a surrogate marker for overall function and hepatic reserve. PHLF was defined according to guidelines of the International Study Group of Liver Surgery (ISGLS)[21] as elevated international normalized ratio (INR) (>1.15) and concomitant hyperbilirubinemia (>1.2 mg/dL) on postoperative day 5 in patients with previously normal values and rising INR and bilirubin in patients with preoperatively elevated values. Grade B/C PHLF was defined according to ISGLS guidelines as laboratory PHLF diagnosis requiring clinical intervention. Textbook outcomes, a composite measure for desirable postoperative outcomes, were defined according to Merath et al.[22] as (1) no prolonged length of hospital stay, (2) no readmission 90 days after discharge, and (3) no 90-day postoperative mortality along with testing for association with BC pathologies.

The cohort was dichotomized at the median age of the cohort (65 years) for univariable analysis. A tumor

TABLE 1 Select patient and clinicopathological characteristics

Patient characteristic	Intrahepatic CCA (n = 86)	Perihilar CCA (n = 103)
Age (years)	65 ± 11.4	66 ± 10.4
BMI (kg/m^2)	26 ± 4.3	25.8 ± 4.7
Sex ratio (F:M), n (%)	49 (57.0): 37 (43.0)	32 (31.1): 71 (68.9)
EBD (stent), n (%)	14 (16.3)	82 (79.6)
PBD, n (%)	1 (1.2)	23 (22.3)
Portal vein embolization, n (%)	8 (9.3)	44 (42.7)
Neoadjuvant chemotherapy, n (%)	3 (3.5)	0 (0.0)
Laparoscopic approach n (%)	5 (5.8)	22 (21.4)
Operative procedure n (%)		
Atypical/anatomical resection/ bisegmentectomy	19 (22.1)	1 (1.0)
Right hepatectomy	15 (17.4)	9 (8.7)
Left hepatectomy	11 (12.8)	11 (10.7)
Extended right hepatectomy	8 (9.3)	18 (17.5)
Extended left hepatectomy	8 (9.3)	26 (25.2)
Right trisectorectomy	6 (7.0)	21 (20.4)
Left trisectorectomy	8 (9.3)	6 (5.8)
Hepatoduodenectomy	0 (0.0)	9 (8.7)
ALPPS	11 (12.8)	2 (1.9)
Lymphadenectomy, n (%)	75 (87.2)	103 (100.0)
Vessel replacement n (%)	54 (62.8)	94 (91.2)
Venous	54 (62.8)	87 (84.5)
Arterial	0 (0.0)	1 (1.0)
Both	0 (0.0)	6 (5.8)
Operation time (minutes)	297.7 ± 99.2	425.4 ± 99.0
Intraoperative blood transfusions (units)	0.8 ± 1.8	1.3 ± 1.7
Intraoperative FFP (units)	1.7 ± 2.8	3.2 ± 3.2
T category, n (%)		
Tis	1 (1.2)	0 (0.0)
T1	24 (28.0)	8 (7.5)
T2	54 (62.8)	57 (56.3)
T3	4 (4.7)	27 (26.2)
T4	2 (2.3)	9 (8.7)
N category, n (%)		
N0	47 (54.7)	58 (56.3)
N1	30 (34.9)	32 (31.1)
N2		12 (11.7)
R category, n (%)		
R0	64 (74.4)	77 (74.8)
R1	9 (10.5)	16 (15.5)
Rx	9 (10.5)	9 (8.7)
(Micro-)vacular invasion, n (%)	33 (38.4)	26 (25.2)
Portal vein infiltration, n (%)	2 (2.3)	5 (4.9)
Hepatic artery infiltration, n (%)	0 (0.0)	9 (8.7)
Lymphovascular invasion, n (%)	21 (24.4)	21 (20.4)
Perineural invasion, n (%)	17 (19.8)	68 (66.0)
Tumor grading, n (%)		

(Continues)

TABLE 1 (Continued)

Patient characteristic	Intrahepatic CCA (n = 86)	Perihilar CCA (n = 103)
G1	1 (1.2)	2 (1.9)
G2	48 (55.8)	72 (69.9)
G2–3	4 (4.7)	1 (1.0)
G3	24 (27.9)	23 (22.3)
G4	2 (2.3)	1 (1.0)
Tumor stage, UICC (8th edition), n (%)		
0	2 (2.3)	0 (0.0)
I	17 (19.8)	6 (5.8)
II	26 (30.2)	36 (35.0)
III	29 (33.7)	44 (42.7)
IV	4 (4.7)	16 (15.5)
Tumor number	2.1 ± 1.6	1.4 ± 0.7
Tumor size	7.6 ± 3.8	3.5 ± 1.8
Cumulative ICU stay, days	3.5 ± 8.6	6.2 ± 15.4
Hospitalization, days	18.1 ± 14.5	25.8 ± 20.6
Postoperative complications, n (%)		
No complications	25 (29.1)	10 (9.7)
Clavien-Dindo I	2 (2.3)	6 (5.8)
Clavien-Dindo II	23 (26.7)	24 (23.3)
Clavien-Dindo IIIa	12 (14.0)	15 (14.6)
Clavien-Dindo IIIb	8 (9.3)	17 (16.5)
Clavien-Dindo IVa	9 (10.5)	12 (11.7)
Clavien-Dindo IVb	0 (0.0)	4 (3.9)
Clavien-Dindo V	7 (8.1)	15 (14.5)
Calculated CCI	44.9 ± 118.9	48.2 ± 32.9
Radiotherapy, n (%)	11 (12.8)	6 (5.8)
Chemotherapy, n (%)	47 (54.7)	40 (38.8)
Gemcitabine	2 (2.3)	5 (4.9)
Gemcitabine + cisplatin	27 (31.4)	19 (18.4)
Other	18 (20.9)	16 (15.5)

Note: Data presented as mean ± SD if not noted otherwise. Pathological categories given from TNM Eighth Edition, UICC stage Eighth Edition. Patients were classified as having received chemotherapy or radiotherapy if they received at least one cycle of the respective adjuvant treatment.

Abbreviations: ALPPS, associating liver partition with portal vein ligation for staged hepatectomy; BMI, body mass index; CCA, cholangiocarcinoma; CCI, comprehensive complication index; EBD, endoscopic biliary drainage; F, female; FFP, fresh-frozen plasma; ICU, intensive care unit; M, male; PBD, percutaneous biliary drainage; UICC, Union Internationale Contre le Cancer.

size of 5 cm for iCCA and 3 cm for pCCA was used to dichotomize the cohort as in previous multicentric experiences and prognostic scores.[23,24]

Statistical analysis

Comparisons between groups of patients were performed with Fisher's exact test and chi-squared test for categorical variables and Mann-Whitney U test for continuous variables. Two-sided testing was performed in all instances.

Primary outcome measures were disease-free survival (DFS), defined as the time between surgery and recurrence or censoring, and overall survival (OS) from surgery until death. Patients were censored at the time of last contact and, for DFS, even if they died without recurrence. Kaplan-Meier survival curves and log-rank tests were used to assess survival. Further, univariable and multivariable Cox regressions were employed for survival analyses and to determine hazard ratios (HRs). Owing to the large number of examined parameters, only clinically significant covariates in univariable analysis were included

in the respective multivariable analysis, with an exclusion of parameters with collinearity. $p < 0.05$ was considered statistically significant. SPSS Statistics (version 23; IBM, Armonk, NY, USA) was used for all statistical analyses.

RESULTS

Study population

Out of all 225 consecutive curative-intent surgeries performed for iCCA (n = 112) and pCCA (n = 113), 189 patients (iCCA = 86, pCCA = 103) met the predefined inclusion and exclusion criteria. Patient characteristics and perioperative outcome data of the cohort were, in part, reported previously[14,15,25,26] (Table 1).

Median time between the CT imaging used for segmentation and liver resection was 2 weeks (range, 0–12 weeks). The final study population was composed of 108 men (57%) and 81 women (43%), with a mean age of 65 (SD, 11) years. Textbook outcome was achieved in 41 (48%) patients with iCCA and 34 (33%) patients with pCCA. Median follow-up was 24 months (25 for patients with iCCA, 22 for patients with pCCA). DFS was 10 months in patients with iCCA and 39 months in patients with pCCA, with 54 (63%) patients with iCCA and 41 (40%) patients with pCCA recurring during the follow-up period. Median OS was 30 months and 29 months for patients with iCCA and pCCA, respectively, and thus slightly different in this subcohort (n = 189) from the overall cohort (n = 225; 25 months for iCCA and 33 months for pCCA). During the observation period, 63% (56/86) of patients with iCCA and 72% (70/103) with pCCA died. Detailed patient characteristics, and perioperative outcome are outlined in Table 1.

BC features in iCCA

In patients with iCCA, the median SMI was 50.3 cm^2/m^2 (range, 48.3 cm^2/m^2) for male and 42.4 cm^2/m^2 (range, 32.4 cm^2/m^2) for female patients. The median SM-RA was 34.5 HU (range, 53.4 HU) for men and 30.1 HU (range, 37.1 HU) for women. Median VFA values were 202.1 cm^2 (range, 505 cm^2) for men and 78.0 cm^2 (range, 352 cm^2) for women. For patients with iCCA, 49% (42/86) had a BMI > 25 kg/m^2 (overweight/obese), 33% (28/86) were classified as sarcopenic, and 66% (57/86) had SM-RA values indicative of myosteatosis. Visceral obesity was noted in 56% (48/86) of patients, while the incidence of sarcopenic obesity was 11% (9/86) (Table 2).

BC and outcome in iCCA

None of the BC pathologies correlated with pathological characteristics (lymph node positivity or lymphovascular, vascular, or perineural invasion) or postoperative complications, as assessed by the incidence of intraoperative transfusions, 90-day CD ≥ 3b complications, 90-day CCI and 90-day mortality, intensive care unit (ICU) and hospital stay, as well as PHLF (Table S1).

BMI ≥ 25 kg/m^2, sarcopenia, and myosteatosis (Table 3; Figure 2) as well as the simultaneous presence of sarcopenia and myosteatosis (data not shown) did not correlate with DFS or OS. When stratifying SMI (sarcopenia), SM-RA (myosteatosis), and VFA in quartiles, no survival trend was observed in any of

TABLE 2 Body composition features of the cohort

Body composition parameter		Intrahepatic CCA (n = 86)	Perihilar CCA (n = 103)
BMI (kg/m^2)	<25 (underweight/ normal)	44 (51.2)	52 (50.5)
	≥25 (overweight/ obese)	42 (48.8)	51 (49.5)
Sarcopenia (skeletal muscle mass, SMI)	No	57 (66.3)	63 (61.2)
	Yes	28 (32.6)	40 (38.8)
Myosteatosis (SM-RA)	No	29 (33.7)	35 (34.0)
	Yes	57 (66.3)	68 (66.0)
Visceral obesity (VFA)	No	38 (44.2)	34 (33.0)
	Yes	48 (55.8)	69 (67.0)
Sarcopenic obesity	No	77 (89.5)	86 (83.5)
	Yes	9 (10.5)	17 (16.5)

Note: Data presented as n (%). Definitions of body composition features are as follows: BMI, weight (kg)/height^2 (m^2); sarcopenia, SMI < 41 cm^2/m^2 in women and <43 cm^2/m^2 in men with BMI < 25 kg/m^2, and <53 cm^2/m^2 in men with BMI ≥ 25 kg/m^2; myosteatosis, <41 HU for patients with BMI < 24.9 kg/m^2 and <33 HU for patients with BMI ≥ 25 kg/m^2; visceral obesity, VFA ≥ 100 cm^2; sarcopenic obesity, BMI > 25 kg/m^2 and SMI ≤ 38.5 cm^2/m^2 in women and ≤52.4 cm^2/m^2 in men, as described.[18]

Abbreviations: BMI, body mass index; CCA, cholangiocarcinoma; SMI, skeletal muscle index; SM-RA, skeletal muscle radiation attenuation; VFA, visceral fat area.

TABLE 3 Univariable analysis of DFS and OS by body composition in iCCA and pCCA

Characteristic	n (%)	Median DFS (95% CI)	HR (95% CI)	*p* value[a]	Median OS (95% CI)	HR (95% CI)	*p* value[a]
iCCA (n = 86)							
Overweight/obesity, BMI (kg/m^2)							
No	43 (50.6)	10 (6.1–13.9)		0.408	31 (18.4–43.6)		0.182
Yes	42 (49.4)	8 (2.6–13.4)			20 (8.8–31.2)		
Reduced skeletal muscle mass (sarcopenia, SMI)							
No	57 (66.3)	8 (4.9–11.1)		0.753	25 (14.2–35.8)		0.336
Yes	28 (32.6)	12 (8.3–15.7)			36 (12.8–59.2)		
Myosteatosis (SM-RA)							
No	29 (33.7)	8 (6.8–9.2)		0.280	29 (11.5–46.5)		0.591
Yes	57 (66.3)	12 (7.8–16.2)			30 (18.2–41.8)		
Visceral obesity (VFA)							
No	38 (44.2)	8 (4.9–11.1)		0.400	32 (24.2–39.8)		0.707
Yes	48 (55.8)	11 (5.8–16.1)			22 (13.7–30.3)		
Sarcopenic obesity							
No	77 (89.5)	10 (6.9–13.1)		0.330	31 (21.5–40.5)	1	0.002
Yes	9 (10.5)	11 (0.0–29.0)			11 (0.0–28.5)	3.193 (1.465–6.962)	
pCCA (n = 103)							
Overweight/obesity BMI, kg/m^2							
No	52 (50.5)	40 (11.3–68.7)		0.696	31 (15.3–46.7)		0.845
Yes	51 (49.5)	31 (0.0–64.6)			28 (13.2–42.8)		
Reduced skeletal muscle mass (sarcopenia, SMI)							
No	63 (61.2)	40 (9.5–70.5)		0.757	31 (12.4–49.6)		0.813
Yes	40 (38.8)	36 (0.0–73.2)			28 (12.5–43.5)		
Myosteatosis (SM-RA)							
No	35 (34.0)	40 (15.7–64.3)		0.902	24 (14.5–33.5)		0.985
Yes	68 (66.0)	36 (5.0–67.0)			31 (23.9–38.1)		
Visceral obesity (VFA)							
No	34 (33.0)	n.a.		0.131	50 (9.5–90.5)		0.072
Yes	69 (67.0)	29 (3.2–54.8)			20 (6.8–33.2)		
Sarcopenic obesity							
No	86 (83.5)	39 (8.0–70.0)		0.812	29 (17.3–40.7)		0.801
Yes	17 (16.5)	55 (0.0–111.2)			29 (11.7–46.3)		

Note: Definitions of body composition features are as follows: BMI, weight (kg)/height^2 (m^2); sarcopenia, SMI < 41 cm^2/m^2 in women and < 43 cm^2/m^2 in men with BMI < 25 kg/m^2, and < 53 cm^2/m^2 in men with BMI ≥ 25 kg/m^2; myosteatosis, < 41 HU for patients with BMI < 24.9 kg/m^2 and < 33 HU for patients with BMI ≥ 25 kg/m^2; visceral obesity, VFA ≥ 100 cm^2; sarcopenic obesity, BMI > 25 kg/m^2 and SMI ≤ 38.5 cm^2/m^2 in women and ≤ 52.4 cm^2/m^2 in men, as described.[18]

Abbreviations: BMI, body mass index; CCA, cholangiocarcinoma; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; iCCA, intrahepatic cholangiocarcinoma; OS, overall survival; pCCA, perihilar cholangiocarcinoma; SMI, skeletal muscle index; SM-RA, skeletal muscle radiation attenuation; VFA, visceral fat area.

[a]Based on log-rank test. $p < 0.05$ is significant.

the quartile groups (Figure S1). Sex-specific analysis of sarcopenia, myosteatosis, and visceral obesity did not yield significant results for DFS and OS (data not shown). While visceral obesity did not correlate with DFS and OS, the presence of sarcopenic obesity was a predictor of shorter OS in patients with iCCA. As such, the nine patients with sarcopenic obesity had a median OS of 11 months compared to 31 months median OS in the 77 patients without sarcopenic obesity (p = 0.002) (Table 3; Figure 2). The total number of events for patients with and without sarcopenic obesity was eight and 48, the 1-year mortality rate was 55.6% (5/9) and 22% (17/77), and the 5-year mortality rate was 88.9% (8/9) and 61% (47/77), respectively.

In multivariable Cox regression analysis, including all respective significant predictors of DFS and OS from univariable analysis (Table 4), sarcopenic obesity did not reach a significant independent predictive effect for

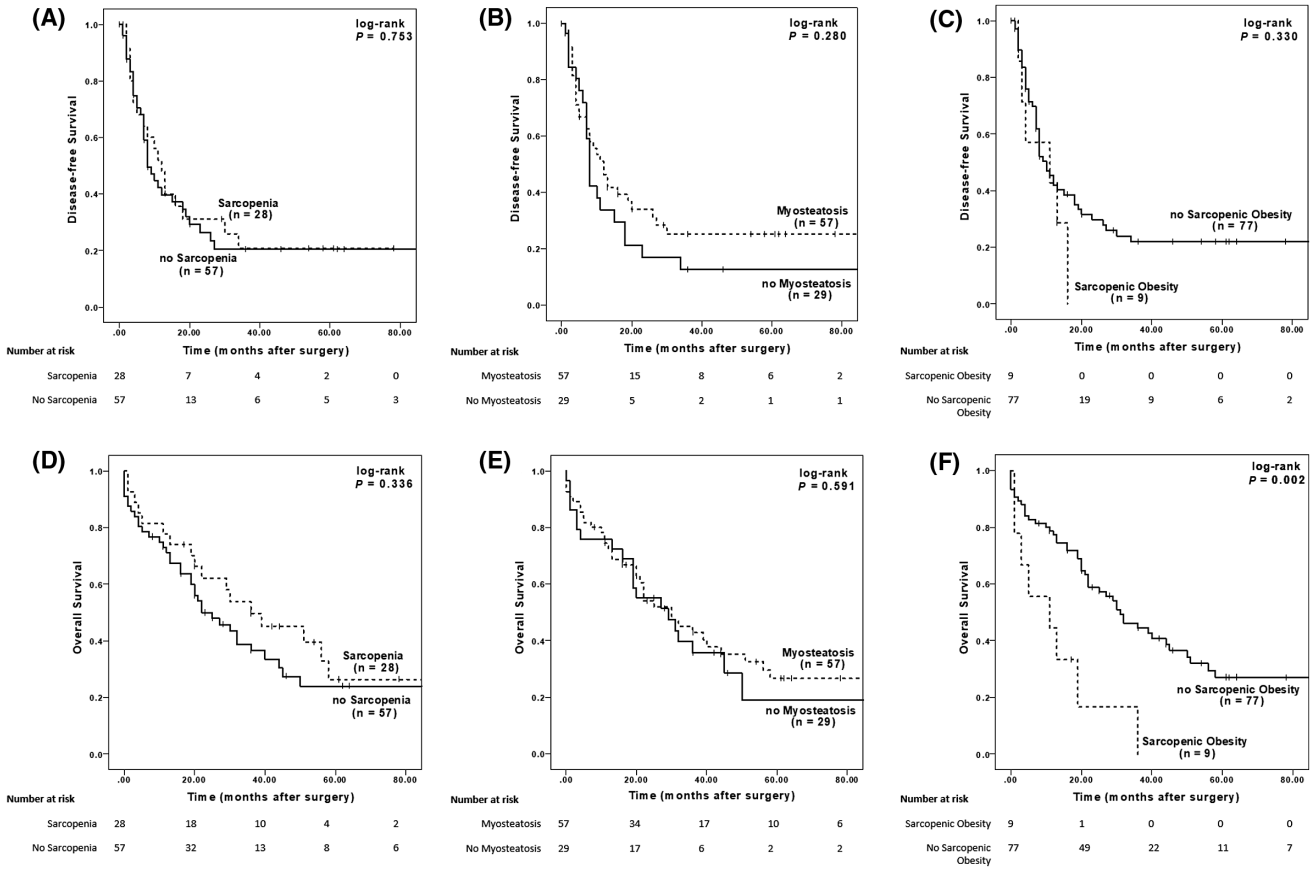

FIGURE 2 DFS and OS in relation to body composition characteristics in patients with intrahepatic cholangiocarcinoma. DFS for (A) sarcopenia, (B) myosteatosis, and (C) sarcopenic obesity. OS for (D) sarcopenia, (E) myosteatosis, and (F) sarcopenic obesity. $p < 0.05$ is significant. DFS, disease-free survival; OS, overall survival.

OS (HR, 1.833; $p = 0.471$). Instead, only UICC stage III/IV (HR, 3.715; $p = 0.037$) was confirmed as an independent predictor of shortened DFS, while lymphovascular invasion (HR, 3.706; $p = 0.036$) was an independent predictor of shortened OS.

BC features in pCCA

In patients with pCCA, the median SMI was 51.7 cm^2/m^2 (range, 46.0 cm^2/m^2) in men and 40.4 cm^2/m^2 (range, 31.4 cm^2/m^2) in women. The median SM-RA was 35.6 HU (range, 36.9 HU) for men and 30.0 HU (range, 36.2 HU) for women. Median VFA values were 171 cm^2 (range, 449 cm^2) for men and 105 cm^2 (range, 275 cm^2) for women. No significant difference in SMI, SM-RA, or VFA values was noted between the two CCA entities (analysis split by sex). For patients with pCCA, 50% (51/103) were considered overweight/obese based on their BMI, 39% (40/103) were considered sarcopenic, and 66% (68/103) were myosteatotic. Visceral obesity was present in 67% (69/103) of patients, and sarcopenic obesity was found in 17% of patients (17/103) (Table 2).

BC and outcome in pCCA

In pCCA, only BMI correlated with postoperative complications. Patients with BMI < 25 kg/m^2 had a higher incidence of PHLF (16/52, 34.5% for BMI < 25 kg/m^2 versus 5/51, 9.8% for BMI ≥ 25 kg/m^2), while patients with BMI ≥ 25 kg/m^2 displayed more frequent 90-day ≥CD3b complications (28/51, 55% versus 18/53, 35%) compared to patients with BMI < 25 kg/m^2 (Table S1). BMI, sarcopenia, myosteatosis, visceral obesity, presence of sarcopenia and myosteatosis (data not shown), and sarcopenic obesity did not correlate significantly with DFS or OS; there was a nonsignificant trend of patients with visceral obesity toward shorter OS (median OS, 20 months versus 50 months in patients without visceral obesity; $p = 0.072$) (Table 3; Figure 3). A nonsignificant trend toward longer DFS in patients with the lowest quartile SM-RA was noted ($p = 0.087$; Figure S2). Sex-specific analysis of the association between sarcopenia, myosteatosis, and visceral obesity with DFS and OS was not significant (data not shown).

In multivariable analysis, including the significant results from univariable analysis (Table 5), preoperative

TABLE 4 Univariable analysis of DFS and OS by clinicopathological characteristics in iCCA

Characteristic	n (%)	Median DFS (95% CI)	HR (95% CI)	*p* value[a]	Median OS (95% CI)	HR (95% CI)	*p* value[a]
Sex							
Male	37 (43.5)	10 (4.9–15.1)		0.792	22 (6.0–38.0)		0.658
Female	48 (56.5)	10 (6.2–13.8)			30 (18.2–41.8)		
Age, years							
≤65	40 (47.1)	8 (4.3–11.7)		0.197	31 (21.7–40.3)		0.100
>65	45 (52.9)	11 (5.6–16.4)			22 (9.6–34.4)		
Cholangitis							
No	77 (90.6)	9 (6.2–11.8)		0.109	29 (20.6–37.4)		0.239
Yes	8 (9.4)	n.a.			n.a.		
PVE							
No	77 (90.6)	11 (8.9–13.1)		0.644	22 (14.3–29.7)		0.986
Yes	8 (9.4)	9 (5.2–12.8)			30 (20.8–39.2)		
EBD							
No	71 (83.5)	8 (5.6–10.4)		0.127	36 (10.6–61.4)		0.548
Yes	14 (16.5)	26 (3.6–48.4)			29 (20.2–37.8)		
Albumin, g/L							
≤42	28 (32.9)	8 (3.9–12.2)		0.360	20 (0.0–45.8)		0.413
>42	56 (65.9)	12 (7.6–16.4)			31 (22.0–40.0)		
AST, U/L							
≤40	49 (57.6)	13 (4.3–21.7)		0.551	25 (12.5–37.5)		0.638
>40	35 (41.2)	8 (5.5–10.5)			36 (11.8–60.2)		
ALT, U/L							
≤40	39 (45.9)	15 (6.5–23.5)		0.767	32 (13.5–50.5)		0.900
>40	45 (52.9)	8 (3.7–12.3)			25 (14.1–35.9)		
GGT, U/L							
≤100	35 (41.2)	10 (5.9–14.1)		0.851	29 (19.3–38.7)		0.917
>100	49 (57.6)	11 (6.3–15.6)			32 (10.3–53.7)		
Bilirubin, mg/dL							
≤1	66 (77.6)	11 (6.6–15.4)		0.618	30 (20.1–39.9)		0.820
>1	17 (20.0)	8 (6.4–9.6)			50 (0.1–100.0)		
Alkaline phosphatase, U/L							
≤100	26 (30.6)	12 (5.6–18.4)		0.688	22 (19.0–25.0)		0.622
>100	58 (68.2)	10 (7.2–12.8)			31 (23.5–38.5)		
Platelet count, 1/nL							
≤200	24 (28.2)	12 (1.7–22.3)		0.934	20 (3.0–37.0)		0.066
>200	60 (70.6)	10 (7.0–13.0)			32 (21.3–42.7)		
INR							
≤1	46 (54.1)	10 (4.8–15.2)		0.319	25 (13.4–36.6)		0.912
>1	38 (44.7)	9 (1.8–12.5)			30 (17.6–46.4)		
Hemoglobin, g/dL							
≤12	21 (24.7)	11 (2.8–19.2)		0.333	19 (0.0–43.5)		0.068
>12	63 (74.1)	10 (5.4–14.6)			32 (21.7–42.3)		
CRP, mg/L							
≤10	46 (51.1)	11 (5.8–16.2)		0.108	31 (17.9–44.1)		0.061
>10	38 (44.7)	8 (4.3–11.7)			29 (14.0–44.0)		

TABLE 4 (Continued)

Characteristic	n (%)	Median DFS (95% CI)	HR (95% CI)	*p* value[a]	Median OS (95% CI)	HR (95% CI)	*p* value[a]
Operative time, minutes							
≤300	49 (57.6)	11 (7.9–14.1)		0.884	25 (16.9–33.1)		0.958
>300	36 (42.4)	8 (6.4–9.6)			32 (21.5–42.5)		
Blood transfusions							
No	60 (70.6)	11 (6.2–15.8)		0.402	29 (18.5–39.5)		0.212
Yes	25 (29.4)	8 (0.2–15.8)			30 (11.4–48.6)		
FFP transfusions							
No	54 (63.5)	10 (7.0–13.0)		0.805	29 (21.1–36.9)		0.718
Yes	31 (36.5)	8 (1.1–14.9)			32 (12.3–51.7)		
R status							
R0	62 (72.9)	10 (6.2–13.8)		0.475	31 (16.3–45.7)		0.163
R1/Rx	18 (21.2)	8 (5.8–10.3)			22 (2.4–41.6)		
Microvascular invasion							
No	46 (54.1)	8 (4.0–12.0)		0.236	31 (13.9–48.1)		0.645
Yes	32 (37.6)	10 (7.0–13.0)			30 (18.5–41.5)		
Perineural invasion							
Pn0	22 (25.9)	10 (5.7–14.3)		0.370	36 (25.7–46.3)	1	0.031
Pn1	17 (20.0)	12 (2.6–21.4)			19 (8.4–29.6)	2.354 (1.041–5.324)	
Lymphovascular invasion							
No	57 (67.1)	8 (4.9–11.1)		0.367	40 (25.2–54.8)	1	0.000
Yes	21 (24.7)	10 (0.6–19.4)			4 (1.1–6.9)	3.929 (2.158–7.151)	
Tumor grading							
G1/G2	48 (56.5)	8 (4.0–12.0)		0.708	32 (21.3–42.7)		0.348
G3/G4	25 (29.4)	9 (5.1–12.9)			20 (0.0–41.6)		
Tumor stage (UICC)							
I/II	42 (49.4)	18 (9.5–26.5)	1	0.001	45 (26.8–63.2)	1	0.001
III/IV	33 (38.8)	6 (1.5–10.5)	2.607 (1.448–4.691)		16 (4.0–28.0)	2.597 (1.472–4.581)	
pT category							
pT1–2	71 (83.5)	11 (7.9–14.1)		0.284	30 (17.6–42.4)		0.202
pT3–4	12 (14.1)	7 (4.1–9.9)			22 (0.0–57.5)		
N category							
pN0	45 (52.9)	12 (3.1–20.9)	1	0.005	45 (28.1–61.9)	1	0.000
pN1	30 (35.3)	6 (2.2–9.8)	2.253 (1.241–4.092)		16 (4.7–27.3)	2.855 (1.602–5.087)	
Tumor number							
Single	49 (55.1)	15 (6.7–23.3)	1	0.007	36 (22.6–49.4)	1	0.009
Multiple	35 (39.3)	8 (7.0–9.0)	2.043 (1.182–3.533)		22 (19.4–24.6)	1.998 (1.165–3.426)	
Tumor size							
≤5 cm	21 (24.4)	15 (4.4–25.6)		0.058	21 (0.0–44.3)		0.697
>5 cm	62 (72.1)	8 (5.5–10.5)			30 (23.4–36.6)		
ICU time, days							
Mean, SD	3.5±8.6		0.923 (0.997–0.930)	0.923		1.041 (1.019–1.065)	0.000

(Continues)

TABLE 4 (Continued)

Characteristic	n (%)	Median DFS (95% CI)	HR (95% CI)	*p* value[a]	Median OS (95% CI)	HR (95% CI)	*p* value[a]
Hospitalization, days							
Mean, SD	18.1 ± 14.5		1.016 (0.998–1.035)	0.087		1.029 (1.013–1.045)	0.000
CCI							
≤40	54 (63.5)	11 (7.2–14.8)		0.437	32 (24.3–39.7)		0.059
>40	31 (36.5)	8 (5.0–11.0)			22 (4.1–39.9)		
Adjuvant therapy							
No	33 (38.8)	27 (1.4–52.6)	1	0.002	22 (12.2–31.8)		0.953
Yes	50 (58.8)	7 (5.7–8.3)	2.543 (1.350–4.789)		31 (25.3–36.7)		
Tumor recurrence							
No	29 (34.1)				58 (0.0–149.4)		0.269
Yes	54 (63.5)				27 (19.7–34.2)		

Note: The cohort was dichotomized at the median age of the cohort.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CCI, comprehensive complication index; CI, confidence interval; CRP, C-reactive protein; DFS, disease-free survival; EBD, endoscopic biliary drainage; FFP, fresh-frozen plasma; GGT, gamma-glutamyltransferase; HR, hazard ratio; iCCA, intrahepatic cholangiocarcinoma; ICU, intensive care unit; INR, international normalized ratio; OS, overall survival; PBD, percutaneous biliary drainage; pT, pathological tumor stage; PVE, portal vein embolization; UICC, Union Internationale Contre le Cancer.

[a]Based on log-rank test. $p < 0.05$ is significant.

hemoglobin ≤12 g/dL (HR, 2.448; $p = 0.05$) and fresh-frozen plasma transfusions (HR, 3.331; $p = 0.020$) were independent predictors of shortened DFS. Tumor grading 3–4 (HR, 1.930; $p = 0.045$) and CCI > 40 (HR, 3.060; $p = 0.001$) independently predicted OS (Table 6).

DISCUSSION

Intrahepatic and perihilar CCA are rare and aggressive malignancies with high rates of recurrence, even after extensive and high-risk major liver resections.[17] In this study, we analyzed preoperative CT scans to determine the incidence and the prognostic value of BC alterations in a large and homogeneous Western cohort of patients with iCCA and pCCA. The two tumor entities were analyzed separately for all outcome measures due to their inherent differences in prognosis and etiology. Alterations of BC were highly prevalent, with 50% of all patients being overweight or obese, 34% of patients sarcopenic, 66% myosteatotic, 62% displaying visceral obesity, and 14% of the overall cohort with sarcopenic obesity. We saw no relevant association of BC pathologies with pathological markers of aggressive tumor biology or with perioperative outcome parameters. While being overweight, sarcopenic, myosteatotic, or viscerally obese was not associated with altered DFS or OS, patients with iCCA with sarcopenic obesity were at an increased risk for inferior OS (11 months survival with sarcopenic obesity compared to 31 months OS in the remaining cohort; HR, 3.193; log-rank $p = 0.002$). This effect was not sustained in the multivariable analysis, possibly due to the relatively low number of patients at risk and events in the sarcopenic obesity group despite the high probability of death (n = 9 patients, eight events).

Our observation that sarcopenia, while not being predictive for the entire cohort, had a relevant prognostic value in a subset of patients who were overweight and obese with iCCA has been similarly noted in patients with pancreatic adenocarcinoma.[7] The hypothesis that low lean body mass combined with obesity results in lower performance status and lower OS in patients with tumors has been brought forward by Prado et al.[27] who delineated sarcopenia as an independent risk factor in individuals with obesity with gastrointestinal and pulmonary malignancies. In sarcopenic obesity, two highly prevalent risk factors come together—an aging population and a global obesity epidemic. In this regard, the traditional focus on isolated BMI measurement for the diagnosis of cachexia/muscle wasting is currently evolving toward more detailed assessments, including functional tests and imaging techniques.[28] The isolated analysis of BMI in patients with cancer, including patients with gastrointestinal malignancies, has shaped the so-called obesity paradox, the observation that while patients who are overweight or class I obese (BMI, 30 to <35 kg/m^2) are at a higher risk for cancer, their risk for overall mortality is lower than in normal-weight patients.[29,30] This phenomenon can be explained by, first, a BMI bias, namely, that BMI does not distinguish muscle mass and quality on one side and adipose mass and distribution on the other; second, by the fact that patients who are overweight and obese typically have higher overall muscle mass.[31] Approximately one half

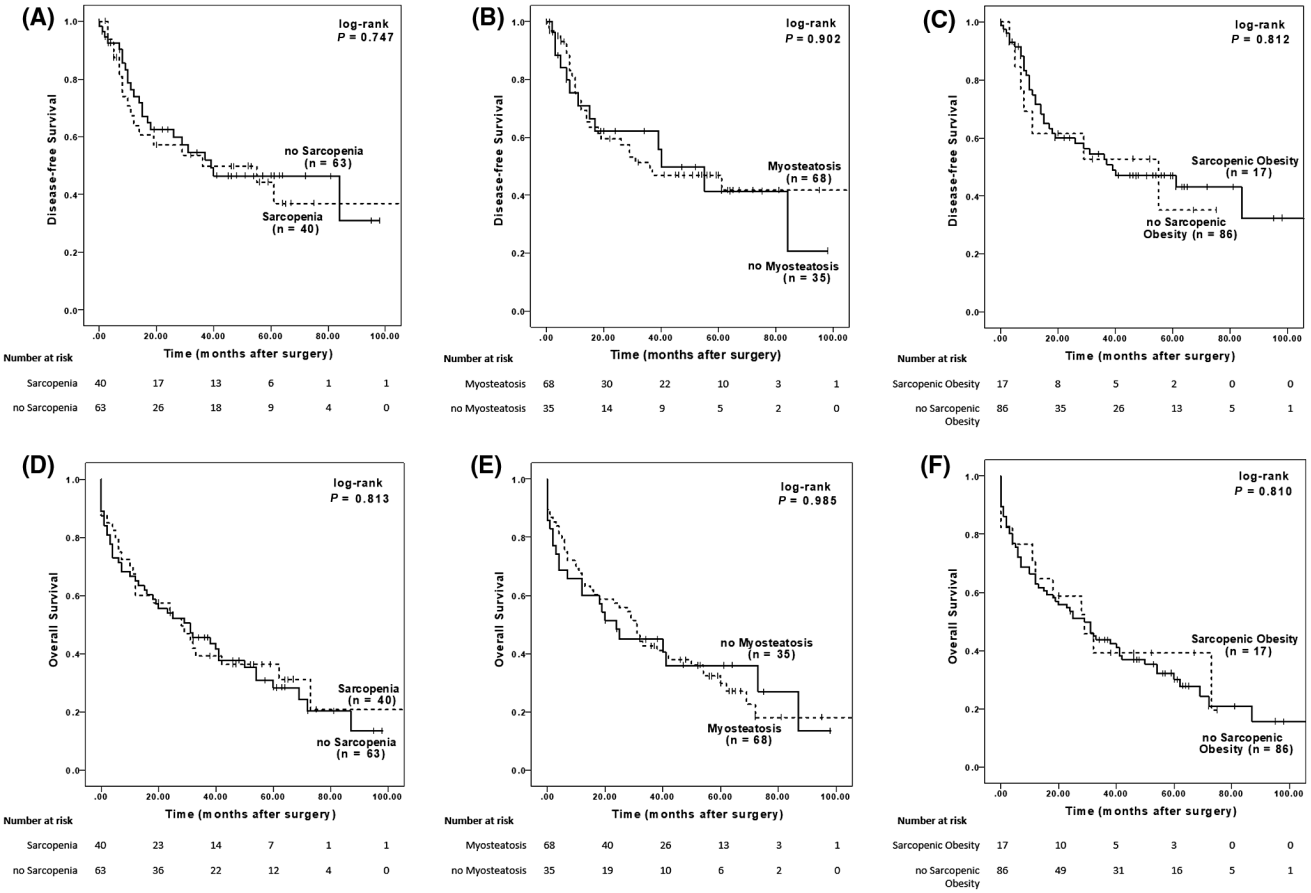

FIGURE 3 DFS and OS in relation to body composition characteristics in patients with perihilar cholangiocarcinoma. DFS for (A) sarcopenia, (B) myosteatosis, and (C) sarcopenic obesity. OS for (D) sarcopenia, (E) myosteatosis, and (F) sarcopenic obesity. DFS, disease-free survival; OS, overall survival.

of our study population was overweight or obese at the time of operation, making it unlikely that these patients would routinely attract clinical attention as being malnourished. Thus, raising the attention to muscle wasting that is masked by excessive adipose tissue may allow for prognostic patient selection and risk stratification as well as facilitate therapeutic interventions, such as nutritional counseling and support and physical prehabilitation.[28] The routine assessment of BC has been incorporated in the 2019 European Association for the Study of the Liver guidelines on clinical nutrition in chronic liver disease, with a recommendation to include sarcopenia evaluation, ideally on available CT scans, into the nutritional assessment.[8] Based on the data from this and future studies, a similar evaluation for sarcopenic obesity may be warranted in patients with iCCA.

Reasons for sarcopenia and sarcopenic obesity in the general population are largely age and lifestyle associated. Nerve cell reduction, decreased concentrations of anabolic hormones (growth hormone, testosterone, insulin-like growth factor), impaired regeneration, as well as decreased and dysfunctional protein synthesis are typical hallmarks of aging.[32] Additionally, patients with cancer exhibit a hypermetabolic state with a systemic inflammatory response that promotes nuclear factor kappa B pathway-mediated muscle degradation and cachexia.[33] Similarly, patients with obesity also have higher levels of inflammatory cytokines, such as interleukin (IL)-6, C-reactive protein, IL-1RA, and soluble IL-6R, and patients with these biochemical changes in turn have lower muscle strength.[34] Thus, sarcopenic obesity in patients with cancer can be viewed as a condition that arises against the background of a severely dysregulated multifactorial metabolic deregulation and a systemic inflammatory response. A recent murine CCA model of targeted Kirsten rat sarcoma viral oncogene homolog (KRAS) activation and loss of p53 recapitulated these hallmarks of sarcopenia and inflammation in the absence of weight loss.[35]

Few studies have examined the role of BC in CCA. A single-center study in surgically treated pCCA suggested an independent prognostic value of sarcopenia and low bone mineral density.[36] In a mixed cohort of 117 patients with curative or palliative regimens for iCCA, pCCA, dCCA, or gallbladder carcinoma, sarcopenia and myosteatosis were independent prognostic

TABLE 5 Univariable analysis of DFS and OS by clinicopathological characteristics in pCCA

Characteristic	n (%)	Median DFS (95% CI)	HR	*p* value[a]	Median OS (95% CI)	HR	*p* value[a]
Sex							
Male	71 (68.9)	39 (12.6–65.4)		0.642	31 (18.6–43.4)		0.332
Female	32 (31.1)	26 (0.0–84.5)			20 (6.1–33.9)		
Age, years							
≤65	44 (42.7)	40 (12.6–67.4)		0.789	29 (7.2–50.8)		0.686
>65	58 (56.3)	37 (0.0–77.3)			31 (19.2–42.8)		
BMI, kg/m^2							
≤25	52 (50.5)	40 (11.3–68.7)		0.696	31 (15.3–46.7)		0.845
>25	51 (49.5)	31 (0.0–64.6)			28 (13.2–42.8)		
Cholangitis							
No	63 (61.2)	31 (21.3–40.7)		0.283	31 (14.4–47.6)		0.206
Yes	36 (35.0)	61 (n.a.)			29 (17.5–40.5)		
PVE							
No	44 (42.7)	40 (12.5–67.5)		0.998	31 (21.6–40.4)		0.746
Yes	59 (57.3)	36 (0.0–83.3)			25 (9.0–41.0)		
EBD							
No	21 (20.4)	n.a.		0.055	n.a.		0.089
Yes	82 (79.6)	36 (13.1–58.9)			28 (17.9–38.1)		
PTCD							
No	79 (76.7)	39 (9.3–68.7)		0.907	31 (22.8–39.2)		0.711
Yes	23 (22.3)	36			29 (0.0–59.3)		
Albumin, g/L							
≤42	68 (66.0)	29 (7.0–51.0)		0.365	20 (7.4–32.6)		0.453
>42	35 (34.0)	55 (25.5–84.5)			38 (27.2–48.7)		
AST, U/L							
≤40	37 (35.9)	84 (20.7–147.3)		0.315	31 (12.2–49.8)		0.788
>40	66 (64.1)	31 (5.5–56.5)			29 (19.5–38.5)		
ALT, U/L							
≤40	18 (17.5)	40 (0.0–84.0)		0.767	23 (8.4–37.6)		0.438
>40	85 (82.5)	39 (10.1–67.9)			31 (18.0–44.1)		
GGT, U/L							
≤100	8 (7.8)	61 (13.1–108.9)		0.507	32 (0.0–70.8)		0.838
>100	95 (92.2)	37 (10.2–63.8)			29 (18.1–39.9)		
Bilirubin, mg/dL							
≤1	45 (43.7)	84 (25.2–142.8)		0.298	32 (27.1–36.9)		0.513
>1	57 (55.3)	36 (4.5–67.5)			25 (8.4–42.0)		
Alkaline phosphatase, U/L							
≤100	4 (3.9)	n.a.		0.239	n.a.		0.113
>100	99 (96.1)	37 (9.6–64.4)			28 (17.8–38.2)		
Platelet count, 1/nL							
≤200	13 (12.6)	n.a.		0.525	4 (0.5–7.5)		0.462
>200	90 (87.4)	39 (11.0–67.0)			31 (23.1–38.9)		
INR							
≤1	38 (36.9)	61 (n.a.)		0.160	50 (22.8–77.2)		0.141
>1	65 (63.1)	31 (8.7–53.3)			24 (8.0–39.6)		

TABLE 5 (Continued)

Characteristic	n (%)	Median DFS (95% CI)	HR	*p* value[a]	Median OS (95% CI)	HR	*p* value[a]
Hemoglobin, g/dL							
≤12	38 (36.9)	14 (6.1–21.9)	3.022 (1.621–5.633)	0.000	15 (5.9–24.1)	1.753 (1.093–2.811)	0.016
>12	65 (63.1)	84 (35.0–133.0)	1		32 (21.3–42.7)	1	
CRP, mg/L							
≤10	42 (40.8)	61 (n.a.)		0.243	32 (27.1–36.9)		0.188
>10	61 (59.2)	36 (20.8–51.2)			23 (5.0–41.0)		
Operative time, minutes							
≤360	30 (29.1)	18 (0.0–56.3)		0.914	31 (0.0–74.1)		0.176
>360	73 (70.9)	39 (12.0–66.0)			29 (15.8–42.1)		
Blood transfusions							
No	56 (54.4)	84 (43.1–124.9)	1	0.002	54 (21.3–86.7)	1	0.002
Yes	47 (45.6)	14 (3.6–24.4)	2.520 (1.353–4.692)		12 (6.6–17.4)	2.057 (1.288–3.288)	
FFP							
No	39 (37.9)	n.a.	1	0.004	69 (22.8–115.2)	1	0.002
Yes	64 (62.1)	29 (9.9–48.1)	2.662 (1.328–5.333)		15 (4.1–25.9)	2.163 (1.288–3.635)	
R status							
R0	78 (75.7)	55 (17.1–92.9)		0.475	31 (17.8–44.2)		0.196
R1/Rx	24 (23.3)	36 (0.2–71.8)			18 (1.2–34.8)		
MVI							
No	71 (68.9)	84 (21.9–146.1)		0.114	38 (25.9–50.1)		0.334
Yes	26 (25.2)	29 (12.9–45.1)			18 (0.0–38.0)		
Perineural invasion							
Pn0	15 (14.6)	n.a.		0.241	69 (11.9–126.1)		0.120
Pn1	68 (66.0)	36 (22.0–50.0)			20 (2.8–37.2)		
LVI							
No	74 (71.8)	61 (33.5–88.5)	1	0.004	41 (23.6–58.4)	1	0.005
Yes	21 (20.4)	15 (7.2–22.8)	2.810 (1.342–5.883)		12 (0.1–23.9)	2.190 (1.241–3.866)	
Tumor grading							
G1/G2	74 (71.8)	84 (19.2–148.8)	1	0.026	41 (23.5–58.5)	1	0.000
G3/G4	24 (23.3)	10 (0.0–51.4)	2.288 (1.073–4.877)		6 (1.2–10.8)	2.937 (1.738–4.964)	
Tumor stage UICC							
I/II	42 (40.8)	84 (28.0–140.0)	1	0.016	54 (33.1–74.9)	1	0.002
III/IV	60 (58.3)	29 (5.5–52.5)	1.482 (1.066–2.061)		13 (4.5–21.5)	2.173 (1.306–3.614)	
pT category							
pT1–2	66 (64.1)	55 (16.8–93.2)		0.081	40 (19.7–60.3)	1	0.001
pT3–4	36 (35.0)	15 (4.8–25.2)			10 (5.0–15.0)	2.145 (1.324–3.474)	
N category							
pN0	57 (55.3)	84 (30.0–138.2)	1	0.001	50 (28.0–72.0)	1	0.002
pN1	44 (42.7)	6 (0.0–37.2)	2.790 (1.475–5.276)		13 (2.2–23.8)	2.064 (1.271–3.352)	

(Continues)

TABLE 5 (Continued)

Characteristic	n (%)	Median DFS (95% CI)	HR	*p* value[a]	Median OS (95% CI)	HR	*p* value[a]
Tumor number							
Single	74 (71.8)	39 (10.6–67.4)		0.934	31 (20.7–41.3)		0.723
Multiple	25 (24.2)	19 (n.a.)			18 (4.0–32.1)		
Tumor size							
≤3 cm	48 (46.6)	n.a.	1	0.001	54 (29.5–78.5)	1	0.005
>3 cm	43 (41.7)	12 (3.0–21.0)	2.496 (1.286–4.842)		13 (4.0–22.0)	2.262 (1.376–3.719)	
ICU time, days							
Mean, SD	6.2 ± 15.4		1.010 (0.978–1.043)	0.542		1.022 (1.011–1.033)	0.000
Hospitalization, days							
Mean, SD	25.8 ± 20.6		1.013 (0.997–1.030)	0.112		1.014 (1.002–1.025)	0.020
CCI							
≤40	50 (48.5)	84 (35.0–133.0)	1	0.015	69 (41.4–96.6)	1	0.000
>40	61 (59.2)	17 (0.0–38.8)	2.130 (1.136–3.994)		7 (0.0–14.1)	3.099 (1.890–5.081)	
Adjuvant therapy[b]							
No	62 (60.2)	84 (n.a.)	1	0.000	25 (0.0–56.2)		0.204
Yes	41 (39.8)	19 (5.5–32.6)	3.174 (1.617–6.229)		29 (22.0–36.0)		
Tumor recurrence							
No	60 (58.3)				60 (15.7–104.3)		0.003
Yes	41 (39.8)				25 (15.0–35.0)		

Note: The cohort was dichotomized at the median age of the cohort.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CCI, comprehensive complication index; CI, confidence interval; CRP, C-reactive protein; DFS, disease-free survival; EBD, endoscopic biliary drainage; FFP, fresh-frozen plasma; GGT, gamma-glutamyltransferase; HR, hazard ratio; ICU, intensive care unit; INR, international normalized ratio; LVI, lymphovascular invasion; MVI, microvascular invasion; n.a., not applicable; OS, overall survival; PBD, percutaneous biliary drainage; pCCA, perihilar cholangiocarcinoma; pT, pathological tumor stage; PTCD, percutaneous transhepatic cholangiography; PVE, portal vein embolization; UICC, Union Internationale Contre le Cancer.

[a] (Header row, behind *p*): based on log rank test, for continuous variables (ICU stay, Hospital stay) based on Cox regression analysis.

[b] (Behind adjuvant therapy): all patients receiving at least one cycle of chemotherapy were considered in this category.

factors for survival while the prognostic value of sarcopenic obesity was not investigated in this heterogeneous cohort.[37] Similarly, a recent study of 75 palliative CCA cases suggested a prognostic role of both sarcopenia and myosteatosis, as assessed by L3 CT SMI and SM-RA, but without differentiating between CCA subtypes.[38] In a mixed cohort of 76 patients with intrahepatic and extrahepatic biliary cancer, including gallbladder carcinoma, sarcopenia predicted OS in male patients,[39] an observation that was not replicated in our cohort. In comparison, our larger cohort of only patients with iCCA and pCCA failed to show a prognostic value of sarcopenia and myostatosis for DFS or OS, potentially due to the absence of distal CCA cases in the cohort. Similarly, in comparison to a palliative cohort, patients undergoing major liver surgery are highly preselected for their functional status, which impairs comparability of our data to studies with patients receiving palliative care.[37,38]

As with all clinical outcome studies, this analysis has the following potential limitations: first, the retrospective single-center nature of the study requiring prospective multicentric validation; second, the relatively small group of patients at risk for each BC pathology owing to the rarity of the disease and the split analysis for iCCA and pCCA; third, the lack of functional assessment, such as handgrip strength, which would require prospective patient recruitment and may be biased due to preoperative patient selection of patients fit enough to undergo surgery; and fourth, the relatively advanced disease at the time of surgical treatment (e.g., >70% of patients with iCCA were staged as ≥T2). Nevertheless, to our knowledge, the present study comprises the largest and most homogeneous CCA cohort focusing on BC analysis in patients with surgical iCCA and pCCA and the first investigation of sarcopenic obesity in CCA, revealing a potential prognostic value of this specific BC profile for OS in iCCA. We accordingly see added value in extrapolating

TABLE 6 Multivariable Cox regression analysis of prognostic factors for disease-free and overall survival in iCCA and pCCA

	Disease-free survival		Overall survival	
Prognostic factor	**Hazard ratio (95% CI)**	***p* value[a]**	**Hazard ratio (95% CI)**	***p* value[a]**
iCCA				
Perineural invasion	n.s.[b]		1.378 (0.456–4.164)	0.570
Lymphovascular invasion	n.s.[b]		3.706 (1.093–12.573)	0.036
Lymph node invasion	1.503 (0.439–5.142)	0.516	0.901 (0.116–11.605)	0.901
UICC stage III/IV	3.715 (1.081–12.765)	0.037	1.652 (0.185–14.730)	0.653
Sarcopenic obesity	n.s.[b]		1.833 (0.353–9.501)	0.471
pCCA				
Hemoglobin ≤ 12 g/dL	2.448 (1.000–5.990)	0.050	0.940 (0.471–1.876)	0.860
Blood transfusions	1.443 (0.533–3.906)	0.470	1.139 (0.539–2.409)	0.733
FFP transfusions	3.331 (1.207–9.194)	0.020	1.510 (0.700–3.256)	0.293
Lymphovascular invasion	1.630 (0.682–3.897)	0.272	1.599 (0.769–3.326)	0.209
Lymph node invasion	1.689 (0.530–5.387)	0.376	1.493 (0.675–3.306)	0.322
Grading 3–4	1.463 (0.602–3.555)	0.401	1.930 (1.014–3.671)	0.045
UICC stage III/IV	1.225 (0.388–3.873)	0.730	2.062 (0.921–4.615)	0.078
Tumor number	1.298 (0.510–3.306)	0.584	1.159 (0.561–2.393)	0.690
CCI > 40	1.534 (0.667–3.527)	0.314	3.060 (1.589–5.893)	0.001
Tumor recurrence	n.a.		1.522 (0.781–2.965)	0.217

Note: Due to multicollinearity, the following variables were not included in the multivariable analysis: adjuvant treatment (patient selection for therapy was associated with pathological risk factors [nodal status, R status] in the pre-BILCAP era), T category (collinearity with UICC staging), ICU and hospital stay (collinearity with transfusions and CCI), tumor size (collinearity with UICC staging).

Abbreviations: BILCAP, Capecitabine Compared With Observation in Resected Biliary Tract Cancer; CCA, cholangiocarcinoma; CCI, comprehensive complication index; CI, confidence interval; FFP, fresh-frozen plasma; iCCA, intrahepatic cholangiocarcinoma; ICU, intensive care unit; n.a., not applicable; n.s., not significant; pCCA, perihilar cholangiocarcinoma; UICC, Union Internationale Contre le Cancer.

[a]$p < 0.5$ is significant.

[b]Not significant in univariable analysis (log-rank test).

BC data from routinely performed CTs in patients with iCCA, with a focus on sarcopenic obesity.

AUTHOR CONTRIBUTIONS

Study design by the initiating study team: Zoltan Czigany, Georg Lurje, Ulf Peter Neumann. Data collection and analysis: Sarah Eischet, Isabella Lurje, Zoltan Czigany, Jan Bednarsch, Tom Florian Ulmer, Peter Isfort, Pavel Strnad, Ulf Peter Neumann, Georg Lurje. Image analysis: Sarah Eischet, Zoltan Czigany. Manuscript draft: Isabella Lurje, Zoltan Czigany, Georg Lurje, Sarah Eischet. Further authors substantially contributing to the final version of the manuscript: Sarah Eischet, Isabella Lurje, Jan Bednarsch, Tom Florian Ulmer, Peter Isfort, Christian Trautwein, Frank Tacke, Ulf Peter Neumann, Georg Lurje. All authors have read and approved the final version of the manuscript. This study had no involvement by the funders in study design, data collection, data analysis, manuscript preparation, or decision to publish.

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CONFLICT OF INTEREST

The authors of this manuscript have no conflict of interest to declare.

ETHICAL APPROVAL

This study was conducted in accordance with the current version of the Declaration of Helsinki and good clinical practice guidelines (International Conference on Harmonization, Good Clinical Practice). Approval was granted by the institutional review board (EK 341/21).

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SUPPORTING INFORMATION

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2.5 Die metabolisch assoziierte Fettlebererkrankung (MASLD) als onkologische Ätiologie ist im intrahepatischen Cholangiokarzinom mit pathologischer Körperzusammensetzung assoziiert

Body Composition is Associated with Disease Etiology and Prognosis in Patients Undergoing Resection of Intrahepatic Cholangiocarcinoma.
Cancer Medicine, 2023, Sep;12(17):17569–17580

Abstract Zitat (98):
***"Background**: Body composition alterations are frequent in patients with cancer or chronic liver disease, but their prognostic value remains unclear in many cancer entities.*
***Objective**: We investigated the impact of disease aetiology and body composition after surgery for intrahepatic cholangiocarcinoma (iCCA), a rare and understudied cancer entity in European and North American cohorts.*
***Methods**: Computer tomography-based assessment of body composition at the level of the third lumbar vertebra was performed in 173 patients undergoing curative-intent liver resection for iCCA at the Department of Surgery, Charité – Universitätsmedizin Berlin. Muscle mass and -composition as well as subcutaneous and visceral adipose tissue quantity were determined semi-automatically. (Secondary) sarcopenia, sarcopenic obesity, myosteatosis, visceral and subcutaneous obesity were correlated to clinico-pathological data.*
***Results:** Sarcopenia was associated with post-operative morbidity (intraoperative transfusions [p = 0.027], Clavien-Dindo ≥ IIIb complications [p = 0.030], post-operative comprehensive complication index, CCI [p < 0.001]). Inferior overall survival was noted in patients with myosteatosis (33 vs. 23 months, p = 0.020). Fifty-eight patients (34%) had metabolic (dysfunction)-associated fatty liver disease (MAFLD) and had a significantly higher incidence of sarcopenic (p = 0.006), visceral (p < 0.001) and subcutaneous obesity (p < 0.001). Patients with MAFLD had longer time-to-recurrence (median: 38 vs. 12 months, p = 0.025, log-rank test). Multivariable cox regression analysis confirmed only clinical, and not body, composition parameters (age > 65, fresh frozen plasma transfusions) as independently prognostic for overall survival.*
***Conclusion:** This study evidenced a high prevalence of MAFLD in iCCA, suggesting its potential contribution to disease etiology. Alterations of muscle mass and adipose tissue were more frequent in patients with MAFLD."*

Während im HCC die weltweite Übergewichts-Epidemie mit hoher Prävalenz von Fettlebererkrankung/MASLD als Krankheitsätiologie breit anerkannt und als kritische

gesundheitspolitische Problematik in den Guidelines internationaler Gesellschaften formuliert ist (13), so ist aufgrund der relativen Seltenheit des CCAs die Rolle der Fettlebererkrankung in dieser Tumorentität nur begrenzt untersucht (99). Hinsichtlich der Körperzusammensetzung ist über Patient*innen mit MASLD zwar bekannt, dass sowohl Fett- als auch Muskelmasse erhöht sind (100), die Auswirkung der metabolischen Syndrom/MASLD-assoziierten Körperzusammensetzung im onkologischen Kontext, in dem vielmehr Mangelernährung und Sarkopenie (niedrige Muskelmasse) im Kontext einer konsumierenden Gesamtsituation prognostisch ungünstig sind, ist allerdings unbekannt. Basierend auf den Beobachtungen der vorherigen, unter 2.4 angeführten Studie, die eine negative prognostische Rolle des sarkopenen Übergewichts dokumentierte, hypothetisierten wir, dass Patienten mit MASLD-iCCA ebenfalls eine vulnerable Gruppe mit Zusammenkommen von onkologischer und metabolischer Lebererkrankung darstellen.

Um die Rolle der MASLD in diesem onkologischen Kontext zu erforschen, analysierten wir in einem zweiten, größeren europäischen Kollektiv (Charité Universitätsmedizin Berlin) die Krankheitsätiologie, Körperzusammensetzung und die Prognose von Patient*innen mit iCCA. Neben der CT-basierten Analyse der Körperzusammensetzung mit Definition der Pathologien Sarkopenie, viszerale, subkutane und sarkopene Obesität sowie Myosteatose, erhoben wir anhand der klinischen Historie und des malignomfreien Leberanteils des Resektats, ob eine hepatische Vorerkrankung bestand.

Ein Drittel unserer Kohorte erfüllte neben dem iCCA die diagnostischen Kriterien einer MASLD, und etwa ein weiteres Drittel hatte eine Fibrose oder Zirrhose, während ein Drittel der iCCA in histologisch gesundem Lebergewebe entstand. Vergleichen mit allen anderen Patient*innen in unserer Kohorte, hatten Patient*innen mit MASLD eine signifikant höhere Inzidenz von sarkopener, viszeraler und subkutaner Obesität, sowie einem Trend zu einer geringeren präoperative Leberfunktion (LiMAx, Humedics GmbH, Berlin). Trotz dieser potenziell prognostisch ungünstigen Konstellation von Leberfunktion und Body Composition hatten Patient*innen mit MASLD-iCCA ein signifikant längere Zeit bis zur Rekurrenz, im Median 38 Monate, verglichen mit 12 Monaten bei Patient*innen mit nicht-MASLD Tumorätiologie, die jedoch in der Multivariablen Analyse gemeinsam mit klinisch-pathologischen Kriterien nicht als unabhängiger prognostischer Faktor bestätigt werden konnte.

Eine weitere Erkenntnis aus dieser größeren Studie war, dass die Sarkopenie signifikant mit perioperativer Morbidität assoziiert war, beispielsweise mit dem Bedarf für intraoperative Transfusionen und der Inzidenz und Schwere postoperativer Komplikationen. Dies geht einher mit der Beobachtung, dass eine geringere Muskelmasse mit einer allgemein geringeren funktionellen Reserve einhergeht.

Zusammenfassend konnte in dieser Pilotstudie gezeigt werden, dass die MASLD-iCCA Ätiologie einhergeht mit typischen Veränderungen der Körperzusammensetzung, ohne in diesem Kollektiv unabhängig die Krankheitsprognose zu bestimmen. Zukünftige Fragestellungen im Setting des MASLD-iCCAs beinhalten die metabolische, funktionelle und immunologische Situation dieser Patient*innen, die angesichts der weltweiten Übergewichts-Epidemie eine zunehmende Proportion der iCCA-Ätiologien darstellen wird.

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RESEARCH ARTICLE

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Body composition is associated with disease aetiology and prognosis in patients undergoing resection of intrahepatic cholangiocarcinoma

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Abstract

Background: Body composition alterations are frequent in patients with cancer or chronic liver disease, but their prognostic value remains unclear in many cancer entities.

Objective: We investigated the impact of disease aetiology and body composition after surgery for intrahepatic cholangiocarcinoma (iCCA), a rare and understudied cancer entity in European and North American cohorts.

Methods: Computer tomography-based assessment of body composition at the level of the third lumbar vertebra was performed in 173 patients undergoing curative-intent liver resection for iCCA at the Department of Surgery, Charité – Universitätsmedizin Berlin. Muscle mass and -composition as well as subcutaneous and visceral adipose tissue quantity were determined semi-automatically. (Secondary) sarcopenia, sarcopenic obesity, myosteatosis, visceral and subcutaneous obesity were correlated to clinicopathological data.

Results: Sarcopenia was associated with post-operative morbidity (intraoperative transfusions [$p = 0.027$], Clavien–Dindo ≥ IIIb complications [$p = 0.030$], post-operative comprehensive complication index, CCI [$p < 0.001$]). Inferior overall survival was noted in patients with myosteatosis (33 vs. 23 months, $p = 0.020$). Fifty-eight patients (34%) had metabolic (dysfunction)-associated fatty liver disease (MAFLD) and had a significantly higher incidence of sarcopenic ($p = 0.006$), visceral ($p < 0.001$) and subcutaneous obesity ($p < 0.001$). Patients with MAFLD had longer time-to-recurrence (median: 38 vs. 12 months, $p = 0.025$, log-rank test). Multivariable cox regression analysis confirmed only clinical, and not body, composition parameters (age > 65, fresh frozen plasma transfusions) as independently prognostic for overall survival.

Conclusion: This study evidenced a high prevalence of MAFLD in iCCA, suggesting its potential contribution to disease aetiology. Alterations of muscle mass and adipose tissue were more frequent in patients with MAFLD.

KEYWORDS
liver cancer, MAFLD, NAFLD, visceral obesity

1 | INTRODUCTION

Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver cancer after hepatocellular carcinoma (HCC).[1] The incidence and mortality of iCCA is rising in western countries, despite an overall declining cancer mortality over the last three decades.[2] While many cases of iCCA in Europe arise sporadically, known risk factors include chronic inflammation, cholelithiasis and cirrhosis, while liver fluke infections strongly contribute to iCCA incidence in Asian regions.[3] Generally, the aetiology and disease course of iCCA are understudied in western cohorts.

Recently, obesity and non-alcoholic fatty liver disease (NAFLD), or, according to recent pathophysiological classifications, metabolic (dysfunction)-associated fatty liver disease (MAFLD), have been recognized as an iCCA aetiology and suggested as an adverse prognostic factor for overall survival.[4,5] MAFLD is estimated to affect 25% of the global population and constitutes an important aetiology of end-stage and malignant liver disease.[6] A rising incidence and disease severity is projected for the population worldwide, including the USA, China and Europe.[7] Patients with fatty liver disease furthermore carry a high overall risk of adverse cardiovascular events, other oncological diseases and musculoskeletal disorders.

Body composition—the quantity of skeletal muscle and the quantity and distribution of adipose tissue—of patients with obesity fulfilling non-alcoholic steatohepatitis (NASH) criteria was characterized by various groups, suggesting that patients typically have a concomitant increase of both adipose tissue and skeletal muscle mass.[8] It is currently unclear whether these observations can be translated into oncological settings, where the typical phenotype of cachexia and sarcopenia is the most frequently described alteration. Our group recently reported that sarcopenic obesity may hold value for overall survival in iCCA in a different patient cohort from a German tertiary centre.[9] This publication, despite a lower sample size, showed a high prevalence of body composition pathologies in patients with iCCA and perihilar CCA.[9]

In gastrointestinal malignancies, an association of body composition with metabolic function, systemic inflammatory processes and overall prognosis has been suggested previously.[10,11] Consequently, nutritional and rehabilitative strategies to maintain and increase muscle mass have been incorporated into guidelines for these patients.[12] It is currently unclear whether patients suffering from MAFLD-iCCA show alterations in their body composition, and whether these changes affect disease prognosis. We therefore aimed to delineate prognostic roles of disease aetiology and body composition in iCCA. Furthermore, we examined the relationship between underlying liver disease and pathologies in the computed tomography (CT)-based composition and quantity of muscle and adipose tissue distribution in a European cohort of surgically treated patients with iCCA.

2 | METHODS

All consecutive patients undergoing curative-intent hepatectomy for iCCA between March 2010 and December 2020 at the Department of Surgery, Charité – Universitätsmedizin Berlin were retrospectively evaluated for study inclusion. Inclusion criteria were defined as, (a) pathological and radiological diagnosis of iCCA under the exclusion of perihilar and distal tumour subtypes and mixed iCCA-HCC, (b) available CT scans including the third lumbal vertebra within 3 months prior to operation. Patients with only other available imaging modalities, such as magnetic resonance imaging (MRI)-based abdominal staging, were not included in this study.

This study was approved by the ethics committee of the Charité – Universitätsmedizin Berlin (EA1/105/21) and conducted in accordance with the Declaration of Helsinki and the good clinical practice (ICH-GCP) guidelines. Informed consent was waived in agreement with the ethics committee due to retrospective, pseudonymized study design and analysis of available clinical data. Clinical data were retrieved from patients' records and from a prospectively managed institutional database. Recurrence and survival data were obtained from the Charité outpatient clinic and from local outpatient hepatologists and oncologists.

Post-hepatectomy liver failure (PHLF), as a potential result of a diminished overall energy reserve, was defined as based on post-operative (Day 5) international normalized ratio (INR) together with hyperbilirubinemia as previously described.[13] The presence of MAFLD, as opposed to just

the presence of steatosis in our exploratory analysis, was defined based on the pathophysiology-centred 2020 consensus statement (any presence of pathology-proven hepatic steatosis plus Type 2 diabetes or a body mass index [BMI] ≥25 kg/m^2 or the presence of more than two metabolic abnormalities).[14] Histological steatosis was routinely assessed on Haematoxylin-Eosin staining and reported in the non-tumourous area of the resected specimen by a surgical pathologist. In contrast to previous NAFLD criteria, this pathophysiology-centred approach uses positive inclusion criteria and does not rely on patient-reported alcohol consumption,[14] consistent with the paradigm shift towards (a) relevant alcohol consumption being often reported inaccurately (and not routinely investigated prior to oncological surgery as opposed to liver transplant recipients with unclear consumption status) (b) the copresence and thus the substantial etiological overlap of the metabolic syndrome and relevant alcohol consumption[15] (c) the more accurate reflection on the pathophysiological aspects of the metabolic syndrome as drivers of liver disease.[16]

2.1 | Image and clinical data analysis

As previously described,[9,17] an axial CT image at the level of the third lumbar vertebra from the most recent CT image before surgery was analyzed semi-automatically with 3D Slicer[18] and the Workstation SlicerCIP extension, body composition module (version 4.10.2). Attenuation values from −29 to 150 Hounsfield units (HU) defined skeletal muscle.[19] The spinal muscle area (SMA) included the psoas major, spinal (erector spinae, quadratus lumborum), transversus abdominis, external and internal oblique, and rectus abdominis muscles. Attenuation values from −150 to −50 HU indicated visceral adipose tissue, while −190 to −30 HU defined subcutaneous adipose tissue (subcutaneous fat area [SFA]). Skeletal muscle radiation attenuation (SM-RA) in HU within the muscle area was recorded to assess myosteatosis. Muscle and adipose tissue indices (skeletal muscle index [SMI]; subcutaneous fat index [SFI]) were calculated by normalizing the SMA and SFA to the patients' height (area[cm]^2/height[m]^2). The same trained investigator performed the segmentation analysis while being blinded to patients' outcomes (MDP).

Cut-offs for body composition pathologies were derived from large multicentric oncological cohorts to avoid overfitting to the present dataset.[20] Primary sarcopenia is defined as low muscle mass and low muscle strength,[21] while our assessment of sarcopenia relied only on the definition of low muscle mass with the following sex-specific cut-off: SMI <52.4 cm^2/m^2 for men and <38.5 cm^2/m^2 for women.[22] Hereafter, this CT-based diagnosis will be referred to as 'sarcopenia'. The cut-off for myosteatosis was <41 HU if the BMI was <25 kg/m^2 and <33 HU for patients whose BMI equalled or exceeded 25 kg/m^2.[20] The adaptation to BMI is based on the fact that an accumulation of inter- and intramyocellular fat is significantly dependent on the overall amount of body fat and can only be considered pathological in the context of BMI. A visceral fat area (VFA) exceeding 100 cm^2 indicated visceral obesity,[19] while the SFI was dichotomized at the upper tertile of the cohort (71.89 cm^2/m^2).[23] Sarcopenic obesity was defined as sarcopenia plus a simultaneous BMI ≥25 kg/m^2.[9]

2.2 | Statistical analysis

The primary end point of the present study was oncological and overall survival in patients with iCCA depending on their body composition. The SPSS Statistics 24 software (IBM Corp.) was used for statistical analyses and GraphPad Prism 9 (GraphPad Software) was used for visualization of correlation matrices. Categorical variables were presented as number (frequency, %) and compared with the Mann–Whitney *U* test, while continuous variables (normally distributed) were displayed as mean ± standard deviation and compared with the Pearson's chi-square test. Non-normally distributed data were presented as median and range. For comparisons between 3 or more variables, the ANOVA with post hoc Bonferroni correction was used for continuous variables, and the Kruskal–Wallis test for categorical variables. Spearman *r* correlation was calculated and plotted for the association of MAFLD and body composition parameters. A two-sided *p*-value of ≤0.05 was regarded as statistically significant, unless corrected with the Bonferroni method. Median time to recurrence (TTR) and overall survival (OS) were presented with 95% confidence intervals (CI). For TTR, the time between operation and recurrence was calculated, and patients were censored if they died or were lost to follow-up. For OS, the time from operation to death (from any cause) was calculated, and patients were censored at the time of their loss to follow-up. Survival differences between groups were compared using the Kaplan–Meier curves and the log-rank test, hazard ratios (HR) were calculated with univariable and multivariable survival analysis. Statistically significant covariates in univariable analysis that are stable over time, and under exclusion of parameters with suspected collinearity were included in the multivariable analyses.

3 | RESULTS

Of 236 patients with iCCA who were operated at the Department of Surgery, Charité – Universitätsmedizin

Berlin within the study period, 173 patients (73%) met the inclusion criteria. The remaining patients either underwent MRI preoperatively or CT images were unavailable for body composition analyses (Figure S1). The study cohort was composed of 87 men (50%) and 86 women (50%) with a mean age of 64 years.

Based on the medical history, tumour aetiology was unknown in most cases (149/173, 86%), while 16 patients reported elevated alcohol consumption (9%), 2 patients (1%) had a previously diagnosed primary sclerosing cholangitis and 6 patients (4%) had chronic viral hepatitis. Histological data on underlying liver disease were available in 156/173 (90%) patients, with histological data on the grade of steatosis available in 147/173 (85%) patients. Histologically, an absence of liver disease was reported only in 44 (26%) of patients, while 46 (27%) patients were diagnosed with liver fibrosis, 8 (5%) with cirrhosis. In our cohort, 58 (34%) patients fulfilled MAFLD criteria and another 17 (10%) patients had steatosis without fulfilling MAFLD criteria.

Most patients underwent extensive liver resections, with 67 (39%) patients undergoing left or right hepatectomy and 92 (53%) patients undergoing extended hemihepatectomy. The remaining 14 patients (8%) were treated with anatomical or nonanatomical resections or bisegmentectomies. Detailed patient and tumour characteristics are shown in Table 1. A total of 31 patients (18%) fulfilled the criteria of PHLF, while 34 patients (20%) had severe (≥Clavien-Dindo 3b) post-operative complications (Table S1). The 90-day mortality was 12% (21/176) (Table S2).

3.1 | Body composition

The median period between CT imaging used for analysis and the operation was 16 days. The median BMI was 24.8 kg/m^2 (range: 13.4–41.1 kg/m^2), with 90 (50%) patients classified as overweight (BMI 25–30 kg/m^2, $n = 53$, 31%) or obese (BMI ≧30 kg/m^2, $n = 33$, 19%). Sarcopenia—deduced from a reduced SMI—was present in 30 (17%) patients, while 9 (5%) patients had a simultaneous BMI ≧25 kg/m^2, thus fulfilling the definition of sarcopenic obesity. Visceral obesity was present in 104 (60%) patients, while 43 (25%) patients had subcutaneous obesity. Myosteatosis, reflective of reduced muscle radiodensity and fatty infiltration, was found in 45% (78/173) of the cohort. Sarcopenia was associated with higher rates of intraoperative transfusions (18/30 vs. 54/142, $p = 0.027$), with a higher rate of severe (≥Clavien-Dindo, CD IIIb) complications (10/30 vs. 23/142, $p = 0.030$) and a higher post-operative comprehensive complication index (CCI, 46 vs. 30, $p < 0.001$, Table S1).

TABLE 1 Patient and tumour characteristics of the iCCA cohort ($n = 173$).

Patient characteristics	
Age (years)	65.0 (23.0–83.0)
BMI	24.9 (13.4–41.1)
Sex ratio (F:M), *n* (%)	87 (50): 86 (50)
Aetiology by predominant histology *n* (%)	
Fibrosis	46 (27)
F1	16 (35)
F2	11 (24)
F3	7 (15)
Cirrhosis/F4	8 (5)
MAFLD	58 (34)
F0	5 (9)
F1	16 (28)
F2	9 (16)
F3	3 (5)
MAFLD-cirrhosis /F4	4 (7)
Steatosis w/o MAFLD criteria	17 (10)
No known liver disease	44 (26)
Endoscopic biliary drainage, *n* (%)	20 (12)
Percutaneous biliary drainage, *n* (%)	4 (2)
Portal vein embolization, *n*, (%)	23 (13)
Neoadjuvant chemotherapy, *n*, (%)	16 (9)
Operative approach *n*, (%)	
Conventional	147 (85)
Laparoscopic	12 (7)
Robotic	14 (8)
Operative procedure *n*, (%)	
Atypical/anatomical resection/ bisegmentectomy	14 (8)
Right hepatectomy	39 (23)
Left hepatectomy	28 (16)
Extended right hepatectomy	61 (35)
Extended left hepatectomy	31 (18)
Lymphadenectomy, *n* (%)	165 (95)
Operative time (min)	280.0 (86.0–704.0)
T category, *n* (%)	
T1	68 (39)
T2	69 (39)
T3	23 (13)
T4	13 (8)
Largest tumour diameter (mm)	60.0 (7.0–230.0)
Number of hepatic tumours	1.0 (1.0–7.0)
N category, *n* (%)	
N0	81 (47)
N1	59 (34)

TABLE 1 (Continued)

Patient characteristics	
R category, *n* (%)	
R0	129 (75)
R1	40 (23)
R2	1 (1)
(Micro-) vacular invasion, *n* (%)	28 (16)
Lymphovascular invasion, *n* (%)	45 (26)
Perineural invasion, *n* (%)	35 (20)
Tumour grading, *n* (%)	
G1	6 (4)
G2	111 (64)
G3	44 (25)
Tumour stage, UICC (8th ed), *n* (%)	
I	21 (12)
II	69 (40)
III	59 (34)
IV	24 (14)
Cumulative ICU stay, days	2.0 (0.0–44.0)
Post-operative complications, *n* (%)	
No complications	21 (12)
Clavien–Dindo I	13 (8)
Clavien–Dindo II	60 (35)
Clavien–Dindo IIIa	45 (26)
Clavien–Dindo IIIb	17 (10)
Clavien–Dindo Iva	7 (4)
Clavien–Dindo IVb	1 (1)
Clavien–Dindo V	9 (5)
Post-operative CCI	29.6 ± (0.0–100.0)

Abbreviations: BMI, body mass index; CCI, comprehensive complication index; G, Grade; iCCA, intrahepatic cholangiocarcinoma; ICU, intensive care unit; MAFLD, metabolic dysfunction-associated fatty liver disease; N, node; R, rest; T, tumour; UICC, Union Internationale Contre le Cancer.

Note: Data presented as median and range if not noted otherwise.

3.2 | Body composition and MAFLD

While most patient characteristics were equally distributed across body composition pathologies, MAFLD was significantly associated with pathological changes in body composition compared to without underlying liver disease, such as a significantly increased BMI (mean: 29.1 vs. 24.6 kg/m^2, $p < 0.001$), VFI / visceral obesity (mean: 85.0 vs. 38.1 cm^2/m^2, $p > 0.001$) and SFI/subcutaneous obesity (mean: 79.6 vs. 58.6 cm^2/m^2, $p = 0.006$). Patients with predominant fibrosis/cirrhosis did not show a significantly different body composition than iCCA patients without underlying liver disease. (Table 2). When pooling all non-MAFLD patients, and comparing their body composition to the one of patients with MAFLD, the latter had a higher median SMI (55 cm^2/m^2 vs. 51 cm^2/m^2, $p = 0.017$) and a similar incidence of sarcopenia (8/58, 14% vs. 18/89, 20% $p = 0.591$), but at the same time, a significantly higher number of patients with sarcopenic obesity was in the MAFLD group (6/58, 10% vs. 0/89, 0% $p = 0.006$). The incidence of both visceral (52/58, 90% vs. 36/89, 40% $p < 0.001$) and subcutaneous obesity (32/58, 55% vs. 13/89, 15% $p < 0.001$) was higher in the MAFLD group compared to patients without fulfilled MAFLD criteria. On average, patients with MAFLD had a lower median SM-RA value than patients with other aetiologies (36 HU vs. 41 HU, $p = 0.005$), without translating into a higher incidence of myosteatosis due to the BMI-adjustment of the diagnostic cut-off (Figure 1). Furthermore, patients with MAFLD in our cohort were significantly older (median age: 67 vs. 61 years, $p = 0.005$), had a lower liver function capacity (LiMAx, Humedics GmbH) test (median LiMAx: 384 µg/kg/h vs. 462 µg/kg/h, $p = 0.008$) and a preoperatively lower CRP (median: 20 vs. 27, $p = 0.033$) than patients with other iCCA aetiologies. While prognostically important tumour-associated factors (lymphovascular invasion, lymph node invasion) were equally distributed between patients with MAFLD and patients without liver disease, a significantly lower proportion of MAFLD patients received post-operative chemotherapy (16/58, 39% vs. 22/44, 63%). The presence of MAFLD did not impact perioperative outcomes (Table S1). There was no significant difference in body composition across fibrosis stages F1–F4, regardless of aetiology (ANOVA, data not shown).

3.3 | Time to recurrence and overall survival

The median follow-up time was 20 months postoperatively, with a median TTR of 18 months and a median OS of 28 months. During the observation period, 92 (53%) patients recurred and 113 (65%) died. The most frequent sites of recurrence were the liver (83/92, 90%), the lungs (27/92, 29%), peritoneum (22/92, 24%), lymph nodes (21/92, 23%) and the bones (16/92, 17%) with most patients experiencing simultaneous recurrence at several sites. A total of 63 (34%) patients received adjuvant chemotherapy, predominantly with gemcitabine/cisplatin or capecitabin regimens. The selection criteria for adjuvant chemotherapy in this cohort prior to the publication of the Capecitabine compared with observation in resected biliary tract cancer (BILCAP) trial in 2019[25] were based on the presence of oncological risk factors (lymphovascular/ nodal invasion, R status) together with overall performance status.

The most frequent histological findings in the non-tumourous liver showed differences in TTR: patients with predominant steatosis had a trend towards longer TTR

TABLE 2 Preoperative patient characteristics stratified by the main clinicopathological aetiologies.

	Entire cohort ($n=173$)	No liver disease ($n=44$)	MAFLD ($n=58$)[a]	p=*	Fibrosis/cirrhosis ($n=54$)	p=*
Age (years)	63.9 ± 11.6	62.8 ± 10.9	67.1 ± 9.9	0.186	60.9 ± 13.0	1.000
Sex ratio (F:M), *n*,(%)	87 (50): 86 (50)	24 (54): 20 (46)	26 (46): 32 (55)	0.331**	26 (48): 28 (52)	0.529**
LiMAX value (μg/kg/h)	428.3 ± 134.4	433 ± 125.4	384 ± 125.5	0.443	416. ± 132.1	1.000
PVE	23 (13)	7 (16)	10 (17)	0.698**	6 (11)	0.900**
Preoperative laboratory values						
CA19.9 (kU/L)	4213.9 ± 24172.4	850.5 ± 2938.6	6409.4 ± 30996.3	1.000	4565.2 ± 25501.4	1.000
CEA (kU/L)	7.3 ± 21.8	13.6 ± 37.2	8.4 ± 21.2	1.000	3.2 ± 2.3	0.485
Total bilirubin (mg/dL)	0.6 ± 0.7	0.7 ± 0.8	0.6 ± 0.7	1.000	0.6 ± 0.6	1.000
Albumin (g/L)	32.4 ± 16.4	32.6 ± 16.0	31.8 ± 17.8	1.000	31.7 ± 17.3	1.000
ALT (U/L)	44.7 ± 62.7	47.8 ± 83.4	43.2 ± 34.5	1.000	47.1 ± 76.9	1.000
AST (U/L)	44.2 ± 44.7	43.4 ± 49.0	39.4 ± 20.6	1.000	52.0 ± 62.3	1.000
GGT (U/L)	228.1 ± 314.6	240.9 ± 342.8	214.7 ± 396.8	1.000	249.9 ± 209.2	1.000
CRP (mg/dL)	22.2 ± 38.1	23.4 ± 27.3	19.5 ± 45.1	1.000	20.7 ± 40.9	1.000
Body composition						
BMI	25.8 ± 4.9	24.6 ± 4.6	29.1 ± 4.0	**<0.001**	24.1 ± 5.0	1.000
Female ($n=87$)	25.1 ± 5.2	23.7 ± 4.8	27.5 ± 4.7	**<0.001**	22.8 ± 5.2	0.274
Male ($n=86$)	26.5 ± 4.5	25.7 ± 4.2	27.8 ± 4.2	0.075	24.7 ± 5.3	0.174
Overweight/obesity (BMI ≧ 25), *n* (%)	86 (50)	16 (36)	52 (90)	**<0.001****	17 (32)	0.611**
SMI (cm^2/m^2)	52.4 ± 10.5	51.5 ± 7.7	54.7 ± 10.9	0.369	52.9 ± 11.9	1.000
Female ($n=87$)	46.7 ± 7.4	47.3 ± 6.1	46.5 ± 7.6	0.443	45.9 ± 8.2	0.364
Male ($n=86$)	58.1 ± 10.1	56.5 ± 6.4	58.4 ± 10.6	0.556	59.0 ± 13.1	0.860
Sarcopenia, *n* (%)	30 (17)	6 (14)	8 (14)	0.928**	9 (17)	0.714**
Sarcopenic obesity, *n* (%)	9 (5)	1 (2)	6 (10)	0.117**	2 (4)	0.697**
VFI (cm^2/m^2)	53.7 ± 47.9	38.1 ± 32.9	85.0 ± 59.3	**<0.001**	37.9 ± 31.5	1.000
Female ($n=87$)	46.2 ± 56.2	28.1 ± 30.4	68.5 ± 73.6	**<0.001**	28.4 ± 29.9	0.837
Male ($n=86$)	61.3 ± 36.4	50.1 ± 32.4	80.0 ± 33.5	**0.003**	40.2 ± 32.3	0.268
Visceral Obesity, *n* (%)	104 (60)	19 (43)	52 (90)	**<0.001****	24 (44)	0.900**
SFI (cm^2/m^2)	64.5 ± 34.2	58.6 ± 30.1	79.6 ± 33.9	**0.006**	56.0 ± 35.2	1.000
Female ($n=87$)	73.6 ± 38.6	62.4 ± 33.8	88.8 ± 33.0	**0.002**	61.8 ± 45.4	0.665
Male ($n=86$)	55.3 ± 26.4	54.0 ± 25.1	59.8 ± 28.3	0.589	48.0 ± 26.8	0.302
Subcutaneous obesity, *n* (%)	43 (25)	10 (23)	36 (62)	**0.006****	11 (20)	0.939**
SM-RA	38.5 ± 9.3	39.1 ± 9.6	36.1 ± 8.4	0.338	40.9 ± 9.9	1.000
Female ($n=87$)	37.7 ± 10.0	39.6 ± 10.0	34.8 ± 8.5	0.098	40.5 ± 11.4	0.665
Male ($n=86$)	39.3 ± 8.6	38.5 ± 9.3	38.1 ± 8.1	0.707	41.6 ± 8.1	0.392
Myosteatosis, *n* (%)	78 (45)	20 (46)	27 (47)	0.912**	22 (41)	0.639**
Risk factors/adjuvant treatment						
Lymphovascular invasion	45 (26)	12 (30)	13 (25)	0.606**	16 (36)	0.487**
Lymph node positivity	59 (34)	18 (44)	14 (30)	0.170**	17 (44)	0.978**
Adjuvant chemotherapy, *n* (%)	54 (49)	22 (63)	16 (39)	0.038**	16 (46)	0.150**

TABLE 2 (Continued)

	Entire cohort ($n=173$)	No liver disease ($n=44$)	MAFLD ($n=58$)[a]	p=*	Fibrosis/cirrhosis ($n=54$)	p=*
Recurrence, n (%)	92 (53)	28 (60)	29 (50)	0.513**	30 (60)	0.900**
Documented tumour-related death, n (%)	114 (62)	29 (62)	40 (68)	0.539**	29 (58)	0.710**

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CA, carbohydrate antigen; CEA, carcinoembryonic antigen; CRP, c-reactive protein; GGT, gamma glutamyl transferase; HU, Hounsfield units; LiMAx, liver function capacity test; MAFLD, metabolic dysfunction-associated fatty liver disease; SFI, Subcutaneous fat index; SMI, skeletal muscle index; SM-RA, skeletal muscle radiation attenuation; VFA, visceral fat area; VFI, visceral fat index.

Note: Data presented as mean and standard deviation if not noted otherwise. Body composition features were defined as follows and as partly described previously[9,24]: BMI – weight[kg]/height^2[m^2], sarcopenia—SMI < 52.4 cm^2/m^2. for men and <38.5 cm^2/m^2 for women, myosteatosis—<41 HU if BMI <25 kg/m^2 and <33 HU if BMI ≥25 kg/m^2, visceral obesity if VFA ≧100 cm^2, subcutaneous obesity derived from the subcutaneous fat index, dichotomized at the upper tertile of the cohort (71.89 cm^2/m^2), sarcopenic obesity defined as BMI >25 kg/m^2 and SMI ≦38.5 cm^2/m^2 in women and ≦52.4 cm^2/m^2 in men. Bold values indicate p values regarded as statistically significant.

[a]Histological liver steatosis data were available in 147/173 (85%) patients.

*Based on ANOVA with post hoc Bonferroni correction, with p values given for comparisons to the no Liver Disease column. Bonferroni correction for three groups resulted in a level of significance $\alpha=0.016$.; **Based on chi-square test, with p values given for comparisons to the no Liver Disease column.

($n=75$, median 22 months, 95% CI: 0.1–43.9 months), compared to patients with fibrosis or no liver disease in the resected specimen (fibrosis: $n=44$, 15 months, 95% CI: 10.4–19.6 months and no liver disease: $n=44$, 12 months, 95% CI: 2.3–21.7 months, respectively, Figure S2, $p=0.127$). In patients with available data on liver steatosis ($n=147$), the MAFLD criteria were subsequently applied because patients without liver disease and without steatosis, but with fibrosis/cirrhosis grouped together in the survival analyses. Subsequently, we divided the cohort of patients with available histopathological data on steatosis into patients with MAFLD ($n=58$) and without MAFLD ($n=89$). A significant difference in TTR was noted between patients with MAFLD aetiology (median TTR: 38 months, 95% CI: 14.7–61.3) and patients without MAFLD (median TTR: 12 months, 95% CI: 8.0–16.0, $p=0.025$, Figure 2). The times of OS were similar between groups.

Univariable analysis showed a significantly shorter OS of patients with myosteatosis (33 vs. 23 months, HR 1.5, log-rank $p=0.020$, Table 3), while other clinical variables associated with TTR and OS are listed in Table S3.

Multivariable analysis of all significant parameters from univariable analysis showed an independent prognostic value of lymphovascular invasion, vascular invasion and lymph node invasion for TTR, while an age >65 years and fresh frozen plasma transfusions were independently prognostic for OS (Table 4). Accordingly, an independent prognostic role of MAFLD for TTR and subcutaneous obesity and myosteatosis for OS was not confirmed.

4 | DISCUSSION

Postoperative recurrence of iCCA with impaired long-term survival are frequent and severe occurrences after curative-intent surgery, despite surgical and perioperative advances in the field.[26] In this context, due to the comparative rarity of iCCA and the challenges of curative-intent liver resections, prognostic biomarkers are severely lacking. Recently, next-generation sequencing revealed molecular subtypes of iCCA that carry prognostic and predictive potential,[27] while the pathological criteria of lymph node and lymphovascular invasion are well-characterized prognostic factors across European and Asian patient collectives.[26,28] At the same time, patient- or host-centred determinants of prognosis remain relatively unexplored in this rare tumour entity.[29] Accordingly, we investigated tumour aetiology together with body composition in a large, homogenous European cohort of patients undergoing curative-intent liver resection for iCCA.

In this study, we were able to identify that a categorical body composition parameter —sarcopenia—was associated with perioperative outcome, but none of the body composition categories held independent prognostic value in multivariable TTR and survival analysis. As such, this study pointed towards an association of sarcopenia with perioperative morbidity, such as intraoperative transfusions, Clavien–Dindo ≥IIIb complications and an elevated post-operative CCI. While this observation is novel to iCCA, it is shared with curative-intent surgery for other gastrointestinal malignancies, such as HCC and pancreatic adenocarcinoma,[30,31] and is a result of malnutrition, systemic inflammation and overall catabolic processes.[32] Furthermore, we noted inferior OS in univariable analysis in patients with myosteatosis, without observing differences when splitting the analysis by gender. Similarly, a study in a smaller palliative cohort across different CCA subtypes recently suggested a prognostic role of both myosteatosis and sarcopenia for overall survival.[33]

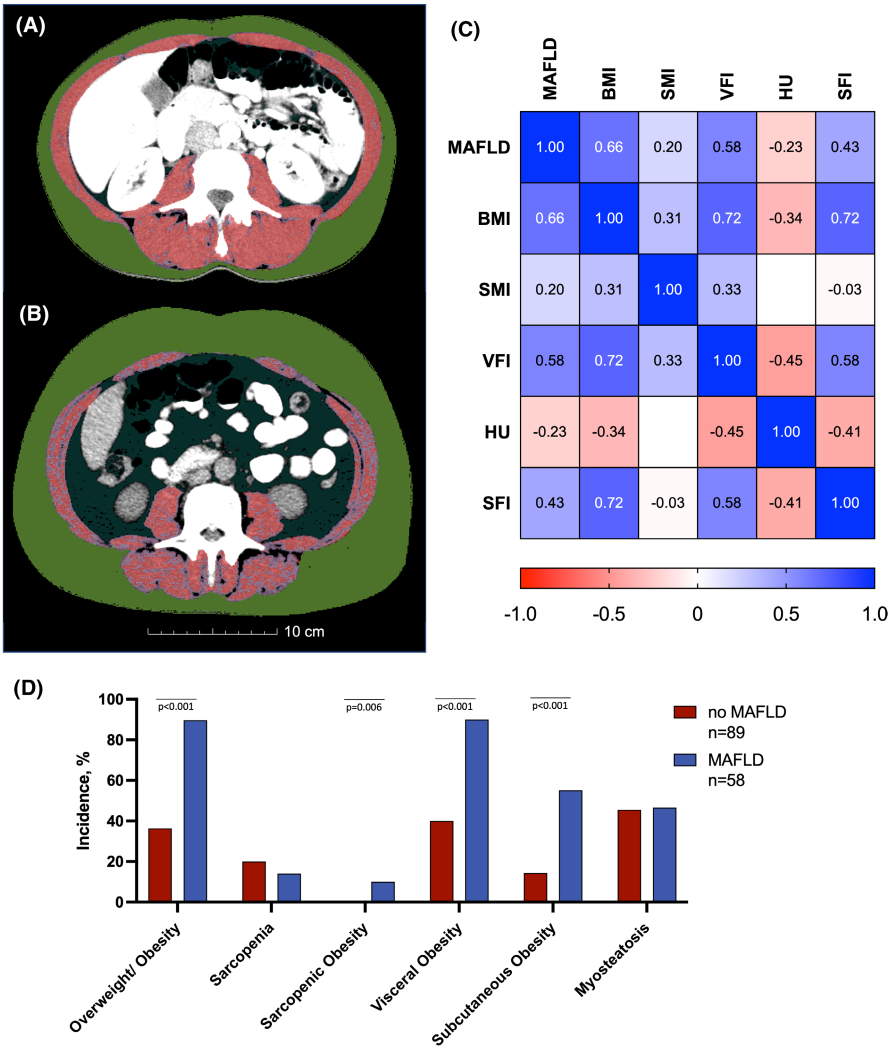

FIGURE 1 Typical physiological body composition versus typical metabolic dysfunction-associated fatty liver disease (MAFLD)-associated body composition. Compared to physiological body composition (A), the typical MAFLD-associated changes of body composition depicted here (B) are an increased subcutaneous fat index (subcutaneous fat in light green), increased visceral fat area (visceral fat area, dark green/petrol), normal spinal muscle index (skeletal muscle index, red, no sarcopenia) and lower Hounsfield units-values (grey intramuscular areas), indicative of intramuscular fat accumulation. (C) Spearman *r* correlation matrix of MAFLD and body composition parameters ($n = 173$ patients included for all columns except MAFLD [$n = 147$]) (D) Distribution of body composition pathologies between non-MAFLD and MALFD patients with intrahepatic cholangiocarcinoma; chi-square test with only significant values shown.

In the present study, a third of our cohort fulfilled the 2020 consensus MAFLD criteria.[14] This subgroup had a higher incidence of body composition alterations, namely, sarcopenic, visceral and subcutaneous obesity, than patients without liver disease or with predominant fibrotic alterations. Recently, an association of NASH and elevated BMI as well as bioelectrical impedance analysis-assessed fat mass and skeletal muscle mass was published in patients undergoing bariatric surgery,[8] but data from oncological settings have not yet been reported. Because our cohort was composed of curatively treated patients without signs of systemic disease and with adequate preoperative performance status, severe cachexia, as observed in advanced gastrointestinal cancers, was less prevalent than it would be expected in palliative cohorts.

Patients with MAFLD had significantly longer TTR than patients without or with other underlying liver disease, while their OS was similar to the overall cohort. The aetiology of iCCA is relatively little studied in European/western cohorts and large meta-analyses are oftentimes skewed towards South Asian/Pacific populations with a predominant aetiological role of *Opisthorchis viverrini*.[3,34] Across various CCA studies, the presence of cirrhosis, as well as hepatitis B and C, was not associated with shorter disease-free survival,[34] and the results on OS in patients with cirrhosis or chronical hepatitis B infection are disparate, with an apparent trend towards shorter OS in these patients.[35,36] To date, the vast majority of iCCA studies investigating clinical prognostic factors do not report on either body composition, steatosis, or MAFLD as an iCCA aetiology or as a prognostic factor.[34,37] Recently, an Italian study reported that patients fulfilling NASH criteria had an inferior OS after iCCA surgery but did not observe a difference in TTR between aetiologies. In fact, it remained unclear in this setting whether the accelerated mortality in the NASH group derived from cancer-related deaths or other causes, such as increased cardiovascular mortality.[5]

The role of MAFLD as a tumour aetiology is more apparent and better characterized in HCC, for which the estimated annual incidence in patients with NASH cirrhosis is from 0.5% to 2.6%.[38] Recent years have brought a deeper understanding of NASH-HCC mechanisms, delineating impaired immune surveillance,[39] autoreactive

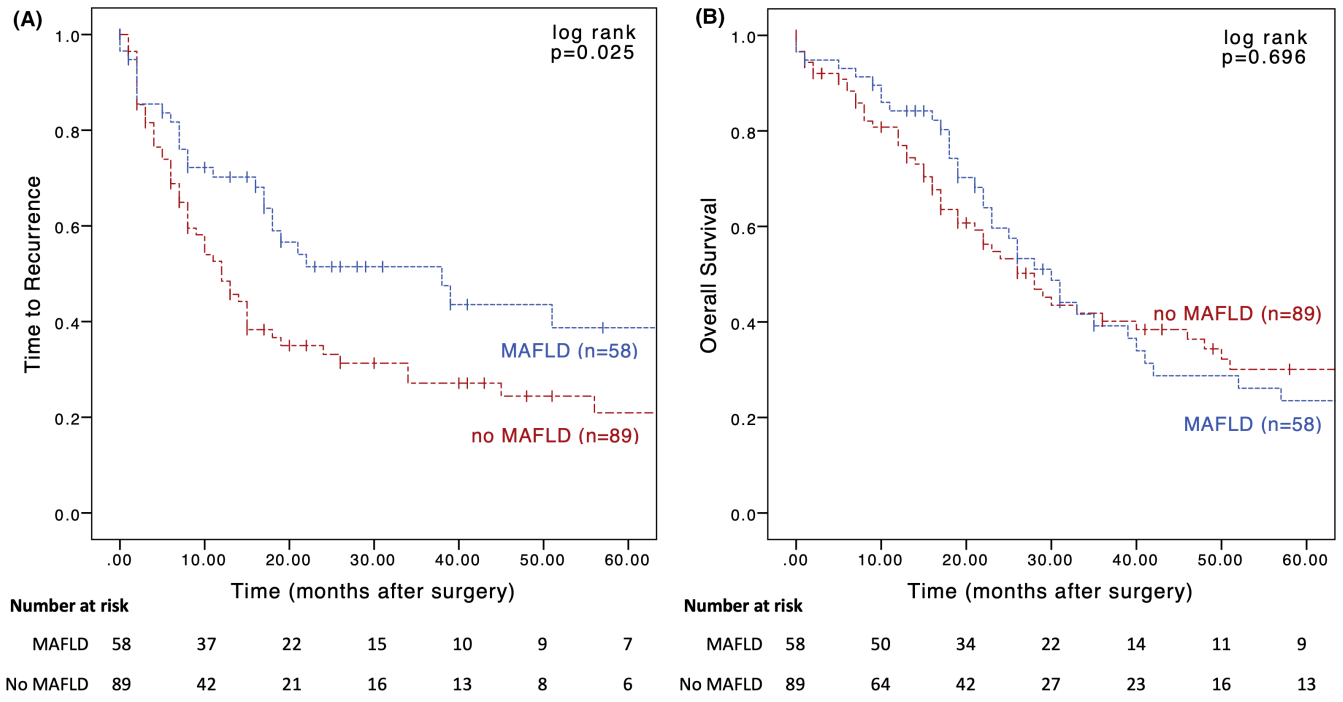

FIGURE 2 Time to recurrence (A) and overall survival (B) for patients with and without metabolic dysfunction-associated fatty liver disease.

TABLE 3 Univariable analysis of time to recurrence and overall survival by body composition in iCCA ($n = 173$).

	n (%)	Median TTR (95% CI)	Hazard ratio	*p*-Value*	Median OS (95% CI)	Hazard ratio (95% CI)	*p*-Value*
BMI							
<25 kg/m^2	87 (50)	14 (7.7–20.3)	-	0.264	33 (23.0–43.0)	-	0.350
≧25 kg/m^2	86 (50)	22 (15.2–28.8)	-		25 (18.6–31.4)	-	
Sarcopenia/ reduced skeletal muscle mass							
No	142 (82)	18 (12.6–23.4)	-	0.262	29 (24.3–33.7)	-	0.495
Yes	30 (17)	n.a.	-		32 (7.6–56.4)	-	
Sarcopenic obesity							
No	163 (94)	18 (11.3–24.7)	-	0.775	30 (24.7–35.3)	-	0.092
Yes	9 (5)	15 (1.6–28.4)	-		16 (5.0–27.0)	-	
Visceral obesity							
No	69 (40)	13 (10.2–15.8)	-	0.053	36 (14.4–57.6)	-	0.275
Yes	104 (60)	26 (6.3–45.7)	-		26 (20.7–31.3)	-	
Subcutaneous obesity							
No	130 (75)	15 (9.4–20.6)	-	0.268	31 (23.4–38.6)	-	0.317
Yes	43 (25)	22 (14.0–30.0)	-		23 (16.9–29.1)	-	
Myosteatosis							
No	95 (55)	16 (11.5–20.6)	-	0.363	33 (22.3–43.7)	1	**0.020**
Yes	78 (45)	22 (13.5–30.5)	-		23 (18.4–27.6)	1.547 (1.065–2.246)	

Abbreviations: BMI, body mass index; CI, confidence interval; iCCA, intrahepatic cholangiocarcinoma; OS, overall survival; TTR, time to recurrence.

Note: Body composition features were defined as follows and as partly described previously[9]: BMI—weight [kg]/height^2[m^2], Sarcopenia—52.4 cm^2/m^2. for men and 38.5 cm^2/m^2 for women, Myosteatosis—<41 HU if BMI <25 kg/m^2 and <33 HU if BMI ≥25 kg/m^2, visceral obesity if VFA ≧100 cm^2, subcutaneous obesity derived from the subcutaneous fat index, dichotomized at the upper tertile, sarcopenic obesity defined as BMI >25 kg/m^2 and SMI ≦38.5 cm^2/m^2 in women and ≦52.4 cm^2/m^2 in men. Bold values indicate *p* values regarded as statistically significant.

*Based on log-rank test.

TABLE 4 Multivariable analysis of time to recurrence and overall survival.

	Time to recurrence (TTR)[a]		Overall survival (OS)[b]	
	Hazard ratio (95% CI)	***p*-Value**	**Hazard ratio (95% CI)**	***p*-Value**
MAFLD	0.622 (0.358–1.079)	0.091	-	-
Lymphovascular invasion	1.925 (1.076–3.445)	**0.027**	-	-
Vascular invasion	1.807 (1.002–3.259)	**0.049**	-	-
Lymph node invasion	3.912 (1.594–9.598)	**0.003**	1.260 (0.798–1.991)	0.322
Grade 3–4	1.237 (0.691–2.214)	0.475	1.314 (0.845–2.043)	0.225
UICC stage III/IV	1.403 (0.604–3.256)	0.431	-	-
FFP transfusions	-	-	2.043 (1.294–3.224)	**0.002**
R1 status	-	-	1.561 (0.951–2.563)	0.078
Myosteatosis	-	-	1.526 (0.993–2.345)	0.315
INR >1	-	-	1.306 (0.835–2.040)	0.715
Age >65	-	-	1.600 (1.036–2.471)	**0.034**

Abbreviations: CCI, comprehensive complications index; CI, confidence interval; CRP, c-reactive protein; FFP, fresh frozen plasma; ICU, intensive care unit; INR, international normalized ratio; MAFLD, metabolic dysfunction-associated fatty liver disease; OS, overall survival; R, rest status; TTR, time to recurrence; UICC, Union Internationale Contre le Cancer.

Note: Parameters with potential collinearity were excluded from the analysis. This pertains to perioperative haemoglobin (collinearity with transfusions), intraoperative blood transfusions (collinearity with FFP transfusions), CRP levels (collinearity with MAFLD aetiology), cumulative days on ICU (collinearity with CCI), adjuvant chemotherapy (patient selection in part based on prognostically relevant pathological parameters), subcutaneous obesity (collinearity with Myosteatosis), CCI (collinearity with death). Parameters that were not assumed to be stable over time (recurrence) were also excluded. Bold values indicate *p* values regarded as statistically significant.

[a]115 patients with complete data included.

[b]149 patients with complete data included.

immune cells,[40] systemic inflammation,[41] oxidative stress due to dysregulated lipid metabolism and dysbiosis.[42] In contrast, fatty liver disease as a contributor to iCCA has only been characterized superficially. In this context, our study contributes to a growing body of evidence that patients with MAFLD-iCCA may constitute a distinct population for whom further metabolic, functional and immunological characteristics remain to be clarified.

The present study has a considerable sample size for European single-centre iCCA studies, with a homogenous surgically-treated patient collective. Nevertheless, the following shortcomings limit our conclusions: besides the exploratory single-centre design with a retrospective evaluation of body composition and MAFLD, the patient collective had, in part, post-operative adjuvant treatment based on negative pathological outcome factors, which was the recommended protocol before the universal recommendation for adjuvant chemotherapy from the BILCAP trial.[25] Furthermore, we did not examine potential prognostic differences in tumour biology, such as gene variants, genetic mutations or molecular subtypes with a documented prognostic role in CCA and did not clarify their distribution across tumour aetiologies.[27,43]

In conclusion, we aimed to link the complex pathophysiology of disease aetiology and body composition by investigating liver disease of iCCA patients together with CT-derived fat and muscle parameters in the largest of these patient cohort to date. Herein, we found that MAFLD is frequent in iCCA patients, may hold potential prognostic value for time to recurrence and is significantly associated with alterations of body composition. In conjunction with the systemic changes observed in both MAFLD and in body composition pathologies, these findings illustrate the necessity of exploring systemic metabolic, performance and immunological changes in MAFLD-iCCA patients in future studies.

AUTHOR CONTRIBUTIONS

Isabella Lurje: Conceptualization (supporting); formal analysis (lead); funding acquisition (supporting); investigation (lead); methodology (supporting); visualization (supporting); writing – original draft (lead). **Deniz Uluk:** Conceptualization (equal); formal analysis (supporting); funding acquisition (supporting); project administration (equal); software (equal); supervision (supporting); writing – review and editing (equal). **Sandra Pavicevic:** Data curation (supporting); funding acquisition (supporting); project administration (supporting); writing – review and editing (equal). **Minh Duc Phan:** Conceptualization (supporting); data curation (lead); formal analysis (supporting); investigation (supporting); software (lead); visualization (equal); writing – review and editing (equal). **Dennis Eurich:** Writing – review and editing (equal). **Uli Fehrenbach:** Resources (supporting); writing – review

and editing (equal). **Dominik Geisel:** Resources (supporting); writing – review and editing (equal). **Timo Alexander Auer:** Resources (supporting); writing – review and editing (equal). **Uwe Pelzer:** Resources (supporting); writing – review and editing (equal). **Dominik Paul Modest:** Writing – review and editing (equal). **Nathanael Raschzok:** Writing – review and editing (equal). **Igor Maximilian Sauer:** Resources (supporting); writing – review and editing (equal). **Wenzel Schöning:** Resources (supporting); writing – review and editing (equal). **Frank Tacke:** Funding acquisition (supporting); resources (supporting); writing – review and editing (equal). **Johann Pratschke:** Resources (supporting); writing – review and editing (equal). **Georg Lurje:** Conceptualization (equal); funding acquisition (lead); project administration (equal); resources (lead); supervision (lead); writing – review and editing (equal).

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None.

CONFLICT OF INTEREST STATEMENT

Georg Lurje reports receiving travel support and speakers' fees from Astellas Pharma, XVIVO, Bridge to Life, Aferetica S.R.L outside the submitted work.

DATA AVAILABILITY STATEMENT

Supplementary Data is published with the manuscript. Further data can be provided upon reasonable request to the corresponding author.

ETHICS STATEMENT

The present research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The study protocol was approved by the institute's committee on human research (Charité – Universitätsmedizin Berlin [EA1/105/21]), informed consent was waived due to the retrospective, anonymized analysis of readily available clinical data.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Diskussion und Ausblick

Im Gegensatz zu anderen gastrointestinalen Malignomen wie das Kolorektale Karzinom, in denen bereits vor 15 Jahren prognostische und prädiktive Wirtsfaktoren wie Genpolymorphismen beschrieben wurden (101), besteht insbesondere im CCA ein Mangel an präoperativ verfügbaren prognostischen Faktoren. Gut beschriebene klinisch-pathologische Prognosefaktoren, wie lymphovaskuläre Invasion und geringe Tumordifferenzierung sind oftmals erst im Resektat identifizierbar, und bieten so nur begrenzten Vorteil in der präoperativen Risikostratifikation (102, 103). Ziel der hier zusammengefassten Arbeiten war es, Patient*innen-zentrierte Determinanten für das Outcome nach Leberteilresektion in kurativer Intention zu finden. Hierfür wurden zwei Patient*innen-zentrierte Ansätze exploriert, die bereits im präoperativen Verlauf, auch im Rahmen klinischer Standardsituationen, anhand von Blut und Schnittbildgebung untersucht werden können.

(1) Funktionelle Genpolymorphismen sind in der Bevölkerung vorkommende Varianten von Genen, die je nach Lokalisation, die Proteinexpression oder -funktion beeinflussen. Unser Ziel war es, den Einfluss Genpolymorphismen in zentralen Mediatoren des TME im Hinblick auf das onkologische und allgemeine Outcome zu erforschen.
(2) Den Einfluss der Körperzusammensetzung von Patient*innen mit iCCA, im Kontext von Krankheitsätiologie und Prognose. Wir untersuchten neben der Inzidenz von Körperzusammensetzungs-Pathologien auch den Stellenwert des metabolischen Syndroms als iCCA-Ätiologie, in der Ausprägung der MASLD.

Im ersten Teil der Arbeit (2.1-2.3) wurde der prognostische Einfluss von Genpolymorphismen in einem Pathway-Basierten Ansatz erforscht, da SNPs insbesondere in Prozessen der Tumor-Wirt Wechselwirkung, wie der Immunologie und Angiogenese relevant sind (104). Die Ergebnisse der Studien 2.1-2.3, in denen im HCC und iCCA der IL-8 SNP rs4073 und im pCCA der IL-8 Rezeptor CXCR1 signifikant mit dem Outcome assoziiert waren, unterstreichen die Wichtigkeit des IL-8-Signalings im CCA und HCC. Das proinflammatorische Chemokin IL-8 wird physiologischerweise von Makrophagen, Endothel-und Epithelzellen als Reaktion auf Schaden oder Infektionen produziert, wodurch die Chemotaxis und Aktivierung von Neutrophilen und Phagozyten stattfindet (105). Die intrazellulären Effekte von IL-8 in Immunzellen und Endothelzellen werden über die Chemokinrezeptoren CXCR1 und 2 vermittelt, die bei Bindung ihrer Liganden phosphoryliert und internalisiert werden, und ihre Signale über spezifische G-Protein-gekoppelter Rezeptorkinasen vermitteln (106).

In soliden Tumoren werden die malignen Zellen selbst ebenfalls zu einer Quelle von IL-8, wodurch eine Attraktion von MDSCs mediiert wird (84). Neben der Chemotaxis führt das CXCR1 und CXCR2 Signaling in MDSCs zur Formation von neutrophilen extrazellulären Fallen (neutrophil extracellular traps, NET) aus granulozytären MDSCs, wodurch der Tumor vor zytotoxischen Lymphozyten abgeschirmt wird und Metastasierung sowie Immuntherapie-Resistenz begünstigt wird (107). Weiterhin fördert IL-8 durch Signaling in Endothelzellen direkt ihre Proliferation, Migration und Überleben und induziert somit direkt die Neoangiogenese (105).

Unsere Auswahl der analysierten Einzelnukleotid-Polymorphismen fand als Pathway-basierter Ansatz statt, mit einem Fokus auf Tumor-Wirt Wechselwirkungen im TME. Zuvor waren insbesondere im CCA zuvor noch keine Daten hinsichtlich der prognostischen Rolle von SNPs veröffentlicht. Der Pathway- beziehungsweise Hypothesenzentrierter Ansatz erlaubte allerdings nicht die breite Untersuchung sämtlicher funktioneller SNPs oder die Dokumentation neuer Funktionen bisher wenig untersuchter Polymorphismen. Genomische Ansätze in unabhängigen Patient*innenkollektiven sind hier zukunftsweisend, sowohl um unsere prognostischen Beobachtungen zu untermauern, als auch um eine breitere, weniger gebiaste Genauswahl zu untersuchen.

Innerhalb epidemiologisch-onkologischer Studien sind genome-wide association studies (GWAS) zur Untersuchung der Inzidenz von Tumoren der Goldstandard. Hierbei wird – meist anhand der Daten großer Register – auf genomischer Ebene, zunächst ohne Pathway-Ansätze zu verfolgen, nach Assoziationen zwischen Genotyp und Krankheitsauftreten gesucht. Selbst große Registries wie die UK Biobank mit ca. 500.000 Teilnehmer*innen verfügen allerdings lediglich über die Information, ob ein CCA aufgetreten ist und über den Überlebensstatus der Patient*innen, nicht aber über Art der Therapie und Therapieansprechen (108). Somit sind GWAS elegante Tools für die Untersuchung von Risiko-Genen für das Auftreten von Tumoren – beispielsweise der PNPLA3 (rs738409)-Polymorphismus als Risikofaktor für die Progression einer Lebererkrankung und Inzidenz des HCCs (109, 110) – geben aber nur begrenzt Aufschluss über die prognostische Relevanz nach Auftreten der onkologischen Erkrankung.

Die Auswahl der analysierten Genpolymorphismen basierte neben der Häufigkeit des selteneren Allels in der Zielpopulation und der biologischen Funktion des Allels, darauf, dass die ausgewählten Proteine eine Mediatorfunktion zwischen malignen Zellen und Wirtszellen haben, und somit eine Veränderung der Proteinexpression in somatischen Zellen zu einem prognostischen Effekt führen kann. Zwar sind für die ausgewählten Genpolymorphismen die systemischen Effekte auf Proteinexpression dokumentiert, aber die Effekte im TME primärer Lebertumoren gänzlich unbekannt – ein wichtiger Aspekt, den es in zukünftigen Arbeiten zu untersuchen gilt. Beispielsweise ist über den *IL-1B* (rs1143634)

Polymorphismus bekannt, dass er zu einer erhöhten Produktion des proinflammatorischen IL-1β, ohne Veränderung der Proteinstruktur führt, sowohl an Orten der Infektion (93), als auch in Monozyten *in vitro* (89, 111). Gleichzeitig bleibt unklar, ob im TME des CCA der SNP zu erhöhter monozytärer IL-1β-Produktion führt, und ob eine experimentelle Abrogation der monozytären IL-1β-Produktion *in vitro* einen Effekt auf CCA-Zellen ausübt. Somit zeigen unsere Daten eine wichtige mechanistische Fragestellung zukünftiger Studien auf.

Im zweiten Teil dieser Habilitationsschrift (2.4-2.5) wurde die Körperzusammensetzung von Patient*innen mit CCA analyisert. Unsere Annahme war, dass der Informationsgehalt routinemäßiger klinischer Bildgebung, die konventionellerwese in diesen Patient*innen nur hinsichtlich Tumorgröße- und Lokalisation sowie Operabilität evaluiert werden, hinsichtlich Krankheitsprognose weiter gesteigert werden kann. Wichtige Ergebnisse unserer Studien waren, (1) dass Veränderungen der Körperzusammensetzung im CCA hochprävalent sind, (2) dass die sarkopene Obesität eine besonders vulnerable Patient*innengruppe mit schlechtem postoperativem Allgemeinüberleben identifiziert, sowie (3) dass die MASLD in etwa 30% der Patient*innen mit iCCA auftritt, potenziell mit verändertem krankheitsfreiem Überleben und mit Veränderungen der Körperzusammensetzung assoziiert ist.

Ein progredienter Verlust von Muskelmasse als Teil des physiologischen Alterungsprozesses, beispielsweise durch den abnehmenden Einfluss von Sexualhormonen, wird als primäre Sarkopenie bezeichnet, und hochprävalent in einer alternden Bevölkerung, wie in den meisten europäischen und nordamerikanischen Ländern (112). Die sekundäre Sarkopenie eine reduzierte Muskelmasse durch systemische Erkrankungen, wie systemische Inflammation (113), im Unterschied zur Kachexie können andere Kompartimente, wie die Fettspeicher, aber weiter bestehen. Die Kachexie als blickdiagnostisch und anamnestisch leicht identifizierbares Warnsignal sind ein wesentlicher Bestandteil der klinischen Patient*innenevaluation, für das klare Behandlungsrichtlinien im onkologischen Kontext existieren (114). Gleichzeitig führt die weltweite Übergewichtsepidemie dazu, dass ein steigender Anteil der Patient*innen einen eingeschränkten Performance-Status hat, der durch ihr Gewicht maskiert wird (115). Hier tritt die primäre oder sekundäre Sarkopenie in Abwesenheit von Untergewicht und augenscheinlicher Auszehrung ein, sodass konventionelle Maße wie der BMI nicht ausreichen, um die Körperzusammensetzung und Performance ausreichend einzuschätzen.

Typisch für das CCA ist die Induktion eines systemisch-inflammatorischen Zustands, der Muskelverlust ohne drastische Abnahme des Gesamt-Körpergewichts, zur Folge haben kann (116). Konkrete Beispiele hierfür sind die Muskeldepletion mit gleichzeitiger Fetteinlagerung, wie bei der Myosteatose oder der sarkopenen Obesität. Eine der ersten Beschreibungen des Phänomens des Verlustes von Muskelmasse bei gleichzeitigem

Übergewicht oder Adipositas, zeigte bei Patient*innen mit Lungen-und Gastrointestinalen Tumoren eine Ätiologie-übergreifende Assoziation der Trias aus einer Gewichtsverlust-Anamnese, niedriger Muskelmasse und Übergewicht/Adipositas mit ungünstiger Prognose (115). Auch hier, ähnlich wie in unseren Studien, konnten die Autor*innen keinen negativen prognostischen Wert von isoliertem Übergewicht ohne begleitenden Muskelverlust feststellen (96, 115).

Während die exakten Gründe hierfür weiterhin unbekannt sind, gehen diese Beobachtungen einher mit dem sogenannten „Obesity Paradox“, welches postuliert, dass Übergewicht zwar einen Risikofaktor onkologischer Erkrankungen darstellt, aber onkologische Patient*innen mit Übergewicht und Adipositas tendenziell eine günstigere Langzeitprognose als Unter-und Normalgewichtige (117). Diese Daten beruhen ausschließlich auf der BMI-Analyse, nicht aber auf der Untersuchung der Körperzusammensetzung. Somit führt wahrscheinlich einerseits, der prognostisch ungünstige Einfluss von Kachexie in der Gruppe der Untergewichtigen, und gleichzeitig die sehr geringe Gruppengröße der Menschen mit sarkopener Obesitas zu einer Verzerrung der BMI-Prognostizierung. Muskelmasse und Körpergewicht verhalten sich oft proportional, sodass die meisten Patient*innen mit geringerem BMI auch eine geringere Muskelmasse haben, sodass die Terminologie „BMI“-Paradox für dieses Phänomen ebenfalls vorgeschlagen wurde (118).

Im allgemeinen onkologischen Kontext scheint das Vorhandensein von ausreichend funktioneller Muskulatur vor allem die adäquate Reaktion auf Stress, beispielsweise im Rahmen von konsumierender Erkrankung, Chemotherapie oder Operation zu ermöglichen. Onkologische Patient*innen mit geringer Muskelmasse erfahren eine höhere Chemotherapie-Toxizität (119) und schwerere Komplikationen nach onkologischen Resektionen (120). In unserer Body-Composition Studie (2.5) konnten wir ebenfalls aufzeigen, dass die Sarkopenie hochgradig mit dem Auftreten postoperativer Komplikationen, beispielweise einem höheren Transfusionsbedarf und Morbidität assoziiert war (98).

Hinsichtlich onkologischer Prognose waren vor der Publikation unserer Studien nur limitierte Daten im CCA publiziert, insbesondere nicht in Patient*innenkollektiven mit frühen Krankheitsstadien und kaum in europäischen Kollektiven, in denen sich nicht nur Krankheitsätiologie, sondern auch die Body-Composition-Kriterien signifikant von den asiatischen unterscheiden. Eine japanische Studie berichtete in einer chirurgischen Kohorte von 74 Patient*innen von einer Assoziation von erhöhtem BMI zu verkürztem Überleben, ohne die Muskelmasse oder Fettverteilung detaillierter aufzuschlüsseln. Interessanterweise fand sich in diesem Kollektiv außerdem bei Übergewichtigen Patient*innen eine erhöhte 18F-FDG-Aufnahme des Tumors in der Positronen-Emissions-Tomografie (PET), was mit verkürztem onkologischen Überleben und einem immunsuppressiven TME assoziiert war (121). Eine weitere japanische Studie zeigte eine signifikante Assoziation zwischen niedriger

Muskelmasse und verkürztem Gesamtüberleben für das chirurgisch therapierte pCCA (122). Im Vergleich zu europäischen und amerikanischen Kollektiven, war in dieser Studie der mediane Skelettmuskelindex wesentlich niedriger, beispielweise im Vergleich zu unserem Kollektiv aus Studie 2.4 (45.7 und 36.7 cm^2/m^2 *versus* 50.3 cm^2/m^2 und 42.4 cm^2/m^2 in Männern und Frauen). Die wenigen publizierten europäisch-amerikanischen Kohorten sind überwiegend so klein, dass sämtliche CCA-Lokalisationen eingeschlossen sind. Beispielsweise zeigte eine Studie in 117 Patient*innen mit iCCA, pCCA, dCCA und Gallenblasenkarzinom mit sowohl kurativen als auch palliativen Therapieansätzen, dass Sarkopenie und Myosteatose einen unabhängigen prognostischen Wert hatte (123). In einem chirurgischen Kollektiv sämtlicher CCA-Lokalisationen war die präoperative Sarkopenie in männlichen Patienten mit verkürztem Gesamtüberleben assoziiert. Weder die Sarkopenie noch die Myosteatose zeigten einen unabhängige signifikante Assoziation mit Prognose in unseren Kollektiven, was, zum Einen, an der Präselektion der Kohorte auf chirurgisch therapierbare Fälle mit relativ erhaltener Funktionalität liegen kann, zum Anderen an unserer Nutzung präexistierender diagnostischer Cutoffs aus großen onkologischen Studien ohne Anpassung des Cutoffs an die Studienpopulation, um ein Overfitting an die Daten zu vermeiden.

Die Tatsache, dass sarkopene Obesitas nur eine kleine Subgruppe mit geringer Eventzahl in Studie 2.4 und 2.5 identifizierte, führte wahrscheinlich mit dazu, dass diese in der multivariablen Analyse eine zu kleine Gruppe darstellte, um trotz hochgradiger Überlebensunterschiede als unabhängiger prognostischer Faktor zu gelten. Gerade bei der Untersuchung einer so kleinen Risikogruppe wird zukünftig die multizentrische Ausweitung der Body-Composition Analysen wegweisend sein.

Die in unserer Studie gewählte retrospektive CT-basierte Analysemethode ist auch für die potenzielle Anwendung im zukünftigen klinischen Alltag geeignet, weist aber gleichzeitig intrinsische Limitationen auf: Parameter der Muskelfunktion, wie die Muskelkraft, korrelieren zwar hochgradig mit der fettfreien Muskelmasse (124), müssen aber separat erhoben werden. Beispiele hierfür sind die standardisierte Messung der Greifkraft der Hand (handgrip strength) mittels eines Dynamometers (125) oder die Funktionsmessung der unteren Extremität, wie Geh-oder Aufstehtests (126). Mittlerweile sind in großen onkologischen Kohorten der prognostische Wert der Handgrip strength für die onkologische- und Gesamtmortalität aufgezeigt worden (127). Studien im onkologischen Setting, die sowohl CT-als auch funktionelle Daten kombinieren, können hier als komplementäres prognostisches Design zukunftsweisend sein.

Nicht nur subkutanes, intramuskuläres und viszerales Fett reflektieren im Rahmen der Übergewichtsepidemie eine metabolische Dysfunktion, sondern auch die MASLD als eine der häufigsten Lebererkrankungen weltweit führt zu signifikanter Morbidität und Mortalität (10).

Ein Drittel unseres in 2.5 publizierten Patient*innenkollektivs mit MASLD-Diagnose, was ungefähr der europäischen MASLD-Prävalenz der Normalbevölkerung entspricht. Interessanterweise findet sich in asiatischen iCCA-Kollektiven eine MASLD-Prävalenz von unter 10% (121). Wir beobachteten, dass Patient*innen mit MASLD in unserem Kollektiv signifikant mehr pathologische Body Composition-Veränderungen als Patient*innen ohne begleitende Lebererkrankungen oder Patient*innen mit Fibrose. Neben einem Trend zu höherer medianer Muskelmasse, waren Patient*innen der MASLD Gruppe öfter übergewichtig oder adipös, mit signifikant größerer Masse viszeralen und subkutanen Fetts. Ebenso war die Inzidenz der Sarkopenen Obesität in dieser Gruppe höher.

In der MASLD ist ein Phänotyp mit niedriger Muskelmasse und gleichzeitiger Muskelverfettung vorbeschrieben, der hochgradig mit kardiovaskulären und metabolischen Komorbidität assoziiert ist (79). Eine Limitation unserer Daten ist, dass wir – abgesehen vom Kontext postoperativer Komplikationen – aufgrund des retrospektiven Studiendesigns keine metabolischen oder kardiovaskulären Aspekte der MASLD-Progression in der MASLD-Body Composition Studie (Abschnitt 2.5) analysiert haben. Somit bleibt der Stellenwert von MASLD-assoziierten Komplikation wie Diabetes Typ 2, kardiovaskulären Events und anderen Malignomen im Kontext des iCCA in dieser Studie ungeklärt.

Die in dieser kumulativen Arbeit aufgeführten Studien wurden anhand dreier chirurgischer Kohorten zweier deutscher akademischer Zentren, dem Uniklinikum RWTH Aachen (HCC und iCCA/pCCA) und der Charité Universitätsmedizin Berlin (iCCA) durchführt. Hervorzuheben gilt hier die Größe und Homogenität der analysierten Patient*innenkollektive, insbesondere der CCA-Kollektive. Aufgrund der Seltenheit des CCAs in Europa und der oftmals palliativen Situation bei Erstdiagnose, sind Kollektive mit frühen Krankheitsstadien meist klein und nur hochspezialisierten Zentren vorbehalten. Die Lokalisation und Ausdehnung der Tumore – beispielsweise beim pCCA in der Gallengangsgabelung (Klatskin Typ IIIa-IV) – erfordert ausgedehnte Resektionen in potenziell vorerkranktem Lebergewebe. Beispielsweise unterzogen sich in der unter 2.1 erläuterten Studie über ein Viertel der Patient*innen einer Trisektorektomie, also der Entfernung von ca. 75% des Lebergewebes, während in der unter 2.3 erwähnten Studie 50% der Patient*innen eine histologisch bestätigte Leberzirrhose hatte.

Rigorose Auswahlkriterien, wie der Ausschluss von CCA-HCC Mischtumoren, Tumoren mit neuroendokriner Differenzierung, und im Falle des pCCAs der Ausschluss neoadjuvant vorbehandelter Patient*innen, zielten auf eine möglichst große Homogenität der analysierten Kohorten. Patient*innen, die mit dem “Associating Liver Partition and Portal vein Ligation for Staged hepatectomy” (ALPPS)-Verfahren operiert wurden, wurden ebenfalls von Studie 2.1 ausgeschlossen, da dieses Verfahren im pCCA ein prognostisch ungünstiges Rescue-Verfahren nach Versagen konventioneller Leberhypertrophie-Induktion darstellt

(128). Des Weiteren sind das in Arbeit 2.1 und 2.4 beschriebene pCCA-Kollektiv mit einer hochstandardisierten hilären *en-bloc* Resektionstechnik mit einer *no-touch* Technik im Leberhilus reseziert worden (129). Bei diesem Verfahren wird der oft chronisch-inflammatorisch adhärente Leberhilus nicht disseziert, sondern im Rahmen einer ausgedehnten Resektion der Portalvene, des Gallengangs und gelegentlich der Leberarterie, gemeinsam mit einer ausgedehnten Lymphadenektomie entfernt (129). Somit sind die beschriebenen Kollektive Gegenstand retrospektiver Arbeiten, allerdings wurde versucht, durch präzise onkologische und chirurgisch-technische Einschlusskriterien retrospektive Einflussgrößen zu minimieren.

Dennoch zeigt auch unser Kollektiv weitere intrinsische Limitationen auf: ein Großteil der Patient*innen wurden in der prä-BILCAP Ära operiert, also vor der 2019 publizierten Evidenz für eine allgemeine Empfehlung einer adjuvanten Chemotherapie für das CCA (37). Die Patient*innenselektion für die postoperative Chemotherapie fand unter Berücksichtigung onkologischer Risikofaktoren wie des Lymphknotenstatus statt. Entsprechend unterscheiden sich die Ergebnisse zum onkologischen- und Gesamtüberleben etwas von Kohorten, die nach 2019 therapiert wurden.

Insgesamt zeigen die hier zusammengefassten Daten, dass Wirtsfaktoren in primären Lebertumoren einen prognostischen Wert haben, und das Potenzial haben, bereits präoperativ Patient*innen mit hohem Risiko für schlechtes Outcome zu identifizieren. Die aufgeführten Body-Composition Ergebnisse zeigen eine hohe Vulnerabilität ausgewählter Kohorten, deren Outcome potenziell durch Interventionen wie Rehabilitation und Ernährungsberatung profitieren könnten. Des Weiteren besteht durch die Analyse von Wirtsfaktoren in Populationen mit hohen postoperativen lokalen Rezidivraten des CCAs und HCCs das Potenzial, selektive Populationen engmaschiger zu kontrollieren, um sie im Falle eines früher Rezidivs potenziell kurativ zu therapieren, oder ausgewählte Patient*innen einer zusätzlichen adjuvanten Therapie im Rahmen zukünftiger Studien zuzuführen. Zusammenfassend zeigt diese Arbeit, dass neben klinisch etablierten Tumor-zentrierten prognostischen Faktoren, beispielsweise Lymphknoteninvasion und Mutationsstatus, auch genetische und physische Wirtsaspekte die Prognose nach der Resektion primärer Lebertumoren beeinflussen.

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Danksagung

Meine Danksagung wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht

Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Datum Unterschrift