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Analiza mikrośrodowiska guza oraz parametrów laboratoryjnych jako potencjalnych czynników prognostycznych u pacjentów chorych na raka wątrobowokomórkowego.

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

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Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa 2025 r.

Słowa kluczowe:

1. czynniki predykcyjne - predictive factors
2. czynniki prognostyczne - prognostic factors
3. immunoterapia - immunotherapy
4. leczenie systemowe - systemic treatment
5. mikrośrodowisko guza - tumor microenvironment
6. rak wątrobowokomórkowy - hepatocellular carcinoma
7. resekcja wątroby - liver resection
8. rokowania - prognosis
9. transplantacja wątroby - liver transplantation

Nazwa i numer projektu badawczego

1. Narodowe Centrum Nauki, grant Miniatura Nr 2020/04/x/nz5/00718;
2. Warszawski Uniwersytet Medyczny, grant Młodego Badacza Nr 2/W11/1/M/MBS/N/21.

Dedykacje

Chciałbym wyrazić moją głęboką wdzięczność i szacunek dla mojego promotora, prof. dr hab. n. med. Rafała W. Steca, za nieocenione wsparcie i przewodnictwo podczas całego procesu tworzenia tej rozprawy. Pańska wiedza, cierpliwość i zaangażowanie były dla mnie nieocenionym źródłem inspiracji i motywacji. Dziękuję za wiarę we mnie oraz za wszystkie cenne rady, które pomogły mi osiągnąć ten ważny etap w mojej karierze naukowej.

Dziękuję mojemu promotorowi pomocniczemu, dr n. med. Leszkowi Krajowi za motywację i cenne wskazówki, które pomogły mi w realizacji tej rozprawy. Pańska pomoc była nieoceniona.

Chciałbym wyrazić moją wdzięczność rodzinie za zrozumienie, miłość i cierpliwość podczas całego procesu przygotowywania tej rozprawy. Wasza wiara we mnie oraz nieustające wsparcie były dla mnie źródłem siły i motywacji. Dziękuję za wszystko, co dla mnie zrobiliście.

Wykaz publikacji stanowiących pracę doktorską

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Łącznie Impact Factor 18,225 oraz 380 punktów MEiN

Spis treści

Wykaz stosowanych skrótów

Skrót	Rozwinięcie	Znaczenie
AFP	Alpha-Fetoprotein	Alfa-fetoproteina
ALBI	Albumin-Bilirubin Index	Wskaźnik albumina-bilirubina
BCLC	Barcelona Clinic Liver Cancer	Klasyfikacja zaawansowania HCC BCLC
BMDSC	Bone Marrow-Derived Stem Cells	Komórki macierzyste pochodzenia szpikowego
BSC	Best Supportive Care	Opieka paliatywna
CD	Cluster of Differentiation	Klaster różnicowania
CI	Confidence Interval	Przedział ufności
EMA	European Medicines Agency	Europejska Agencja Leków
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor	Czynnik stymulujący kolonie granulocytarno-makrofagowe
HCC	Hepatocellular Carcinoma	Rak wątrobowokomórkowy
HR	Hazard Ratio	Współczynnik ryzyka
IFN-γ	Interferon gamma	Interferon gamma
IHC	Immunohistochemistry	Immunohistochemia
IL	Interleukin	Interleukina
LT	Liver Transplantation	Przeszczepienie wątroby
M-CSF	Macrophage Colony-Stimulating Factor	Czynnik stymulujący kolonie makrofagów
MVA	Microwave Ablation	Ablacja z wykorzystaniem mikrofal
NLR	Neutrophil-to-Lymphocyte Ratio	Stosunek neutrofili do limfocytów
OS	Overall Survival	Całkowite przeżycie

PALBI	Platelet-Albumin-Bilirubin Index	Wskaźnik płytek, albuminy i bilirubiny
PD-1	Programmed Death 1 (receptor)	(Receptor) programowanej śmierci komórki 1
PD-L1	Programmed Death Ligand 1	Ligand programowanej śmierci komórki 1
PH test	Proportional Hazards test	Test proporcjonalności hazardów (dla modelu Coxa)
PLR	Platelet-to-Lymphocyte Ratio	Stosunek płytek krwi do limfocytów
PLT	Platelets	Płytki krwi
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses	Wytyczne dla przeglądów systematycznych PRISMA
RFA	Radiofrequency Ablation	Ablacja prądem o częstotliwości radiowej
RFS	Recurrence-Free Survival	Przeżycie wolne od nawrotu
SBRT	Stereotactic Body Radiotherapy	Radioterapia stereotaktyczna
TACE	Transarterial Chemoembolization	Przeztętnicza chemoembolizacja
TAM	Tumor-Associated Macrophages	Makrofagi towarzyszące nowotworowi
TAN	Tumor-Associated Neutrophils	Neutrofile związane z nowotworem
TARE	Transarterial Radioembolization	Radioembolizacja przez tętnicę
TIL	Tumor-Infiltrating Lymphocytes	Limfocyty naciekające guz
TME	Tumor Microenvironment	Mikrośrodowisko guza
TNF-α	Tumor Necrosis Factor Alpha	Czynnik martwicy nowotworu alfa

UCSF	University of California, San Francisco	Kryteria kwalifikacji do przeszczepu wątroby Uniwersytet Kalifornijski, San Francisco
VEGF	Vascular Endothelial Growth Factor	Czynnik wzrostu śródbłonka naczyniowego
WBC	White Blood Cells	Białe krwinki

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Streszczenie

Rak wątrobowokomórkowy (hepatocelllular carcinoman, HCC) należy do najczęściej diagnozowanych nowotworów i jest czwartą przyczyną zgonów z przyczyn onkologicznych (Globoscan 2020[1]). Częstość występowania HCC różni się w istotny sposób geograficznie – najczęściej choroba jest diagnozowana w Azji oraz Afryce, co ma związek z zakażeniami wirusami hepatotropowymi HCV oraz HBV (Ferlay et al. 2015[2]). Obecnie przewiduje się, że w krajach Europy Zachodniej można spodziewać się wzrostu zapadalności. Pomimo ogólnego postępu w medycynie i opiece onkologicznej, rokowania pacjentów chorujących na HCC pozostają poważne. Terapia HCC może przebiegać w różny sposób, w zależności od stopnia zaawansowania choroby. Rekomendacje European Society for Medical Oncology w ostatnich latach były kilkukrotnie aktualizowane, w związku z pojawiającymi się nowymi opcjami terapeutycznymi.

Rekomendacje leczenia HCC w zależności od zaawansowania choroby.

Obecne rekomendacje zwracają uwagę na podział choroby wynikający z jej zaawansowania na podstawie skali Barcelona Clinic Liver Cancer, BCLC. Bardzo wczesna choroba (stopień 0), charakteryzuje się obecnością pojedynczego guza mniejszego niż 2cm, zachowaną funkcją wątroby (Child-Pugh A) oraz dobrym stanem klinicznym pacjenta (performance status, PS 0). Wczesny etap (BCLC A) jest definiowany jako obecność pojedynczego guza albo 3 zmian do 3 cm, częściowo upośledzona funkcja wątroby (Child-Pugh A-B) oraz dobry stan ogólny. Natomiast choroba o pośrednim zaawansowaniu (BCLC B) jest diagnozowana przy obecności licznych zmian w wątrobie i jednocześnie nadal zachowanej częściowej funkcji wątroby i dobrym stanie pacjenta. O chorobie zaawansowanej (BCLC C) można mówić, jeśli stwierdzane są zmiany przerzutowe poza wątrobą oraz inwazja układu wrotnego; stan pacjenta nadal powinien być dość dobry (PS 1-2). Ciężkie upośledzenie funkcji wątroby (Child Pugh C) oraz gorszy stan ogólny pacjenta (PS 3-4) są wykładnikami terminalnego etapu choroby (BCLC D) (Pons et a. 2005[3]).

W zależności od stopnia zaawansowania choroby, pacjenci powinni być kwalifikowani do różnych metod terapeutycznych. Należy zauważyć, że w 2025 opublikowane zostały nowe zalecenia ESMO, dotyczące leczenia pacjentów chorych na HCC. Zgodnie z nimi pacjenci w stadiach BCLC 0–B mogą być kwalifikowani do resekcji - zalecana u pacjentów z

pojedynczym guzem >2 cm, bez nadciśnienia wrotnego, możliwa jest także u wybranych pacjentów z Child-Pugh B; ablacji (ablacja prądem o częstotliwości radiowej, radiofrequency ablation, RFA/ ablacja z wykorzystaniem mikrofal, microwave ablation, MWA), która może być alternatywą dla resekcji w BCLC 0–A, dla guzów 3–5 cm, lub jako pomost do przeszczepu. Przeszczep wątroby (liver transplantation, LT) może być rozważany jeśli spełnione są kryteria mediolańskie lub Uniwersytetu Klifornijskiego w San Francisco, UCSF. Warto zauważyć, że terapie pomostowe (transarterial chemoembolization, TACE; transarterial radioembolization, TARE; stereotactic body radiotherapy, SBRT) mogą być rozważane przy długim czasie oczekiwania. Co istotne, nie zaleca się stosowania inhibitorów kinazy tyrozynowej (tyrosine kinase inhibitors, TKI) ani immunoterapii po resekcji/ablacji lub LT. Istotne zmiany w nowych rekomendacjach dotyczą leczenia systemowego w stadium zaawansowanej choroby – BCLC C. W ramach leczenia pierwszej linii zalecane schematy obejmują atezolizumab (immunoterapia anty-PD-L1, ligand receptorowy śmierci programowanej 1) w skojarzeniu z bewacyzumabem (anty-VEGF, vascular endothelial growth factor) lub durwalumab z tremelimumabem. Alternatywnie rozważyć można kamrelizumab z rivoceranibem (niezatwierdzone przez EMA, European Medicines Agency, Europejską Agencję Leków oraz FDA, Food and Drug Administration, Agencja Żywności i Leków), podwójną immunoterapię, niwolumab z ipilimumabem (anty-CTLA-4, cytotoxic T lymphocyte-associated antigen 4, zatwierdzone przez EMA; durwalumab lub tislelizumab w monoterapii oraz lenwatynib lub sorafenib przy przeciwwskazaniach do immunoterapii. W leczeniu drugiej linii po sorafenibie można stosować regorafenib, kabozantynib lub ramucyrumab (przy poziomie alfa-fetoproteiney, AFP ≥400 ng/ml), natomiast po immunoterapii lub lenwatynibie sorafenib (off-label).[4]

Mimo, że przeszczepienie wątroby lub resekcja są uważane za radykalne opcje leczenia, u niektórych pacjentów dochodzi do nawrotu choroby. Dlatego istnieje silna potrzeba identyfikacji czynników prognostycznych, pozwalających na dostosowanie leczenia i nadzoru onkologicznego do potrzeb pacjentów i charakterystyki nowotworu. Ponadto, w prawdopodobnie nadchodzącej erze leczenia uzupełniającego i immunoterapii, przy zastosowaniu których obserwuje się odpowiedź na leczenie u części pacjentów, kluczowe znaczenie ma identyfikacja czynników predykcyjnych.

W świetle aktualnej wiedzy uważa się, że mikrośrodowisko nowotworu odgrywa zasadniczą rolę w postępie choroby. Jednym z najważniejszych immunologicznych czynników mikrośrodowiska są makrofagi towarzyszące nowotworowi (tumor-associated macrophages,

TAM). Sugeruje się, że mogą mieć one zastosowanie zarówno jako czynniki prognostyczne jak i predykcyjne.

Mikrośrodowisko guza i jego wpływ na rozwój choroby

Mikrośrodowisko (tumor microenvirnoment, TME) odgrywa kluczową rolę w rozwoju nowotworu. Rozwijające się w nim nieustanne procesy uszkodzenia i regeneracji hepatocytów związane są z rozwojem przewlekłego stanu zapalnego. Złożone wzajemne oddziaływania między hepatocytami a komórkami układu odpornościowego w dużym stopniu zależą od TME. Do TME należy macierz zewnątrzkomórkowa, komórki nowotworowe, fibroblasty oraz komórki układu immunologicznego. Komórki układu odporności obejmują makrofagi związane z nowotworem makrofagi (TAM), limfocyty naciekające nowotwór (tumor-infiltrating lymphocytes, TIL), neutrofile związane z nowotworem (tumor associated neutrophils, TAN) i pochodzące ze szpiku kostnego komórki macierzyste (bone marrow-derived suppressor cells, BMDSC). Wszystkie te typy komórek są zdolne do uwalniania cząsteczek sygnałowych, które wywierają rożnorodne skutki, takie jak wywoływanie odporności, tolerancję komórek nowotworowych i angiogenezę, wzmożoną proliferację lub zapobiegają wzrostowi guza i powstawaniu przerzutów.[5] [6]

W obrębie TME obecne są czynniki wzrostu, cytokiny, zewnątrzkomórkowe białka macierzy oraz enzymy, które mogą sprzyjać lub zapobiegać karcynogenezie. Szczególnie interesujące są TAM. TAM to komórki odpornościowe, które nie tylko są zdolne do regulacji wzrostu guza pierwotnego, ale biorą także udział w przeciwnowotworowej nabytej odpowiedzi immunologicznej, angiogenezie nowotworu i przebudowie macierzy zewnątrzkomórkowej.

TAM charakteryzują się ekspresją cząsteczek CD 68 (claster of differenttaion, CD68). W zależności od charakterystyki, są one dzielone na dwie główne klasy: M1 oraz M2.

M1 TAM są niezbędne do działania prozapalnego i odpowiedzi przeciwnowotworowej. Komórki te mogą być aktywowane przez cytokiny, takie jak interferon-gamma (IFN-gamma), czynnik martwicy nowotworu alfa (TNF-alfa) czynnik wzrostu kolonii granulocytarno-makrofagowych (GM-CSF) lub w odpowiedzi na infekcję bakteryjną. Wytwarzanie interleukiny (IL)-12 i innych cytokin prozapalnych prowadzi do inicjacji odpowiedzi immunologicznej zależnej od Th-1. Makrofagi M1 są również zdolne do działania cytotoksycznego przeciwko komórkom nowotworowym.[7]

Makrofagi M2 Makrofagi M2 są powiązane z progresją nowotworu i są częściej obserwowane w tkance HCC niż makrofagi M1. Markery makrofagów M2 obejmują CD163,

CD204, CD206 i MARCO.[8] M2 TAM mogą być aktywowane przez IL 4, 10, 13 lub przez czynnik stymulujący kolonie makrofagów (M-CSF) i hormony glikokortykoidowe. Makrofagi podobne do M2 prezentują różne fenotypy funkcjonalne i dzielą się dalej na cztery inne typy.[9] [10] [11]

Wyniki kilku badań sugerują, że ekspresja CD68 może mieć negatywną wartość prognostyczną. Z drugiej strony, część badań sugeruje, że jest to pozytywny czynnik prognostyczny. Niedawno opublikowana metaanaliza wykazała, że TAM CD68 nie są powiązane z rokowaniem pacjentów.[12] Dostępne dane mają jednak różne ograniczenia. Większość badań analizujących ekspresję CD68 przeprowadzono wśród pacjentów po resekcji, a tylko w 2 analizowano populacje po przeszczepieniu wątroby, przy czym wyniki jednego badania sugerowały brak wpływu na rokowania (n=206),[13] podczas gdy drugie badanie sugerowało wpływ negatywny (n=88).[14]

W innym opublikowanym badaniu wykazano, że TAM CD68 M1 były powiązane z indukcją ligandu programowanej śmierci 1 (PD-L1) w komórkach HCC, co sugerowało ich rolę w rozwoju nowotworu. Kiedy oceniano ekspresję PD-L1 w HCC razem z CD68, analiza przeżycia wykazała, że obecność PD-L1 na komórkach nowotworowych była skorelowana z progresją nowotworu, podczas gdy ekspresja PD-L1 na makrofagach odgrywała rolę ochronną w rokowaniu pacjentów chorych na HCC. Co więcej, w tym badaniu komórki CD68/PD-L1+ powiązano z aktywowanym mikrośrodowiskiem immunologicznym z intensywnym naciekiem limfocytów T CD8.[15] [16]

Cząsteczki punktów kontrolnych układu odpornościowego kontrolują odpowiedź immunologiczną. Jednym z najważniejszych wydaje się być receptor PD-1, który zazwyczaj jest indukowany na aktywowanych limfocytach T, komórkach NK (natural killer), limfocytach B i komórkach prezentujących antygen. I odwrotnie, PD-L1 jest obecny na komórkach nowotworowych, hepatocytach, komórkach gwiaździstych wątroby, komórkach Kupffera lub komórkach sinusoidalnych wątroby.[17] PD-L2 jest innym znanym ligandem PD-1, który jest obecny na komórkach dendrytycznych.

Oś PD-L1/PD-1 odgrywa ważną rolę w odpowiedzi przeciwnowotworowej. Wiązanie PD-1/PD-L1 prowadzi do tłumienia aktywności komórek odpornościowych przeciwko nowotworowi i rozwoju tolerancji gospodarza. Nadekspresja PD-L1 może być jednym z mechanizmów wykorzystywanych przez nowotwory do „ucieczki” z układu odpornościowego gospodarza.[18] [19]

Dowody z kilku badań wskazują, że u pacjentów z obecną ekspresją PD-L1 na komórkach nowotworowych lub komórkach układu odpornościowego obserwuje się większe prawdopodobieństwo osiągnięcia korzyści systemowej immunoterapii.[20]

Wpływ osi PD-1/PD-L1 na rokowanie i wyniki leczenia HCC oceniano w kilku badaniach. Wyniki okazały się jednak sprzeczne, a leżący u podstaw mechanizm nie jest w pełni poznany. Wykazano, że pacjenci z silną ekspresją PD-L1 i obecnością TILs mają zwykle lepsze rokowania, chociaż jeśli jednocześnie występuje mniejsza ekspresja PD-L1 i galektyny-9 oraz niski poziom CD8+ TIL, rokowanie może być gorsze.[21] Z drugiej strony, wyniki innego badania sugerują że ekspresja PD-L1 przez komórki nowotworowe lub przez obecne w środowisku nowotworu komórki immunologiczne może być powiązana z agresywnością guza.[22] Te sprzeczne wyniki wyraźnie pokazują, że potrzeba dalszych badań środowiska immunologicznego.

Ponieważ immunoterapia anty-PD-1/PD-L1 jest obecnie badana w różnych sytuacjach klinicznych u pacjentów chorujących na HCC, a dane dotyczące wpływu ekspresji PD-1/PD-L1 na rokowanie pacjenta są niespójne, zatem istotna jest ocena częstości ekspresji PD-L1 i nacieków komórek układu odpornościowego w HCC i ocena ich wartości prognostycznej lub predykcyjnej.[23] [24]

Retrospektywna analiza czynników prognostycznych w HCC

Na podstawie powyższych danych zaprojektowano badanie, które miało na celu ocenę molekularnych oraz laboratoryjnych czynników związanych z rokowaniem pacjentów chorych na HCC.

W badaniu oceniano w korelacji do przeżycia całkowitego oraz wolnego od nawrotu choroby następujące parametry: wielkość i liczba guzów nowotworowych, metoda leczenia (resekcja/transplantacja/leczenie systemowe), status zakażenia HBV, HCV; wyjściowe parametry laboratoryjne: AFP, WBC (white blood cel), NLR (neutrophil-to-lymphocyte ratio), PLR (platelet-to-lymphocyte ratio), ALBI (albumin-bilirubin), PALBI (platelet-albumin-bilirubin), bilirubina; ocena ekspresji PD-L1, CD68, TILs.

W pracy przeanalizowano dane ponad 227 kolejnych pacjentów chorych na HCC leczonych w ośrodku akademickim. Analizę statystyczną przeprowadzono dla populacji ogólnej oraz dla dwóch podgrup – po przeszczepieniu wątroby i po resekcji. Trzeciej analizy w grupie pacjentów kwalifikujących się do leczenia systemowego nie przeprowadzono (tych pacjentów wyłączono także z analizy populacji ogólnej ze względu na odmienną charakterystykę

kliniczną). Wynikało to z faktu, że mimo oceny danych ponad 70 chorych leczonych systemowo z powodu HCC, jedynie u kilkunastu chorych dostępny był materiał histopatologiczny, co nie pozwoliło na rzetelną analizę statystyczną. Ponadto stwierdzono znaczną heterogeniczność grupy pod względem zastosowanego leczenia: 12 pacjentów otrzymywało sorafenib, 3 otrzymywało kabozantynib, 1 otrzymywał gemcytabinę z oksaliplatyną i 1 otrzymywał kwas zoledronowy.

Do ostatecznej analizy włączono 111 pacjentów spełniających kryteria włączenia: 52 po przeszczepieniu wątroby, 59 po resekcji. Osiemdziesięciu dwóch pacjentów stanowili mężczyźni, a 29 kobiety; mediana wieku wynosiła 61,7 lat. U ponad 42% (n=48) stwierdzono zakażenie HCV, a u 24% (n=28) zakażenie HBV. Większość pacjentów miała stosunkowo małe guzy (do 50 mm); u 38% pacjentów (n=44) wykryto zmiany o średnicy około 50 mm lub większej.

Mediana okresu obserwacji wyniosła 47,95 miesiąca i wahała się od 0,1 miesiąca (śmierć z powodu powikłań pooperacyjnych) do 138 miesięcy. W czasie obserwacji zdiagnozowano 52 przypadki wznowy nowotworu: 41/59 pacjentów po resekcji (69%) i 11/52 po przeszczepieniu wątroby (21%), a 45 pacjentów zmarło. Mediana czasu przeżycia wolnego od nawrotu (recurrence-free survival, RFS) wynosiła 20,5 miesiąca, a czasu przeżycia całkowitego (overall survival, OS) po leczeniu pierwotnym w przypadku nawrotu choroby 30 miesięcy.

Dodatkową analizę przeprowadzono w dwóch podgrupach: pacjentów po przeszczepieniu wątroby oraz pacjentów po resekcji.

Po jednoczynnikowej analizie statystycznej określono kilka czynników prognostycznych nawrotu raka wątrobowokomórkowego. Najważniejszymi negatywnymi czynnikami prognostycznymi w populacji ogólnej były: wielkość guza, stopień zróżnicowania i obecność inwazji mikronaczyniowej, poziom AFP przed leczeniem oraz poziom bilirubiny. Inne czynniki to ocena wg PALBI i PLT. Ekspresję PD-L1 w tkance nowotworowej powiązano z graniczną istotną wartością p = *0,053*. W analizie wieloczynnikowej jedynie mikroinwazja naczyń (obecna lub nieobecna), wyjściowy poziom AFP i WBC były znamiennymi negatywnymi czynnikami prognostycznymi w populacji ogólnej.

Analiza podgrup wykazała, że wśród pacjentów po przeszczepieniu wątroby wiek, wielkość guza, stopień zróżnicowania, inwazja mikronaczyniowa i ekspresja CD68 były czynnikami negatywnymi w analizie jednoczynnikowej. W analizie wieloczynnikowej statystycznie

istotna okazała się inwazja mikronaczyń oraz stopień zróżnicowania. Podobnie w jednoczynnikowej analizie przeżycia całkowitego jako czynniki prognostyczne zasugerowano AFP, wielkość guza, stopień zróżnicowania i inwazję mikronaczyniową, ekspresję PD-L1 i NLR, podczas gdy w analizie wieloczynnikowej jedynie inwazja mikronaczyniowa i NLR były powiązane ze istotnością statystyczną.

Wśród pacjentów po resekcji wątroby w analizie jednoczynnikowej wykazano, że czynnikami prognostycznymi dla nawrotu choroby są jedynie wiek, AFP i WBC. Analiza wieloczynnikowa nie potwierdziła jednak istotności statystycznej. Analiza jednoczynnikowa w odniesieniu do całkowitego przeżycia sugeruje, że AFP, bilirubina i inwazja mikronaczyń są czynnikami prognostycznymi. Wyniki analizy wielu zmiennych również potwierdziły to spostrzeżenie.

Ekspresję PD-L1 potwierdzono w 4 próbkach w całej populacji. Wśród chorych po przeszczepieniu wątroby stwierdzono to u 2 osób i u obu doszło do wznowy. W jednym przypadku progresję rozpoznano po 87 miesiącach, a ekspresję PD-L1 stwierdzono w 10% komórek, w drugim przypadku wznowę rozpoznano po 10,8 miesiąca, a ekspresję PD-L1 stwierdzono w 100% komórek. Ponadto wśród pacjentów po resekcji zidentyfikowano 2 inne przypadki ekspresji PD-L1: u pierwszego pacjenta potwierdzono ekspresję PD-L1 w 5% komórek nowotworowych i zmarł on z powodu nawrotu choroby 14,75 miesiąca po operacji; u drugiego pacjenta ekspresja ta wynosiła 10%, a 24 miesiące po operacji stwierdzono wznowę. Pacjent zmarł 3 miesiące od rozpoznania wznowy. Mała liczba pacjentów, u których stwierdzono ekspresję PD-L1 powoduje, że należy zachować ostrożność w interpretacji tej obserwacji, chociaż w analizie statystycznej nie wiązało się to z istotnie zwiększonym ryzykiem nawrotu choroby ($p=0,053$) lub zgonu. Jedynie w analizie jednoczynnikowej zasugerowano ją, jako negatywny czynnik prognostyczny u chorych po przeszczepieniu wątroby.

Ekspresję PD-L1 wykryto także na makrofagach towarzyszących nowotworowi (TAM) i/lub limfocytach naciekających guz (TIL) u 55 pacjentów: 17 pacjentów po przeszczepieniu wątroby i 31 po resekcji, w tym 8 leczonych później systemowo. Nie miało to jednak związku z rokowaniem w żadnej z podgrup pacjentów.

We wszystkich próbkach tkanek przeprowadzono barwienie CD68, a obecność makrofagów towarzyszących nowotworowi CD68 potwierdzono w 62 przypadkach w zakresie od 5% do 40%. Analiza wykazała, że CD68 nie wiązało się z ryzykiem nawrotu HCC.

Limfocyty naciekające guz (TIL) zaobserwowano w 19 przypadkach. Obecność TIL nie była związana z rokowaniem w żadnej z podgrup ani w populacji ogólnej.

Zgodnie z wynikami analizy jednoczynnikowej w populacji pacjentów chorych na raka wątrobowokomórkowego, poziom PLT należał również do czynników prognostycznych. Z tego względu przeprowadzono meta-analizę i przegląd systematyczny, które stały się podstawą czwartej publikacji w cyklu. Celem pracy było zbadanie wpływu trombocytopenii na przeżycie pacjentów z rozpoznaniem raka wątrobowokomórkowego oraz ocena związku liczby płytek krwi z cechami kliniczno-patologicznymi nowotworu. W ramach pracy przeprowadzono systematyczny przegląd literatury i metaanalizę zgodnie z wytycznymi PRISMA, przeszukano bazy: PubMed, Web of Science i Scopus. Na podstawie wyszukiwania zidentyfikowano 26 badań (łącznie 9403 pacjentów), które spełniały określone kryteria wyszukiwania. Analizowano współczynniki hazardu (HR) dla przeżycia całkowitego (OS) w zależności od liczby płytek krwi. Przeprowadzone analizy wskazują, że trombocytopenia ($<100 \times 10^9$/L) była związana z gorszym przeżyciem całkowitym: HR (model efektów losowych) =1.30 (95% CI: 1.05–1.63), HR (model wieloczynnikowy)= 1.47 (95% CI: 1.15–1.86). W grupie leczonej z zamiarem radykalnym (np. resekcja, RFA) HR=1.62 (95% CI: 1.25–2.11), natomiast w grupie leczonej paliatywnie (np. TACE, leczenie systemowe) HR=0.81 (95% CI: 0.62–1.05) – co sugeruje możliwy odwrotny trend. Ponadto niska liczba płytek była częstsza u pacjentów z mniejszymi guzami i marskością wątroby, natomiast wyższe wartości PLT korelowały z większymi guzami i mniejszą częstością marskości. Wskazuje to na możliwy udział płytek krwi i ich mediatorów (np. czynnik wzrostu płytek krwi, platelet-derived growth factor, PDGF, serotoniny) w progresji HCC. Liczba płytek krwi może być użytecznym, łatwo dostępnym markerem prognostycznym u pacjentów chorych na HCC, szczególnie w leczeniu radykalnym. Niemniej, potrzebne są dalsze badania prospektywne, szczególnie w grupach leczonych systemowo, z uwzględnieniem dokładnych kryteriów włączenia.

The analysis of the tumor microenvironment and laboratory parameters as potential prognostic factors in patients with hepatocellular carcinoma.

Summary

Hepatocellular carcinoma (HCC) is one of the most frequently diagnosed cancers and is the fourth leading cause of cancer-related deaths (Globoscan 2020). The incidence of HCC varies significantly geographically, with the highest rates in Asia and Africa, which is associated with infections by hepatotropic viruses HCV and HBV (Ferlay et al. 2015). Currently, it is predicted that the incidence in Western European countries will increase. Despite overall progress in medicine and oncology care, the prognosis for HCC patients remains poor. HCC therapy can vary depending on the stage of the disease. The European Society for Medical Oncology (ESMO) recommendations have been updated several times in recent years due to the emergence of new therapeutic options.

Treatment Recommendations for HCC Based on Disease Stage

Current recommendations emphasize the division of the disease based on its stage according to the Barcelona Clinic Liver Cancer (BCLC) scale. Very early disease (stage 0) is characterized by the presence of a single tumor smaller than 2 cm, preserved liver function (Child-Pugh A), and good clinical condition of the patient (performance status, PS 0). The early stage (BCLC A) is defined as the presence of a single tumor or up to 3 lesions up to 3 cm, partially impaired liver function (Child-Pugh A-B), and good overall condition. Intermediate disease (BCLC B) is diagnosed with multiple liver lesions and still preserved partial liver function and good patient condition. Advanced disease (BCLC C) is characterized by metastatic changes outside the liver and portal vein invasion; the patient's condition should still be relatively good (PS 1-2). Severe liver function impairment (Child-Pugh C) and poor overall condition (PS 3-4) are indicators of the terminal stage of the disease (BCLC D) (Pons et al. 2005).

Depending on the stage of the disease, patients should be qualified for different therapeutic methods. It should be noted that in 2025, new ESMO guidelines for the treatment of HCC were published. According to these guidelines, patients in stages BCLC 0–B can be qualified for resection - recommended for patients with a single tumor >2 cm, without portal hypertension, and possible for selected patients with Child-Pugh B; ablation (radiofrequency ablation, RFA/microwave ablation, MWA), which can be an alternative to resection in BCLC 0–A, for tumors 3–5 cm or as a bridge to transplantation. Liver transplantation (LT) can be

considered if the Milan or University of California, San Francisco (UCSF) criteria are met. It is worth noting that bridging therapies (transarterial chemoembolization, TACE; transarterial radioembolization, TARE; stereotactic body radiotherapy, SBRT) can be considered for long waiting times. Importantly, tyrosine kinase inhibitors (TKI) or immunotherapy are not recommended after resection/ablation or LT. Significant changes in the new recommendations concern systemic treatment in advanced disease stage - BCLC C. First-line treatment regimens include atezolizumab (anti-PD-L1 immunotherapy) combined with bevacizumab (anti-VEGF) or durvalumab with tremelimumab. Alternatively, camrelizumab with rivoceranib (not approved by EMA/FDA), dual immunotherapy, nivolumab with ipilimumab (anti-CTLA-4, approved by the European Medicines Agency, EMA); durvalumab or tislelizumab monotherapy, and lenvatinib or sorafenib in case of contraindications to immunotherapy. In second-line treatment after sorafenib, regorafenib, cabozantinib, or ramucirumab (with alpha-fetoprotein level, AFP ≥400 ng/ml) can be used, while after immunotherapy or lenvatinib, sorafenib (off-label) can be considered (Vogel et al. 2025).

Although liver transplantation or resection are considered radical treatment options, some patients experience disease recurrence. Therefore, there is a strong need to identify prognostic factors that allow for the adjustment of treatment and oncological surveillance to the needs of patients and tumor characteristics. Moreover, in the likely upcoming era of adjuvant treatment and immunotherapy, where response is observed only in some patients, identifying predictive factors is crucial.

Tumor Microenvironment and Its Impact on Disease Progression

The tumor microenvironment (TME) plays a key role in tumor development. Continuous processes of hepatocyte damage and regeneration within the TME are associated with the development of chronic inflammation. Complex interactions between hepatocytes and immune cells largely depend on the TME. The TME includes the extracellular matrix, tumor cells, fibroblasts, and immune cells. Immune cells include tumor-associated macrophages (TAM), tumor-infiltrating lymphocytes (TIL), tumor-associated neutrophils (TAN), and bone marrow-derived suppressor cells (BMDSC). All these cell types are capable of releasing signaling molecules that exert various effects, such as inducing immunity, tumor cell tolerance, angiogenesis, increased proliferation, or preventing tumor growth and metastasis (Greten and Grivennikov 2019; Sas et al. 2022).

Within the TME, growth factors, cytokines, extracellular matrix proteins, and enzymes are present, which can contribute to or prevent carcinogenesis. TAMs are particularly interesting.

TAMs are immune cells that not only regulate primary tumor growth but also participate in anti-tumor acquired immune response, tumor angiogenesis, and extracellular matrix remodeling. TAMs are characterized by the expression of CD68 molecules (cluster of differentiation, CD68). Depending on their characteristics, they are divided into two main classes: M1 and M2.

M1 TAMs are essential for pro-inflammatory and anti-tumor responses. These cells can be activated by cytokines such as interferon-gamma (IFN-gamma), tumor necrosis factor-alpha (TNF-alpha), granulocyte-macrophage colony-stimulating factor (GM-CSF), or in response to bacterial infection. The production of interleukin (IL)-12 and other pro-inflammatory cytokines leads to the initiation of a Th-1 dependent immune response. M1 macrophages are also capable of cytotoxic activity against tumor cells (Martinez and Gordon 2014).

M2 macrophages are associated with tumor progression and are more frequently observed in HCC tissue than M1 macrophages. M2 macrophage markers include CD163, CD204, CD206, and MARCO (Larionova et al. 2020). M2 TAMs can be activated by IL-4, IL-10, IL-13, or macrophage colony-stimulating factor (M-CSF) and glucocorticoid hormones. M2-like macrophages exhibit various functional phenotypes and are further divided into four other types (Zhou et al. 2014; Solinas et al. 2009; Zhang and Sioud 2023).

Several studies suggest that CD68 expression may have a negative prognostic value. On the other hand, some studies suggest it is a positive prognostic factor. A recently published meta-analysis showed that CD68 TAMs are not associated with patient prognosis (Zhang et al. 2021). However, the available data have various limitations. Most studies analyzing CD68 expression were conducted among patients after resection, and only two analyzed populations after liver transplantation, with one study suggesting no impact on prognosis (n=206) (Gao et al. 2012), while the other suggested a negative impact (n=88) (Atanasov et al. 2020).

In another published study, CD68 M1 TAMs were associated with the induction of programmed death ligand 1 (PD-L1) in HCC cells, suggesting their role in tumor development. When PD-L1 expression in HCC was evaluated together with CD68, survival analysis showed that the presence of PD-L1 on tumor cells was correlated with tumor progression, while PD-L1 expression on macrophages played a protective role in HCC patient prognosis. Moreover, in this study, CD68/PD-L1+ cells were associated with an activated immune microenvironment with intense CD8 T cell infiltration (Zong et al. 2019; Liu et al. 2018).

Immune checkpoint molecules control the immune response. One of the most important seems to be the PD1 receptor, which is typically induced on activated T cells, NK cells, B cells, and antigen-presenting cells. Conversely, PD-L1 is present on tumor cells, hepatocytes, liver stellate cells, Kupffer cells, or liver sinusoidal cells (Wang et al. 2011). PD-L2 is another known PD-1 ligand present on dendritic cells.

The PD-L1/PD-1 axis plays an important role in the anti-tumor response. PD-1/PD-L1 binding leads to the suppression of immune cell activity against the tumor and the development of host tolerance. PD-L1 overexpression may be one of the mechanisms used by tumors to "escape" the host immune system (Keir et al. 2008; Kudo et al. 2017). Evidence from several studies indicates that patients with PD-L1 expression on tumor cells or immune cells are more likely to benefit from systemic immunotherapy (Garon et al. 2015 1; Lin et al. 2018).

The impact of the PD1/PDL1 axis on prognosis and treatment outcomes in HCC has been evaluated in several studies. However, the results have been conflicting, and the underlying mechanism is not fully understood. It has been shown that patients with strong PD-L1 expression and the presence of TILs generally have better prognoses, although if there is lower PD-L1 expression and galectin-9 and low CD8+ TIL levels, the prognosis may be worse (Sideras et al. 2017). On the other hand, the results of another study suggest that PD-L1 expression by tumor cells or immune cells present in the tumor environment may be associated with tumor aggressiveness (Calderaro et al. 2016). These conflicting results clearly indicate the need for further research on the immune environment.

Since anti-PD-1/PD-L1 immunotherapy is currently being investigated in various clinical settings in HCC patients, and data on the impact of PD-1/PD-L1 expression on patient prognosis are inconsistent, it is essential to assess the frequency of PD-L1 expression and immune cell infiltrates in HCC and evaluate their prognostic or predictive value (Kan and Dong 2015; Sideras et al. 2017).

Retrospective Analysis of Prognostic Factors in HCC

Based on the above data, a study was designed to evaluate molecular and laboratory factors associated with the prognosis of HCC patients. The study assessed the following parameters in correlation with overall survival and recurrence-free survival: tumor size and number, treatment method (resection/transplantation/systemic treatment), HBV, HCV infection status;

baseline laboratory parameters: AFP, WBC, NLR, PLR, ALBI, PALBI, bilirubin; PD-L1, CD68, TILs expression.

The study analyzed data from over 227 consecutive HCC patients treated at an academic center. Statistical analysis was conducted for the general population and two subgroups - post-liver transplantation and post-resection. A third analysis in the group of patients eligible for systemic treatment was not conducted (these patients were also excluded from the general population analysis due to different clinical characteristics). This was because, despite evaluating data from over 70 patients treated systemically for HCC, histopathological material was available for only less than 20 patients, which did not allow for reliable statistical analysis. Additionally, significant heterogeneity of the group in terms of treatment was noted: 12 patients received sorafenib, 3 received cabozantinib, 1 received gemcitabine with oxaliplatin, and 1 received zoledronic acid.

The final analysis included 111 patients meeting the inclusion criteria: 52 post-liver transplantation, 59 post-resection. Eighty-two patients were men, and 29 were women; the median age was 61.7 years. Over 42% (n=48) were HCV-infected, and 24% (n=28) were HBV-infected. Most patients had relatively small tumors (up to 50 mm); in 38% of patients (n=44), lesions of approximately 50 mm or larger were detected.

The median follow-up period was 47.95 months, ranging from 0.1 months (death due to postoperative complications) to 138 months. During the follow-up, 52 cases of tumor recurrence were diagnosed (41/59 patients post-resection (69%) and 11/52 post-liver transplantation (21%)), and 45 patients died. The median recurrence-free survival (RFS) was 20.5 months, and the overall survival (OS) after primary treatment in case of disease recurrence was 30 months.

Additional analysis was conducted in two subgroups: post-liver transplantation and post-resection patients.

Univariate statistical analysis identified several prognostic factors for HCC recurrence. The most important negative prognostic factors in the general population were: tumor size, differentiation grade, presence of microvascular invasion, pre-treatment AFP level, and bilirubin level. Other factors included PALBI and PLT assessment. PD-L1 expression in tumor tissue was associated with a borderline significant p-value of 0.053. In multivariate analysis, only microvascular invasion (present or absent), baseline AFP level, and WBC were significant negative prognostic factors in the general population.

Subgroup analysis showed that among post-liver transplantation patients, age, tumor size, differentiation grade, microvascular invasion, and CD68 expression were negative factors in univariate analysis. In multivariate analysis, microvascular invasion and differentiation grade were statistically significant. Similarly, in univariate overall survival analysis, AFP, tumor size, differentiation grade, microvascular invasion, PD-L1 expression, and NLR were suggested as prognostic factors, while in multivariate analysis, only microvascular invasion and NLR were statistically significant.

Among post-resection patients, univariate analysis showed that age, AFP, and WBC were prognostic factors for disease recurrence. However, multivariate analysis did not confirm statistical significance. Univariate overall survival analysis suggested that AFP, bilirubin, and microvascular invasion were prognostic factors. Multivariate analysis also confirmed this observation.

PD-L1 expression was confirmed in 4 samples in the entire population. Among post-liver transplantation patients, it was detected in 2 individuals, both of whom experienced recurrence. In one case, progression was diagnosed after 87 months, with PD-L1 expression in 10% of cells; in the other case, recurrence was diagnosed after 10.8 months, with PD-L1 expression in 100% of cells. Additionally, among post-resection patients, 2 other cases of PD-L1 expression were identified: in the first patient, PD-L1 expression was confirmed in 5% of tumor cells, and the patient died due to disease recurrence 14.75 months after surgery; in the second patient, PD-L1 expression was 10%, and recurrence was diagnosed 24 months after surgery. The patient died 3 months after recurrence diagnosis. The small number of patients with PD-L1 expression warrants caution in interpreting this observation, although statistical analysis did not show a significantly increased risk of disease recurrence ($p=0.053$) or death. Only in univariate analysis was it suggested as a negative prognostic factor in post-liver transplantation patients.

PD-L1 expression was also detected on tumor-associated macrophages (TAM) and/or tumor-infiltrating lymphocytes (TIL) in 55 patients: 17 post-liver transplantation and 31 post-resection, including 8 later treated systemically. However, this was not associated with prognosis in any patient subgroup.

CD68 staining was performed in all tissue samples, and the presence of CD68 tumor-associated macrophages was confirmed in 62 cases, ranging from 5% to 40%. Analysis showed that CD68 was not associated with the risk of HCC recurrence.

Tumor-infiltrating lymphocytes (TIL) were observed in 19 cases. The presence of TIL was not associated with prognosis in any subgroup or the general population.

According to univariate analysis results in the HCC patient population, PLT level was also a prognostic factor. Therefore, a meta-analysis and systematic review were conducted, forming the basis of the fourth publication in the series. The study aimed to investigate the impact of thrombocytopenia on the survival of patients diagnosed with HCC and assess the relationship between platelet count and clinical-pathological features of the tumor. A systematic literature review and meta-analysis were conducted according to PRISMA guidelines, searching included databases: PubMed, Web of Science, and Scopus. Based on the search, 26 studies (9403 patients) meeting the specified search criteria were identified. Hazard ratios (HR) for overall survival (OS) were analyzed depending on platelet count. The analyses indicate that thrombocytopenia ($<100 \times 10^9$/L) was associated with poorer overall survival: HR (random effects model) =1.30 (95% CI: 1.05–1.63), HR (multivariate model)= 1.47 (95% CI: 1.15–1.86). In the group treated with curative intent (e.g., resection, RFA), HR=1.62 (95% CI: 1.25–2.11), while in the palliative treatment group (e.g., TACE, systemic treatment), HR=0.81 (95% CI: 0.62–1.05) – suggesting a possible reverse trend. Additionally, low platelet count was more common in patients with smaller tumors and liver cirrhosis, while higher PLT values correlated with larger tumors and lower cirrhosis frequency. This indicates a possible role of platelets and their mediators (e.g., platelet-derived growth factor, PDGF, serotonin) in HCC progression. Platelet count may be a useful, easily accessible prognostic marker in HCC patients, particularly in curative treatment. However, further prospective studies are needed, especially in systemically treated groups, considering precise inclusion criteria.

Wstęp

W cyklu publikacji ujęto 4 prace pełnotekstowe dotyczące leczenia pacjentów chorujących na HCC. Pierwszy artykuł to przegląd systematyczny opisujący postępy w leczeniu systemowym oraz zasady leczenia zabiegowego HCC. Druga, przeglądowa publikacja podsumowuje obecny stan wiedzy na temat makrofagów naciekających guz jako istotnego czynnika w rozwoju nowotworu i jego zwalczaniu przez organizm. W trzeciej publikacji przeanalizowano wyniki dotyczące rokowania pacjentów leczonych z powodu HCC w Uniwersyteckim Centrum Klinicznym Warszawskiego Uniwersytetu Medycznego w zależności od charakterystyki molekularnej oraz klinicznej. W związku z wynikami przedstawionymi w trzeciej publikacji, sugerującymi możliwość wykorzystania oceny PLT jako czynnika prognostycznego w HCC, przeprowadzono meta-analizę oraz przegląd systematyczny, które stały się przedmiotem czwartej publikacji włączonej do cyklu.

Wszystkie czasopisma znajdują się w na liście Ministerstwa Nauki i Szkolnictwa Wyższego, MENiSW, (łącznie 380 punktów), a łączny Impact Factor, IF wynosi 18,225. Poniżej przedstawiono podsumowanie każdej z wymienionych publikacji oraz podsumowano analizę kliniczną w kontekście dotychczasowych danych literaturowych.

W pierwszej publikacji, Milestones in the treatment of hepatocellular carcinoma: A systematic review (Critical Reviews in Hematology/Oncology, 2021) przedstawiono dostępne opcje terapeutyczne dla pacjentów, w zależności od zaawansowania choroby, ze szczególnym uwzględnieniem pacjentów leczonych systemowo. Warto zauważyć, że po wielu latach ograniczonych możliwości leczenia, gdy jedynym dostępnym lekiem w tej populacji chorych był sorafenib, w ostatnim czasie zostało przebadanych i zarejestrowanych przez EMA, European Medicines Agency, Europejską Agencję Leków kilka nowych cząsteczek. Wśród nich najważniejszą opcją terapeutyczną wydaje się immunoterapia atezolizumabem (anty-PD-L1) w skojarzeniu z bewacyzumabem (anty-VEGF). Dostępne obecnie dane wskazują jednak, że odpowiedź na to leczenie różni się wśród pacjentów, przy czym czynniki predykcyjne nie są w pełnie zdefiniowane. Sugeruje się, że jednym z kluczowych czynników w rozwoju nowotworu oraz odpowiedzi na leczenie może być mikrośrodowisko guza i obecne w nim nacieki limfocytarne. Warto zauważyć, że ostatnich latach obserwowany jest dynamiczny rozwój tego obszaru i pojawia się coraz więcej opcji terapeutycznych.

Kolejna publikacja, The role of tumor-associated macrophages in hepatocellular carcinoma-from bench to bedside: A review (Journal of Gastroenterology and Hepatology, 2024) przedstawia podsumowanie aktualnej wiedzy dotyczącej makrofagów związanych z nowotworem (tumor-associated-macropahges, TAM). Zgodnie z dostępnymi danymi literaturowymi, komórki te mogą wywierać zarówno wpływ przeciwnowotworowy jak i promujący rozwój guza. Wyniki kilku badań sugerują, że TAM mogłyby służyć jako markery prognostyczne. Z drugiej strony, mogą być one zaangażowane w odpowiedź na leczenie, w tym na immunoterapię. Makrofagi związane z nowotworem mogą być potencjalnym dobrym celem terapii. W publikacji w szczegółowy sposób podsumowano postępy w zakresie wiedzy dotyczącej podstaw charakterystyki molekularnej, wpływu na rokowanie i potencjalnych implikacji klinicznych obecności makrofagów związanych z nowotworem w raku wątrobowokomórkowym. W pracy tej zwrócono również uwagę na populację TAM CD68. Dane dotyczące wpływu obecności tych komórek na rokowanie pacjentów chorych na HCC są ograniczone i niejednoznaczne. Ostatnio zasugerowano, że makrofagi CD68 można wykorzystać jako czynnik prognostyczny w HCC. W metaanalizie badań obejmujących ogółem 4297 pacjentów, zasugerowano, że TAM mogą służyć jako niezależne wskaźniki predykcyjne i cele terapeutyczne w HCC. Warto zauważyć, że obserwowane są różne efekty w zależności od podtypów TAM - istnieją doniesienia naukowe, które nie potwierdzają związku obecności TAM CD68+ z rokowaniem pacjentów, co wskazuje potrzebę dalszych badań w tym obszarze.

Wydaje się również, że istnieje wzajemne oddziaływanie między TAM CD68, a ekspresją ligandu zaprogramowanej śmierci 1 (PD-L1) i odpowiedzią immunologiczną w obrębie TME. Według ostatnich danych wskazano, że TAM CD68 M1 są powiązane z indukcją PD-L1 w komórkach HCC, co sugeruje, że odgrywają one rolę w rozwoju nowotworu.[25] Kiedy oceniano ekspresję PD-L1 w populacji pacjentów chorujących na HCC w korelacji z ekspresją CD68 wykazano, że ekspresja PD-L1 na komórkach nowotworu była związana z progresją choroby, podczas gdy ekspresja na makrofagach wpływała na bardziej korzystne rokowanie. Ponadto stwierdzono, że komórki CD68 PD-L1 mogą być powiązane z aktywowanym immunologiczne mikrośrodowiskiem guza z wysokim poziomem limfocytów T CD8.[26]

Biorąc pod uwagę istotną potrzebę kliniczną zaprojektowano badanie, którego wyniki opublikowano w artykule „Prognostic factors for hepatocellular carcinoma recurrence after liver transplantation or resection - single-center experience” (Heliyon, 2024). Celem pracy była ocena czynników prognostycznych związanych ze zwiększonym ryzykiem nawrotu raka wątrobowokomórkowego (HCC) po radykalnym leczeniu oraz ocena czynników

wpływających na rokowanie i wyniki leczenia systemowego. Jest to retrospektywna, jednoośrodkowa analiza danych dotyczących nawrotów HCC i przeżycia u pacjentów poddanych radykalnemu leczeniu – resekcji lub przeszczepowi. Planowana analiza w trzeciej populacji, poddanej leczeniu systemowemu, nie została wykonana ze względu na brak dostatecznej liczby pacjentów, u których dostępny byłby materiał do oceny histopatologicznej oraz ze względu na różne rodzaje zastosowanego leczenia systemowego. W ramach badania przeanalizowano molekularną charakterystykę nowotworu z uwzględnieniem wyżej wymienionych czynników takich jak ekspresja PD-L1, CD68 oraz obecność nacieków limfocytarnych. Dodatkowo oceniano stopień zaawansowania, wyjściowe wyniki badań laboratoryjnych wraz z parametrami takimi jak NLR, PLR, diagnoza zakażenia wirusowego wątroby. Najważniejszymi czynnikami prognostycznymi wznowy były mikroinwazja naczyniowa (HR=4,54; 95% CI 1,769–11,681; *p < 0,001*), wyjściowa liczba białych krwinek (HR=2,13; 95% CI 1,261–3,567; *p<0,004*) i wyjściowy poziom alfa-fetoproteiny (HR=1,00009; 95% CI 1,000001-1,00002; *p=0,034*). Inwazja mikronaczyniowa była czynnikiem prognostycznym, który istotnie korelował z przeżyciem całkowitym (HR=5,04, 95% CI 2,352–12,413; *p <0,001*). Ekspresję PD-L1 potwierdzono u 4 pacjentów i u wszystkich wystąpił nawrót choroby. Nie stwierdzono jednak istotnego statystycznie związku z OS oraz RFS. U 62 pacjentów potwierdzono obecność makrofagów towarzyszących nowotworowi CD68 w zakresie od 5% do 40%. Analiza wykazała, że ekspresja CD68 nie wiązała się jednak z ryzykiem nawrotu HCC. Wyniki analizy statystycznej potwierdzają, że spośród badanych czynników, inwazja mikronaczyń jest najważniejszym zwiększającym ryzyko nawrotu i zgonu z powodu raka wątrobowokomórkowego, natomiast ekspresja PD-L1 i CD68 nie ma wpływu na OS oraz RFS pacjenta.

W czwartej publikacji analizowano wpływ poziomu płytek krwi na rokowanie pacjentów chorych na HCC. Wyniki tej meta-analizy sugerują, że liczba płytek krwi może pełnić rolę markera prognostycznego w HCC, szczególnie przy wartości PLT $<100 \times 10^3/mm^3$. W ramach tej pracy przeanalizowano 26 badań obejmujących 9403 pacjentów. Wyniki wykazały, że małopłytkowość u pacjentów z HCC była związana z gorszym całkowitym przeżyciem (HR efektu wspólnego = 1,15, 95% CI: 1,06–1,25; HR efektów losowych = 1,30, 95% CI: 1,05–1,63). Ponadto trzy badania wykazały istotne korelacje między wskaźnikami PLT a cechami guza, takimi jak rozmiar, liczba ognisk i etiologia rozwoju HCC. W grupie leczonej radykalnie (np. resekcja, RFA): małopłytkowość wiązała się z gorszym rokowaniem (HR=1.62), natomiast wśród pacjentów leczonych paliatywnie: niższa liczba płytek była związana była z lepszym rokowaniem (HR= 0.81), choć wynik nie był istotny statystycznie.

Warto jednak zwrócić uwagę na wysoka heterogeniczność wyników (I^2 = 86%) co można wiązać z wpływem m.in. rodzaju leczenia i wartości granicznej PLT.

Komentarz

Według obecnej wiedzy jest to pierwsza analiza ekspresji PD-L1 i CD68 w połączeniu z charakterystyką kliniczną pacjentów chorujących na raka wątrobowokomórkowego w populacji Europy Środkowej, która obejmowała pacjentów po przeszczepieniu wątroby. Dane analizowane w badaniu zostały zebrane od niewyselekcjonowanych, kolejnych pacjentów, co można uznać za zaletę. Badanie ma jednak kilka ograniczeń. Po pierwsze, była to analiza retrospektywna, jednoośrodkowa. Ponadto okres obserwacji różnił się u poszczególnych pacjentów. Warto zauważyć, że nawrót zaobserwowano nawet po długim czasie od przeszczepienia – maksymalnie 87 miesięcy; dlatego nie można wykluczyć, że po dłuższej obserwacji zdiagnozowanych może być więcej przypadków nawrotów. Wielkość próby była ograniczona, co można uznać za kolejne ograniczenie.

Ważnym parametrem ocenianym w badaniu jest częstość występowania ekspresji PD-L1 u pacjentów chorych na HCC. W związku z coraz szerzej stosowaną immunoterapią oraz doniesieniami o jej efektywności w zależności stopnia ekspresji PD-L1 w różnych chorobach nowotworowych, istotne z punktu widzenia klinicznego byłoby określenie, czy może być ona wykorzystana w tym celu również w grupie pacjentów chorujących na HCC. Wyniki obecnego badania sugerują jednak niską wartość predykcyjną ze względu na niską częstość występowania – ok 1% populacji.

Wyniki kilku analiz wśród pacjentów po resekcji wykazały różne rokowanie związane z ekspresją CD68. Zidentyfikowano również dwa badania obejmujące populację po przeszczepieniu, w których analizowano ekspresję CD68. W pierwszym badaniu nie stwierdzono związku pomiędzy komórkami CD68 a przeżyciem całkowitym lub nawrotem choroby.[13] Z drugiej strony, w drugim badaniu Atanasov i wsp. wykazano, że TAM CD68 w centralnym obszarze guza były powiązane z gorszym przeżyciem.[14] W naszej analizie, w populacji ogólnej nie wykazano wpływu ekspresji CD68 na ryzyko nawrotu (*p=0,94*). Jednak w jednoczynnikowej analizie danych pacjentów po przeszczepieniu wątroby był to czynnik negatywny (*p=0,002*).
Niniejsza analiza obejmowała także ocenę podstawowych badań laboratoryjnych, komentarze odnośnie wyników badania w kontekście dostępnej literatury przedstawiono poniżej.

W analizie retrospektywnej wykazano, że u pacjentów z AFP ≥500 ng/ml przed przeszczepem ryzyko zgonu było 1,6-krotnie wyższe w porównaniu do grupy pacjentów z AFP ≤20 ng/ml (*p < 0,001*). Analiza Berry et al. sugeruje, że ocena poziomu AFP może przewidywać rokowanie pacjenta, wykazując, że pacjenci z masą nowotworu przekraczającą kryteria mediolańskie (ognisko raka wątrobowokomórkowego o średnicy równej lub niższej niż 5 cm lub nie więcej niż 3 zmiany o średnicy równej lub niższej niż 3 cm każda) mieli dłuższe przeżycie po przeszczepieniu wątroby, jeśli poziom AFP w surowicy wynosił od 0 do 15 ng/ml (AHR = 0,97, 95% CI = 0,66-1,43), podczas gdy pacjenci spełniający kryteria mediolańskie mieli krótsze przeżycie, jeśli ich poziom AFP w surowicy był znacząco podwyższony (dla poziomu AFP w surowicy ≥ 66 ng/ml, AHR = 1,93, 95% CI = 1,74–2,15).[27] [28] W naszym badaniu wykazano w analizie wieloczynnikowej, że poziom AFP ma istotny wpływ przeżycie całkowite po resekcji, oraz na czas do nawrotu w populacji ogólnej; w pozostałych populacjach nie wykazano istotności statystycznej.

W literaturze naukowej istnieją doniesienia, że na rokowanie pacjentów chorych na HCC może mieć zakażenie wirusami hepatotropowymi.[29] W tej analizie ani HBV, ani HCV nie miały wpływu RFS ani OS.

Innym potencjalnie łatwym do zastosowania czynnikiem prognostycznym może być stosunek neutrofilów do limfocytów (NLR) oznaczony przed przeszczepem lub przed operacją. Jednakże dotąd dostępne dane wykazywały niejednoznaczne wyniki. W przeglądzie systematycznym Najjar et al. podwyższony wskaźnik NLR wiązano z gorszym OS po LT w przypadku HCC w 8 z 13 badań, przy czym zgłaszane wskaźniki OS w ciągu 5 lat wahały się od 20% do 62% w grupie z wysokim NLR w porównaniu z 62% do 84% w grupie o niskim NLR. Z drugiej strony, w tej samej analizie, poziomy NLR przed przeszczepem wydawały się silnie powiązane z RFS. Badacze w 11 z 13 badań doszli do wniosku, że wysoki przedoperacyjny NLR był czynnikiem predykcyjnym krótszego RFS po LT, przy HR i 95% CI w zakresie od 1,088 CI: 1,029–1,151 do 67 CI: 11–413 (*p<0,05*).[30] Jak wynika z przedstawionych w obecnej publikacji wyników w analizie wieloczynnikowej dotyczącej przeżycia całkowitego w populacji pacjentów po przeszczepie wątroby – wskaźnik NLR okazał się pozytywnym czynnikiem prognostycznym.
Szacuje się, że nawrót raka wątrobowokomórkowego po przeszczepieniu wątroby dotyczy aż 16–18% pacjentów. Po resekcji radykalnej odsetek nawrotów jest jeszcze wyższy i waha się w przedziale od 10% w pierwszym roku do 70% po 5 latach. W przedstawionym badaniu retrospektywnym, nawrót po resekcji leczniczej zaobserwowano u 41 z 59 pacjentów (69%), a mediana czasu do nawrotu wynosiła 15 miesięcy. Z drugiej strony liczba pacjentów, u

których wystąpił nawrót po LT, wyniosła 11/52 (21%). Warto zauważyć istotną różnicę pomiędzy częstością nawrotów po resekcji i przeszczepieniu wątroby. Obserwacja ta podkreśla potrzebę optymalnego leczenia i wczesnej diagnostyki HCC.
Istotnym problemem po przeszczepieniu wątroby może być karcynogeneza związana z immunosupresją. Obecnie dostępna jest mała liczba publikacji dotyczących ryzyka rozwoju nowotworu u pacjentów po LT z powodu HCC. Warto zauważyć, że w dużym badaniu skandynawskim zaobserwowano 461 nowotworów u 424 osób z 4246 pacjentów z LT podczas średniego okresu obserwacji wynoszącego 6,6 roku.[31] W przedstawionej analizie zdiagnozowano sześć przypadków nowych nowotworów. Dotyczyło to dwóch przypadków raka podstawnokomórkowego, jednego czerniaka, raka płuc i jajnika z przerzutami oraz jednego przypadku chłoniaka z komórek Mantla. Podkreśla to potrzebę odpowiedniego nadzoru i zwiększonej świadomości na temat potencjalnych zagrożeń dla pacjentów.

Przedstawione badanie ujawniło jednocześnie potrzebę poszukiwania czynników prognostycznych oraz predykcyjnych leczenia systemowego, które mogłyby być oceniane bez dostępnego materiału histopatologicznego a np. na podstawie analizy parametrów surowicy krwi. Mimo przeanalizowania danych wszystkich pacjentów leczonych systemowo w danym ośrodku z powodu HCC, jedynie u kilkunastu osób był dostępny materiał do oznaczeń patomorfologicznych. Wynika to z faktu, że ze względu na możliwość rozpoznania HCC na podstawie obrazu tomografii komputerowej, (przy spełnieniu określonych kryteriów radiologicznych oraz klinicznych) u większości osób kwalifikowanych do leczenia systemowego nie był dostępny materiał tkankowy do analizy patomorfologicznej. W dobie rosnącej roli immunoterapii w leczeniu raka wątrobowokomórkowego oraz wprowadzeniu do praktyki klinicznej coraz większej liczby nowych metod leczenia systemowego istnieje potrzeba identyfikacji wiarygodnych czynników prognostycznych i predykcyjnych, które mogłyby znaleźć zastosowanie w praktyce klinicznej. Ta grupa pacjentów wymaga szczególnej uwagi, gdyż obserwacje z badania ImBrave wskazują, że immunoterapia nie jest tak samo aktywna u wszystkich chorych.[32]

Założenia i cel pracy

Celem pracy było:

1. Opisanie charakterystyki molekularnej chorych na HCC leczonych w UCK WUM.

2. Ocena odległych wyników leczenia oraz poszukiwanie czynników prognostycznych związanych z nawrotem HCC po przeszczepieniu wątroby, resekcji leczniczej lub w grupie pacjentów kwalifikowanych do leczenia systemowego.

3. Ocena możliwości zastosowania określonych oznaczeń molekularnych oraz parametrów laboratoryjnych jako czynników prognostycznych u pacjentów chorych na HCC.

Metodologia

Kryteriami włączenia do analizy retrospektywnej były: rozpoznanie raka wątrobowokomórkowego, przeszczepienie wątroby lub resekcja lub kwalifikacja do leczenia systemowego, brak innych aktywnych nowotworów w momencie rozpoznania oraz dostępność tkanki nowotworowej do dodatkowych badań patologicznych. Kryteria przeszczepienia wątroby obejmowały kryteria mediolańskie (jedna zmiana < 5 cm; alternatywnie do trzech zmian, każda < 3 cm; brak objawów pozawątrobowych; wykluczenie inwazji naczyń) i UCSF, (jeden guz ≤6,5 cm, maksymalnie trzy guzki, z których największy wynosi ≤ 4,5 cm i całkowita średnica guza ≤ 8 cm)) lub kryteria Up-to-7.

Retrospektywnie przeanalizowano dokumentację medyczną pacjentów chorych na raka wątrobowokomórkowego leczonych w Uniwersyteckim Centrum Klinicznym Warszawskiego Uniwersytetu Medycznego w latach 2010–2022.

Zebrano podstawowe dane demograficzne i kliniczne, w tym laboratoryjne badania krwi. Zaplanowane laboratoryjne badanie krwi objęte analizą wykonano w ciągu 7 dni przed rozpoczęciem leczenia. Skupiono się na określeniu statusu zakażeń wirusami hepatotropowymi, wyjściowego poziomu AFP, liczbie limfocytów, neutrofilów i płytek krwi, punktacji w skali PALBI, albuminie, bilirubinie, punktacji i stopniu ALBI, PLR, NLR a także wielkości guza i liczbie zmian. Ponadto przeanalizowano charakterystykę molekularną nowotworu.

Do oceny patomorfologicznej pobierano próbki tkanek zgodnie ze standardowymi protokołami operacyjnymi. Wszystkie próbki tkanek zostały ponownie ocenione przez

drugiego niezależnego histopatologa, który potwierdził rozpoznanie HCC, zweryfikował ocenę histologiczną oraz oceniał inwazję mikronaczyniową, która została zdefiniowana jako i obecność komórek nowotworowych w świetle naczyń. Następnie oceniano ekspresję PD-L1 i CD68 w tkance nowotworowej.

Ekspresję PD-L1 oceniano w utrwalonych w formalinie i zatopionych w parafinie tkankach przy użyciu jakościowego testu immunohistochemicznego PD-L1 IHC 28-8 pharmDx (PD-L1 IHC 28-8 pharmDx, Dako Agilent). Zastosowano system wizualizacji EnVision FLEX na urządzeniu Autostainer Link 48 zgodnie z instrukcją producenta. Ekspresję PD-L1 stratyfikowano według ≥ 1%, ≥ 5% lub ≥ 10% ekspresji komórek nowotworowych.

Ekspresję CD68 oceniano przy użyciu mysiego przeciwciała monoklonalnego, które rozpoznaje ludzki antygen i znakuje ludzkie monocyty i makrofagi (IR613 CD68, PG-M1, niesprzężone, FLEX RTU, Agilent Technologies). Wszystkie procedury wykonano zgodnie z instrukcją producenta.

Dane dotyczące obserwacji długoterminowych zbierano podczas wywiadów z pacjentami oraz pozyskiwano z wewnętrznego systemu szpitala UCK WUM.

W końcowej analizie oceniono wartość prognostyczną wybranych cech molekularnych guza lub wstępnych danych klinicznych oraz ich wpływ na czas przeżycia całkowitego i czas wolny od nawrotu HCC.

Analizę statystyczną przeprowadzono przy użyciu oprogramowania Statistica firmy StatSoft. Potencjalne czynniki prognostyczne dla OS i RFS oceniano za pomocą modeli regresji proporcjonalnego hazardu Coxa. Po przygotowaniu modelu Coxa wyniki sprawdzano za pomocą testu hazardu proporcjonalności (test PH). Wartości $p<0,05$ uznano za istotne statystycznie.

Wszystkie próbki zostały anonimowo zakodowane zgodnie z lokalnymi wytycznymi etycznymi, zgodnie z wymogami Deklaracji Helsińskiej. Badanie zostało zaakceptowane przez Komisję Bioetyczną Warszawskiego Uniwersytetu Medycznego pod numerem AKBE/154/2021 w dniu 6 września 2021 roku.

Dodatkowo, przeprowadzono systematyczny przegląd literatury i metaanalizę zgodnie z wytycznymi PRISMA. Celem pracy było zbadanie wpływu na czas przeżycia całkowitego pacjentów z rozpoznaniem raka wątrobowokomórkowego (HCC) oraz ocena korelacji liczby płytek z cechami kliniczno-patologicznymi nowotworu. Przeszukano bazy: PubMed, Web of

Science i Scopus, finalnie uwzględniono 26 badań (28 zestawów danych), obejmujących łącznie 9403 pacjentów.

Critical Reviews in Oncology / Hematology 157 (2021) 103179

Contents lists available at ScienceDirect

Critical Reviews in Oncology / Hematology

journal homepage: www.elsevier.com/locate/critrevonc

Milestones in the treatment of hepatocellular carcinoma: A systematic review.

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ARTICLE INFO

Keywords:
hepatocellular carcinoma
liver cancer
systemic treatment
sorafenib
immunotherapy

ABSTRACT

According to data provided by WHO (World Health Organization), hepatocellular carcinoma (HCC) is the fourth most common cause of cancer-related deaths worldwide. Since the approval of sorafenib in 2008, several trials have assessed other particles for the treatment of HCC, but few have proven to be effective. ESMO (European Society for Medical Oncology) guidelines have been changed several times recently. This systematic review aims to describe both successful and failed trials of systemic treatments for HCC. Methods: We examined randomized, phase III trials of first- and second-line treatments in adults, identifying 23 fully-published trials and 2 reported as abstracts. The latest advances in immunotherapy were also briefly discussed. Conclusions: The landscape of HCC treatment has changed significantly in recent years. Several small molecule inhibitors currently form the core of HCC treatment; however, immunotherapy is now emerging as a promising treatment option.

1. Introduction

According to data provided by World Health Organization (WHO), hepatocellular carcinoma (HCC) is the fourth most common cause of cancer-related deaths worldwide (Globoscan, 2020). Although HCC incidence varies with geographical region, it is predicted to increase in most Western countries. The highest incidence is currently observed in Asia and Africa, where it is associated with viral hepatitis B and C infections (Ferlay et al., 2015).

Since the approval of sorafenib in 2008, several trials have assessed other particles for the treatment of HCC, but few have proven to be effective. Analysis of recent studies lead to the 2018 publication of new ESMO (European Society for Medical Oncology) guidelines, updated in 2019 and 2020 (Vogel et al., 2018). Here we review both successfully completed trials and those that failed to confirm effectiveness of particular molecules in HCC treatment, before briefly summarizing trials assessing the use of immunotherapy in this context.

1.1. Current HCC treatment algorithms

HCC patients may be offered various treatment options depending on their clinical presentation and disease category based on the Barcelona Clinic Liver Cancer (BCLC) staging system. According to the BCLC staging system, HCC can be classified into 5 categories: Very early disease (stage 0), with a single tumor < 2cm, preserved liver function (Child-Pugh A), and overall good clinical presentation (performance status [PS] 0); Early disease (stage A) with either a single tumor or no more than 3 nodules < 3cm, partially impaired liver function (Child-Pugh A or B), and PS 0. Intermediate disease (stage B) with multinodular changes, Child-Pugh A-B and PS 0; Advanced disease (stage C), with portal invasion and extrahepatic spread, Child-Pugh A-B, and moderately impaired clinical condition (PS 1-2); and Terminal disease (stage D), with severely impaired liver function (Child-Pugh C) and poor clinical presentation (PS 3-4) (Pons et al., 2005).

For patients with BCLC stage 0 or A, surgical resection, liver transplantation and ablation are primary treatment options. In some cases, use of radiotherapy may be appropriate, including stereotactic body radiation therapy (SBRT), brachyterapy, or selective internal radiation therapy (SIRT). Patients with BCLC stage B may receive curative treatment (surgery or ablation) or if that is not possible, regional palliative treatment, i.e. transarterial chemoembolization (TACE) or, in selective cases, SIRT. When regional treatment is insufficient, the treatment method of choice is systemic therapy. Systemic therapy is a standard of care for patients with BCLC stage C either with the combination of atezolizumab and bevacizumab, or with sorafenib or lenvatinib as the first-line therapeutic, and regorafenib, cabozantinib, or ramucirumab as

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https://doi.org/10.1016/j.critrevonc.2020.103179
Received 16 June 2020; Received in revised form 7 November 2020; Accepted 11 November 2020
Available online 18 November 2020

the second-line therapeutic according to the ESMO guidelines (Vogel et al., 2018). Several recent changes to treatment recommendations for BCLC stage C patients include immunotherapy. Patients with BCLC stage D are recommended to receive best supportive care (Vogel et al., 2018).

HCC is a neoplasm whose growth depends on pathological vascularization. Cancer cell proliferation and neoangiogenesis are influenced by many factors, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF). These promote angiogenesis and cellular proliferation through numerous intracellular signaling pathways, including RAS (rat sarcoma) / RAF (rapidly accelerated fibrosarcoma) / MEK (mitogen-activated protein/extracellular signal-regulated kinase kinase) / ERK (extracellular signal-regulated kinases), PI3K/ AKT (phosphatidylinositol 3-kinase and protein kinase B) / mTOR (mammalian target of rapamycin), WnT (Wingless and Int-1) / B-catenin, the Hedgehog pathway and the c-MET (mesenchymal-epithelial transition factor) signaling pathway. Contemporary systemic treatments affect the signaling pathways in neoplastic cells by inhibiting tyrosine kinases associated with appropriate receptors (RTK, receptor tyrosine kinase).

2. Methods

This systematic review aims to describe both successful and failed trials of systemic treatments for HCC. The manuscript was prepared according to the PRISMA guidelines.

We examined randomized, phase III trials in the first- and second-line treatment of adults. As sorafenib became the standard of care since its approval in 2008, earlier trials were excluded. Only full publications, in English, were eligible.

2.1. Search methods

Two independent reviewers conducted literature searches in the Scopus and PubMed databases. Search terms were: 'hepatocellular carcinoma' OR 'hepatocellular cancer' AND 'phase III' OR 'phase 3' AND 'randomized'. Additional filters excluded trials published before 2008 and not written in English.

2.2. Study selection

The analysis included only prospective, randomized phase III trials that were published in full. Selected studies were assessing systemic treatments for advanced/unresectable HCC, without any additional treatment modalities. Trials with (neo)adjuvant treatment were excluded. Data regarding overall survival (OS), progression-free survival (PFS)/time to progression (TTP) and toxicity of the treatment had to be available. In any cases of discrepancies or uncertainties, discussion or consultation with a third reviewer was implemented to resolve it. Inclusion and exclusion criteria are summarized in Table 1.

Table 1
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Randomized controlled trial	Reviews
Phase III	Case reports
Advanced/unresectable HCC	Other than phase III RCT, i.e. phase I, II, IV
Adults (>18 y.o.)	
Available data regarding OS, PFS/TTP	Combination with TACE, ablations, radiotherapy/ adjuvant treatment
Available data regarding toxicity	
Available full paper	

OS: overall survival; PFS: progression free survival; TTP: time to progression; RCT: randomized controlled trial; TACE: transarterial chemoemobilization.

3. Results

Two independent reviewers conducted literature searches using the Scopus and PubMed databases on the 20th of April 2020. The initial search yielded 703 records. After excluding duplicates, 552 papers remained. All papers were screened for eligibility criteria by title and abstract. All irrelevant papers were excluded, e.g., those not related to HCC, reviews, non-phase III trials, and those with inappropriate treatment settings etc., leaving 52 potentially relevant papers; among these, 1 was excluded due to lack of desired endpoints, 5 were excluded due to lack of results (only study design) and 23 presented additional analyses of original clinical trials (Fig. 1). Our analysis is based on 23 different randomized clinical trials. Included studies are presented in Tables 2 and 3. The successful trials are described in more detail.

3.1. Sorafenib

Sorafenib is a multikinase inhibitor that decreases tumor cell proliferation and impairs tumor growth by inhibiting angiogenesis. It is approved for the treatment of HCC, renal cell carcinoma and differentiated thyroid carcinoma (Sorafenib SMPC, 2020). Its use in HCC was assessed in two pivotal phase III trials: SHARP (Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol) and the Asia-Pacific trial.

In the SHARP trial, patients with unresectable, advanced HCC and well-preserved liver function (Child-Pugh class A; 95% of cases) were randomly assigned to receive sorafenib (400mg twice daily) or a placebo (Llovet et al., 2008). Primary outcomes included OS and TTP. Median OS was 10.7 months in the sorafenib group and 7.9 months in the placebo group (hazard ratio [HR] in the sorafenib group, 0.69; 95% confidence interval [CI], 0.55-0.87; $P<0.001$), without any significant difference between the two groups in the median TTP (4.1 months vs 4.9 months, respectively, $P=0.77$).

In the Asia-Pacific trial (Cheng et al., 2009), patients with HCC and Child-Pugh class A liver function, who had received no previous systemic treatment were randomly assigned to receive sorafenib (400mg twice daily) or a placebo, in 6-week cycles. Median OS was 6.5 months (95% CI, 5.56-7.56) in the sorafenib group and 4.2 months (3.75-5.46) in the placebo group (HR 0.68, 95% CI, 0.50-0.93; $P=0.014$). Median TTP was 2.8 months (2.63-3.58) in the sorafenib group, and 1.4 months (1.35-1.55) in the placebo group (HR 0.57 [0.42-0.79]; $P=0.0005$).

The most common grade 3-4 adverse events in the sorafenib group were hand-foot reaction, diarrhea and fatigue, while the most common adverse events across all grades included above mentioned adverse events as well as alopecia, rash and hypertension (Cheng et al., 2009). These side effects may correlate with a better response to treatment (Reig et al., 2014; Koschny et al., 2013). The most serious adverse events reported during sorafenib treatment were myocardial infarction, gastrointestinal tract perforation, and bleeding.

The combined results of these 2 trials led to the approval of sorafenib as a first-line systemic treatment for patients with advanced HCC.

3.2. Lenvatinib

Lenvatinib is another tyrosine kinase inhibitor approved for the treatment of HCC and differentiated thyroid carcinoma. It selectively inhibits factors associated with angiogenesis: vascular endothelial growth factor (VEGF) receptors VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4); fibroblast growth factor (FGF) receptors FGFR1, 2, 3 and 4; the platelet derived growth factor (PDGF) receptor PDGFR and KIT; and RET (Lenvatinib SMPC, 2020).

Lenvatinib was evaluated for HCC treatment in the phase III REFLECT trial. Almost 1000 patients with unresectable HCC were randomly assigned to receive either lenvatinib (12mg [body weight >60kg] or 8mg [body weight <60kg]) once daily, orally) or sorafenib (400mg twice daily). The primary endpoint was OS. Median OS in the lenvatinib group was 13.6 months vs 12.3 in the control (sorafenib)

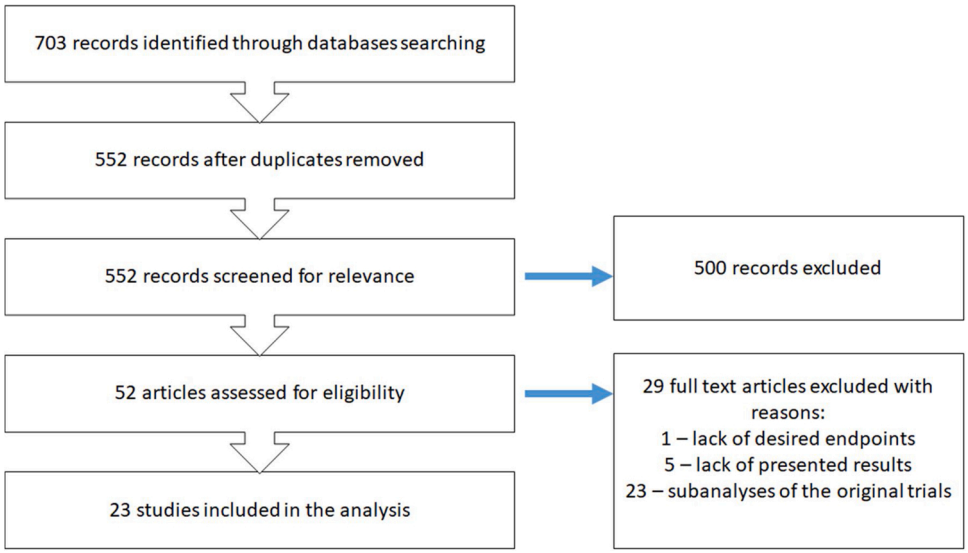

Fig. 1. Preferred reporting items for systematic reviews and meta-analyses flowchart showing study selection.

group (HR=0.92; 95% CI, 0.79-1.06), which met the non-inferiority criteria. Progression free survival (PFS) was significantly higher in the lenvatinib group at 7.3 months (5.6-7.5) vs sorafenib at 3.6 months (3.6-3.7) (HR 0.64 [0.55-0.75]; P<0.00001). Overall response rate (ORR) was higher for Lenvatinib at 18% vs sorafenib at 6.5%.

Hypertension (42%), diarrhea (39%) and decreased appetite (34%) were the most commonly reported adverse events of any grade, followed by fatigue and weight loss (both ca 30%). The most commonly reported grade 3 and 4 adverse events included: hypertension (23%), decreased weight (8%) and increased bilirubin (7%).

This study confirmed non-inferiority of lenvatinib in relation to sorafenib (Kudo et al., 2018).

3.3. Regorafenib

Regorafenib is a multitarget inhibitor approved for the treatment of metastatic colorectal cancer, gastrointestinal tumors, and advanced HCC. It may block several kinases that play important roles in oncogenesis (KIT, RET, RAF-1, BRAF, BRAFV600E), angiogenesis (VEGFR1, -2, -3, TIE2), metastasis (VEGFR3, PDGFR, FGFR) and tumor immunity (CSF1R) (Regorafenib SMPC, 2020).

A single-arm, phase II trial of regorafenib in patients with HCC after sorafenib failure, showed a median TTP of 4.3 months (95% CI 2.9-13.1) and median OS of 13.8 months (95% CI 9.3-18.3) (Bruix et al., 2013).

Regorafenib was then evaluated in the phase III trial RESORCE (Bruix et al., 2013). Patients with Child-Pugh class A liver function whose disease progressed on sorafenib were randomly assigned to groups receiving best supportive care plus either oral regorafenib 160mg (n=374) or a placebo (n=179) once daily during weeks 1-3 of each 4-week cycle. The primary endpoint was OS. Median OS was 10.6 months (95% CI, 9.1-12.1) for the treatment group vs 7.8 months (6.3-8.8) for the placebo group (HR 0.63; 95% CI, 0.50-0.79; one-sided P<0.0001).

The most common grade 3 and 4 adverse events were hypertension (15%), hand-foot skin reaction (13%), fatigue (9%) and diarrhea (3%). The most common adverse events across all grades included palmar-plantar erythrodysaesthesia syndrome, hypertension, decreased appetite, diarrhea and fatigue.

3.4. Cabozantinib

Cabozantinib is a multitarget inhibitor approved for renal cell carcinoma and HCC treatment. It inhibits MET (hepatocyte growth factor receptor protein) and VEGF (vascular endothelial growth factor) receptors and several other kinases involved in tumor growth, metastasis formation, and drug resistance, including AXL, RET, ROS1, TYRO3, MER, the stem cell factor receptor KIT, TRKB, Fms-like tyrosine kinase-3 (FLT3), and TIE (Cabonaztinib SMPC, 2020).

Cabozantinib was assessed in a phase II discontinuation trial, with 41 patients. Patients had a 12-week lead-in treatment period (cabozantinib 100mg/daily) followed by restaging. Those with stabilization were randomly assigned to receive cabozantinib or a placebo, whereas patients with partial responses received cabozantinib (open-label), and progressors discontinued treatment. At week 12, ORR was 5% and disease control rate (DCR) was 66%. Median OS was 11.5 months and median PFS was 5.2 months (Kelley et al., 2017; Schoffski et al., 2017).

The subsequent phase III CELESTIAL trial enrolled patients with HCC after sorafenib treatment failure. Patients were randomly assigned to receive 60mg of cabozantinib (n=470) daily or a placebo (n=237) in a double-blinded manner. Median OS was 10.2 months for the cabozantinib group vs 8 months in the placebo group (HR for death 0.76; 95% CI, 0.63-0.92; P=0.005). Patients that had previously received sorafenib treatment fared slightly better (median OS 11.3 months vs 7.2 months; HR for death 0.70; 95% CI, 0.55-0.88). Median PFS was 5.2 months in the cabozantinib group vs 1.9 months in the placebo group (HR 0.44; 95% CI, 0.36-0.52; P<0.001) (Abou-Alfa et al., 2018a).

The safety profile was consistent with previous trials. The most common grade 3 and 4 adverse events were hand-foot syndrome reaction (17%) and hypertension (16%). Other frequently reported adverse events of any grade included diarrhea, decreased appetite, palmar-plantar erythrodysesthesia and fatigue each affected >40% of patients.

3.5. Ramucirumab

Ramucirumab is approved by (European Medicines Agency) EMA for gastric, colorectal, and non-small-cell lung cancers. It is a monoclonal antibody that interferes with angiogenesis by blocking VEGF-2 receptor binding with VEGF-A,-C and -D. This inhibits further signaling associated with p44/p42 mitogen-activated protein kinases, neutralizing ligand-induced proliferation and human endothelial cell migration (Ramucirumab SMPC, 2020).

In the phase III REACH trial, ramucirumab was administered to patients with advanced HCC who had previously received sorafenib treatment. Results suggested that patients with alpha-fetoprotein ≥400ng/mL or≥ 1.5 the upper limit of normal were more likely to gain clinical benefit from the treatment; in 250 patients with baseline

Table 2
First line treatment trials.

Trial	Design	TTP/PFS [months]	ORR [%]	OS [months]	Grade 3 or 4Toxicities
SHARP NCT00105443 (Llovet et al., 2008)	Sorafenib (n = 299) vs PBO (n = 303)	TTP 5.5 vs 2.8; HR 0.58 (95% CI, 0.45—0.74); P < 0.001, (radiologic) 4.1 vs 4.9 HR 1.08 (95% CI, 0.88- 1.31); P = 0.77 (TTP, symptomatic)	2 vs 1	10.7 vs 7.9; HR 0.69; (95% CI, 0.55-0.87); P<0.001	HSFR 8 vs <1%; Hypertension 2 vs 1%; Abdominal pain 2 vs 1%; Weight loss 2 vs 0%
Asia-Pacific (Cheng et al., 2009)	Sorafenib (n = 150) vs PBO (n = 76)	TTP 2.8 vs 1.4; HR 0.57 (95% CI, 0.42-0.79); P = 0.0005	3.3 vs 1.3	6.5 vs 4.2; HR 0.68 (95% CI, 0.50-0.93); P = 0.014	HSFR 10.7 vs 0%; Diarrhea 6 vs 0%; Fatigue 3.4 vs 1.3%
REFLECT/Study 304 NCT01761266 (Kudo et al., 2018)	Lenvatinib (n = 478) vs Sorafenib (n = 476)	TTP 8.9 vs 3.7; HR 0.63 (95% CI, 0.53-0.73); P<0.0001	24.1 vs 9.2	13.6 vs 12.3; HR 0.92 (95% CI, 0.9-1.06)	Hypertension 23 vs 14%; Weight loss 8 vs 3%; Palmar-plantar erythrodysesthesia syndrome 3 vs 11%; Decreased appetite 5 vs 1%
NCT00241020 (Barbare et al., 2009)	Octreotide LAR (n = 135) vs PBO (n = 137)	PFS 3.37 vs 3.93; (log-rank P = 0.2626, stratified log-rank P = 0.5616)	0 vs 2.9	6.53 vs 7.03; P = 0.34	Diarrhea 0.76 vs 0.75%; Hyperglycemia 6.82 vs 5.26%; Injection-site reaction 1 vs 0%
CALGB 80802 (Alliance) NCT01015833 (Abou-Alfa et al., 2019)	Sorafenib+Doxorubicin (n = 173) vs Sorafenib (n = 173)	TTP 4.7 vs 4.2; HR 0.92 (95% CI, 0.71-1.18); P = 0.49	9.3 vs 5.4	9.3 vs 9.4; HR 1.05 (95% CI, 0.83-1.31); P = .68	Neutropenia 36.8 vs 0.6%; Thrombocytopenia 17.5 vs 2.4%; Fatigue 12. vs 10.1%; Hypertension 4.8 vs 13.6%; HSFR 13.3% vs 14.2%
SEARCH NCT00901901 (Zhu et al., 2015c)	Erlotinib+Sorafenib (n = 362) vs Sorafenib (n = 358)	TTP 3.2 v 4.0; HR 1.135; (95% CI, 0.944-1.366); P = 0.18	6.6 vs 3.9	9.5 v 8.5; HR 0.929 (95%CI, 0.781 - 1.106); P = 0.408	Fatigue 17.7 vs 17.5% ; HSFR 10.2 vs 17.5% ; Ascites 9.7 vs 9.7%; Hypertension 4.7 vs 8.7%; Abdominal pain 6.1 vs 7.1%
LIGHT NCT01009593 (Cainap et al., 2015)	Linifanib (n = 514) vs Sorafenib (n = 521)	TTP 5.4 vs 4.0; HR 0.759 (95% CI, 0.643-0.895); P = 0.001	13 vs 6.9	9.1 vs 9.8; HR 1.046; (95% CI, 0.896-1.221)	Hypertension 20.8 vs 10.6%; Palmar-plantar erythrodysesthesia syndrome 13.7 vs 14.8%; Elevated AST 12.2 vs 12.5%; Diarrhea 12.0 vs 9.2%
BRISK-Fl NCT00858871 (Johnson et al., 2013)	Brivanib (n = 577) vs Sorafenib (n = 578)	TTP 4.2 vs 4.1; HR 1.01 (95%CI, 0.88-1.16); P = 0.8532	12 vs 9	9.5 vs 9.9; HR 1.06; (95% CI, 0.93-1.22)	Hyponatremia 23 vs 9%; Elevated AST 14 vs 17%; Fatigue 15 vs 7%; HFSR 2 vs 15%; Hypertension 13vs 5%
SUN1170 NCT00699374 (Cheng et al., 2013)	Sunitinib (n = 530) vs Sorafenib (n = 544)	TTP 4.1 v 3.8; HR 1.13; One-sided P = 0.8312; Two-sided P = 0.3082	6.6 vs 6.1	7.9 vs 109; HR, 1.30; one-sided P = 0.9990; two-sided P = 0.0014	Thrombocytopenia 29.7 vs 4.7%; Neutropenia 25.7 vs 2.2%; HSFR 13.3 vs 21.2%
EACH trial NCT00471965 (Qin et al., 2013)	FOLFOX4 (n = 184) vs Doxorubicin (n = 187)	PFS 2.93 vs 1.77; HR 0.62 (95% CI, 0.49-0.79); P < 0.001	8.15 vs 2.67	6.40 vs 4.97; HR, 0.80; (95% CI, 0.63-1.02); P = 0.07	Neutropenia 30.60 vs 22.99%; Leukocytopenia 8.74 vs 9.78%; Thrombocytopenia 7.65 vs 6.32%; Anemia 4.91 vs 8.04%; Elevated AST 11.96 vs 12.07%
ISRCTN 64487365 (Dollinger et al., 2010)	Thymostymulin (n = 67) vs PBO (n = 68)	TTP 5.3 vs 2.9; HR 1.13 (95% CI, 0.7-1.8); P = 0.60	33 vs 29	5.0 vs. 5.2; HR 1.04 (95% CI, 0.7-1.6); P = 0.87	Ascites 26 vs 12% ; Renal failure 17 vs 2% ; Pleural effusion 9 vs 1.5%;
PRODIGE 11 NCT01075555 (Jouve et al., 2019)	Sorafenib + Pravastatin (n = 135) vs Sorafenib (n = 134)	TTP 6.1 vs 6.0; HR 1.06 (95% CI, 0.80-1.38); P = 0.698	(-)	10.7 vs 10.5; HR 1.00 (95% CI, 0.79-1.28); P = 0.975	Hepatobiliary disorders 67.1 vs 52.9%; Asthenia 34.2 vs 28.0%; Digestive toxicity 16.1 vs 15.9%
Imbrave150* NCT03434379 (Finn et al., 2020b)	Sorafenib (n = 165) vs Atezolizumab + Bevacizumab (n = 336)	PFS 4.3 vs 6.8; HR, 0.59 (95% 0.47-0.76); P < 0.0001	12 vs 27	13.2 vs NE; HR 0.58 (95% CI, 0.42-0.79); P = 0.006	Any grade 3-4 adverse event 55 vs 57%
CheckMate 459 NCT02576509* (Yau et al., 2019)	Nivolumab (n = 371) vs SOR (n = 372)	3.7 vs 3.8	15 vs 7	16.4 vs 14.7; HR 0.85 (95% CI, 0.72-1.02); P = 0.0752	Any grade 3-4 adverse event 22 vs 49%

PBO: placebo; PFS: progression free survival; TTP: time to progression; OS: overall survival; HR: hazard ratio; CI: confidence interval; HSFR: hand-foot skin reaction.
* only abstracts available.

alpha-fetoprotein ≥400ng/mL (RAM 119; PBO 131), OS HR was 0.67 (95% CI, 0.510.90; p=0.0059) (Zhu et al., 2015a; Zhu et al., 2015b).

The subsequent REACH 2 trial evaluated the use of ramucirumab specifically in patients with elevated alpha-fetoprotein. Almost 300 patients with advanced HCC after sorafenib treatment, with BCLC stage B/C, Child-Pugh class A liver function, and alpha-fetoprotein ≥400ng/mL were randomly assigned to receive ramucirumab (8mg/kg IV every 2 weeks) or a placebo. Median OS was 8.5 months [95% CI, 7.0-10.6] in the ramucirumab group vs 7.3 months [5.4-9.1] in the placebo group (HR 0.710 [95% CI, 0.531-0.949]; P=0.0199). PFS was 2.8 months (2.8-4.1) in the ramucirumab group vs 1.6 months [1.5-2.7] in the placebo group (HR 0.452 [0.339-0.603]; P<0.0001). These results suggest that ramucirumab provides significant clinical benefits in this subgroup of patients.

Safety analysis showed that ramucirumab was generally well tolerated. The most common grade 3 or higher treatment-emergent adverse events were hypertension (13% with ramucirumab vs 5% with placebo), hyponatremia (6% vs 0%) and increased aspartate aminotransferase

Table 3
Second line treatment trials.

Study	Design	TTP/PFS [months]	ORR [%]	OS [months]	Grade 3 or 4 toxicities
CELESTIAL NCT01908426 (Abou-Alfa et al., 2018a)	Cabozantinib (n = 470) vs PBO (n = 237)	PFS 5.2 vs 1.9; HR 0.44 (95% CI, 0.36-0.52); P < 0.001	4 vs <1	10.2 vs 8.0; HR 0.76; (95% CI, 0.63-0.92); P = 0.005.	HSFR 17 vs 0%; Hypertension 16 vs 2%; Elevated AST 12 vs7 %; Fatigue 10 vs 4%; Diarrhea 10 vs 2%
RESORCE NCT01774344 (Bruix et al., 2017)	Regorafenib (n = 379) vs PBO (n = 194)	TTP 3.9 vs 1.5; HR 0.41; (95% CI, 0.34-0.51); One-sided P < 0.0001	11 vs 4	10.6 vs 7.8; HR 0.62 (95% CI, 0.5-0.78); P < 0.0001	Hypertension 15 vs 5%; HFSR 13 vs 1%; Fatigue 9 vs 5%; Diarrhea 3 vs 0%
REACH NCT01140347 (Zhu et al., 2015a; Zhu et al., 2015b)	Ramucirumab (n = 283) vs PBO (n = 282)	TTP 3.5 vs 2.6; HR 0.59 (95% CI, 0.42-0.72); P < 0.001	7 vs <1	9.2 vs 7.6; HR 0.87 (95% CI, 0.72-1.05); P = 0.14	Ascites 5 vs 4%; Hypertension 12 vs 4%; Asthenia 5 vs2%; Malignant neoplasm progression 6 vs 4%; Elevated AST 5 vs 8%; Thrombocytopenia 5 vs <1%; Hyperbilirubinemia 1 vs 5%; Increased blood bilirubin 2 vs 5%
REACH-2 NCT02435433 (Zhu et al., 2019)	Ramucirumab (n = 197) vs PBO (n = 95)	TTP 3 vs 1.6; HR 0.427 (95% CI, 0.313-0.582); P < 0.0001	5 vs 1	8.5 vs 7.3; HR 0.710 (95% CI, 0.531-0.949); P = 0.0199	Hypertension 13 vs 5%; Hyponatremia 11 vs 0%; Elevated AST 3 vs 5%
Keynote 240 NCT02702401 (Finn et al., 2020a)	Pembrolizumab (n = 278) vs PBO (n = 135)	TTP 3.8 vs 2.8; HR 0.688; (95% CI, 0.540-0.877); P = 0.0011	18.3 vs 4.4	13.9 vs 10.6; HR 0.78; (95% CI, 0.611-0.998); P = 0.0238	Elevated AST 13.3 vs 7.5%; Hyperbilirubinemia 7.5 vs 5.2%; Elevated ALT 6.1 vs 3.0%; Anemia 9 vs 3.9%; Diarrhea 2.2 vs 1.4%
BRISK-PS NCT00825955 (Llovet et al., 2013)	Brivanib (n = 263) vs PBO (n = 132)	TTP 4.2 vs 2.7; HR 0.56 (95% CI, 0.42–0.76); P < 0.001	10 vs 2	9.4 vs 8.2; HR, 0.89; (95% CI, 0.69-1.15); P = 0.3307	Hypertension 17 vs 2%; Fatigue 13 vs 1%; Hyponatremia 11 vs 2%; Decreased appetite 10 vs 2%
EVOLVE-1 NCT01035229 (Zhu et al., 2014)	Everolimus (n = 362) vs PBO (n = 184)	TTP 3.0 vs 2.6; HR 0.93 (95% CI, 0.75-1.15)	(not described)	7.6 vs 7.3	Anemia 7.8 vs 3.3%; Asthenia 7.8 vs 5.5%; Decreased appetite 6.1 vs 0.5%
NCT 01287585 (Abou-Alfa et al., 2018b)	ADI-PEG20 (n = 424) vs PBO (n = 211)	PFS 2.6 vs 2.6; HR 1.175 (95% CI, 0.964-1.432); P = 0.075	(not described)	7.8 vs 7.4; HR = 1.02; ((95% CI, 0.847-1.233); P > 0.8	Abdominal pain 4.5 vs 2.4%; Ascites 2.6 vs 3.3%; Peripheral edema 2.4 vs 1.4%
NCT01755767 (Kobayashi et al., 2017)	Tivantinib (n = 226) vs PBO (n = 114)	TTP 2.4 vs 3.0; HR 0.96 (95% CI, 0.74-1.25); P = 0.76	50 vs 50	8.4 vs 8.1; HR, 0.97 (95% CI, 0.75-1.25); P = 0.81	Ascites 7% vs 8%; Anemia 5 vs 6%; Abdominal pain 4 vs 5%; Neutropenia 4 vs 1%
RELIEVE NCT0165569 (Merle et al., 2019)	Doxorubicin nanoparticles 30 mg/sqm (n = 133) or 20 mg/sqm (n = 130) vs standard care (n = 134)	TTP 2.3 vs 2.3; HR 0.96 (95% CI, 0.74-1.23); Two-sided P = 0.74	1 vs 1	9.1vs 9.0; HR 1.00 (95% CI, 0.78-1.28); Two-sided P = 0.99	Neutropenia 10 vs 6%; Asthenia 2% vs 3%; Thrombocytopenia 1% vs 7%; Drug-related death 2 vs 1%
S-CUBE (Kudo et al., 2017)	S1 (n = 223) vs PBO (n = 111)	TTP 2.6 vs 1.4; HR 0.59 (95% CI, 0.46-0.76); P < 0.0001	5 vs 1	11.1 vs 11.2; HR 0.86 (95% CI, 0.67-1.10); P = 0.220	Neutrophil count decreased <14% vs 3%; Lymphocyte count decreased 13 vs <6% White blood cell count decreased 12 vs 1% Platelet count decreased <13 vs 1%

PBO: placebo; PFS: progression free survival; TTP: time to progression; OS: overall survival; HR: hazard ratio; CI: confidence interval; HSFR: hand-foot skin reaction

(3% vs 5%). Three patients in the ramucirumab group died due to treatment-related adverse events: one had acute kidney injury, one had hepatorenal syndrome, and one had renal failure (Zhu et al., 2019). The most common adverse events were fatigue, peripheral oedema, hypertension and decreased appetite, most of which were grade 1-2 and affected 20-30% of patients.

3.6. Pembrolizumab

Pembrolizumab is a humanized monoclonal antibody that blocks interaction of PD-1 (programmed death) receptor with PD-L1 and PD-L2, enhancing the immune system's T-cell response-associated anti-tumor activity (Pembrolizumab SMPC, 2020).

The Keynote 240 (Finn et al., 2020a) trial examined patients with ECOG 0-1 and Child Pugh class A liver function despite advanced HCC, who had previously been treated with sorafenib. Patients were randomly assigned to receive pembrolizumab (200mg every 3 weeks, up to 35 cycles) and best supportive care (n=278) or a placebo with best supportive care (n=135). Primary endpoints included OS and PFS; secondary endpoints were ORR, DOR and treatment safety. The median follow-up was 13.8 months, at which point 10% were still receiving pembrolizumab, and 3% were still receiving the placebo. The median OS in the pembrolizumab group was 13.9 months (95% CI, 11.6-16.0 months) vs 10.6 months (95% CI, 8.3-13.5 months) for the placebo group (HR 0.781; 95% CI, 0.611-0.998; P=0.0238), and the PFS for the pembrolizumab group was 3 months (95% CI, 2.8-4.1) vs 2.8 months (95% CI, 1.6-3); at final analysis (HR 0.718; 95% CI, 0.570-0.904; P=0.0022), those differences did not meet statistical significance. ORR was 18.3% (95% CI, 14.0-23.4%) in the pembrolizumab group and 4.4% (95% CI, 1.6-9.4%) for the placebo group (nominal one-sided P=0.00007). Median DOR in the pembrolizumab group was 13.8 months (1.5-23.6).

Use of pembrolizumab was associated with several adverse events, most commonly increased liver enzymes and blood bilirubin, fatigue, and pruritus (all affected ca 18%). Grade 3 or 4 adverse events were observed in 52.7% of patients receiving pembrolizumab and 46.3% of patients receiving the placebo, most commonly increased AST (13.3%), hyperbilirubinemia (7.5%), and anemia (3.9%).

4. Discussion

After more than a decade of relative stagnation in HCC treatments, rapid changes have occurred in recent years. Several new molecules have been approved, and others are currently under evaluation.

Sorafenib has remained a first-choice treatment for advanced hepatocellular carcinoma despite its limited activity and association with poor tolerance and resistance (Tang et al., 2020). To date there are no reliable, validated predictive biological markers of response to sorafenib, though neutrophil-to-lymphocyte ratio may prove useful (Marisi et al., 2020).

Several studies failed to prove efficacy of their featured molecules in treating advanced HCC. Phase III trials failed to confirm the benefit of brivanib, linifanib, sunitinib or a combination of erlotinib or doxorubicin with sorafenib as first-line treatments. No benefit was seen with ADI-PEG, brivanib, everolimus, s-1, or tivantinib as second-line treatments. Other treatment methods including thymostymulin, octreotide and megestrol also produced no clinically important benefits.

The ability of hepatocytes to detoxify and inactivate exogenous compounds such as drugs, can manifest as resistance to classical chemotherapy. A trial published by Qin et al. compared the FOLFOX chemotherapy protocol (fluorouracil, leucovorin and oxaliplatine) vs doxorubicin: the OS was 6.4 months vs 5 months, but the response rate was only 8% and 2%, respectively (Cheng et al., 2013). The retrospective AGEO study evaluated the GEMOX protocol (gemcitabine with oxaliplatine). ORR was 22% (95% CI, 16-27) and the DCR was 66% (95% CI, 59-72). The median OS was 11 months (95% CI, 9-14), while PFS was 4.5 (95% CI, 4-6). Unfortunately, a major limitation of that study is the lack of a control group (Zaanan et al., 2013).

Immunotherapy is gaining attention in the area of hepatocellular carcinoma therapy. ESMO treatment guidelines were updated several times last year: The first update included pembrolizumab and nivolumab for patients with BCLC stage C disease as first- (nivolumab) or second-line (nivolumab, pembrolizumab) treatments; however, the second update advises that none of these drugs are recommended (Vogel et al., 2018).

There are several limitations of our systematic review. We included only phase III trials, but acknowledged that some phase II trials, such as CheckMate 040 with nivolumab (El-Khoueiry et al., 2017), may also be important; therefore, we briefly mentioned some phase II trials of successful molecules. Results of the 2019 phase III ImBrave150 trial examining atezolizumab with bevacizumab showed very promising results (Finn et al., 2020b). Although not all of the immunotherapy trials have been published as full manuscripts yet, we consider their results very important, so have briefly summarized them below and included them in Table 1.

Nivolumab is a monoclonal antibody that attaches to PD-1 receptors on lymphocytes T, preventing PD-L1- and PD-L2-induced inhibition of those lymphocytes. This in turn results in increased immune response towards cancer. It is already approved to treat patients with many types of cancer and it is currently under investigation in several other cancers (Nivolumab SMPC, 2020).

The phase III CheckMate 459 trial compared nivolumab with sorafenib as a first-line treatment. The trial's 743 patients with advanced HCC were randomly assigned in a 1:1 ratio to receive nivolumab (n=371) or sorafenib (n=372). The primary endpoint was OS; the median OS was 16.4 months for the nivolumab group and14.7 months for the sorafenib group (HR 0.85 [95% CI, 0.72-1.02]; P=0.0752). The ORR was 15% for nivolumab and 7% for sorafenib. Grade 3 or 4 treatment-related adverse events were reported in 22% of patients receiving nivolumab and 49% of patients receiving. Although nivolumab did not achieve statistically significantly improvements in OS, it produced a modest improvement in OS and ORR, with an acceptable safety profile (Yau et al., 2019). Nivolumab continues to be evaluated for HCC treatment in several other clinical settings (Clinicaltrials.gov., 2020a).

Table 4
A selection of current immunotherapy trials examining targeted treatment in combination with immunotherapy.

Trial NCT number	Design	Phase	Trial status
03298451 (Clinicaltrials.gov, 2020b)	Durvalumab + Tremelimumab	3	Active, not recruiting
02519348 (Clinicaltrials.gov, 2020i)	Durvalumab + Tremelimumab or Bevacizumab	2	Active, not recruiting
03482102 (Clinicaltrials.gov, 2020j)	Durvalumab + Tremelimumab + Radiotherapy	2	Recruiting
02821754 (Clinicaltrials.gov, 2020k)	Durvalumab + Tremelimumab + RFA (Radiofrequency ablation) / TACE (Transarterial chemoembolization) /Cryoablation	2	Recruiting
03519997 (Clinicaltrials.gov)	Pembrolizumab + Bavituximab	2	Recruitning
03259867 (Clinicaltrials.gov, 2020m)	Nivolumab/Pembrolizumab + Transarterial Tirapazamine Embolization (TATE)	2	Active, not recruiting
03572582 (Clinicaltrials.gov, 2020n)	Nivolumab + TACE	2	Active, not recruiting
04523662 (Clinicaltrials.gov, 2020o)	Carrelizumab + Apatinib Mesylate+Radiotherapy	2	Not yet recruiting
03222076 (Clinicaltrials.gov, 2020l)	Nivolumab +/- Ipilimumab	2	Active, not recruiting
04183088 (Clinicaltrials.gov., 2020c)	Tislelizumab + Regorafenib	2	Not yet recruiting
03941873 (Clinicaltrials.gov., 2020d)	Sirtavatinib +/- Tislelizumab	1/2	Recruiting
03382886 (Clinicaltrials.gov, 2020p)	Nivolumab + Bevacizumab	1/2	Terminated
02859324 (Clinicaltrials.gov, 2020q)	Nivolumab + CC122	1/2	Completed
03071094 (Clinicaltrials.gov, 2020r)	Nivolumab + PexaVAC (oncolytic virus)	1/2	Active, not recruiting
01658878 (Clinicaltrials.gov, 2020s)	Nivolumab +/- Cabozantinib +/- Ipilimumab	1/2	Active, not recruiting
02325739 (Clinicaltrials.gov, 2020t)	FGF401 +/- PDR001	1/2	Completed
02795429 (Clinicaltrials.gov, 2020u)	PDR001 +/- INC280	1/2	Active, not recruiting
03511222 (Clinicaltrials.gov, 2020v)	Pembrolizumab / Nivolumab + Voloranib	1	Terminated
02705105 (Clinicaltrials.gov, 2020w)	Nivolumab + Mogamulizumab	1	Completed
03418922 (Clinicaltrials.gov., 2020x)	Nivolumab + Lenvatinib	1	Active, not recruiting
03289533 (Clinicaltrials.gov, 2020y)	Avelumab + Axitinib	1	Completed
03006926 (Clinicaltrials.gov, 2020z)	Pembrolizumab + Lenvatinib	1	Active, not recruiting
03347292 (Clinicaltrials.gov, 2020aa)	Pembrolizumab + Regorafenib	1	Active, not recrutinig
02509507 (Clinicaltrials.gov, 2020bb)	Pembrolizumab + Talimogene Laherparepvec (oncolytic virus)	1	Recruiting
02432963 (Clinicaltrials.gov, 2020cc)	Pembrolizumab + p53MVA vaccine	1	Active, not recruiting
02572687 (Clinicaltrials.gov, 2020dd)	Durvalumab + Ramucirumab	1	Active, not recruiting
03539822 (Clinicaltrials.gov, 2020ee)	Durvalumab + Cabozantinib	1	Recruiting
03257761 (Clinicaltrials.gov, 2020ff)	Durvalumab + Guadecitabine	1	Active, not recruiting
02988440 (Clinicaltrials.gov, 2020gg)	PDR001 + Sorafenib	1	Completed

HCC treatment combining various immunotherapies or tyrosine kinase inhibitors is being extensively evaluated in HCC patients. An example is the ImBrave150 (Finn et al., 2020b) trial, which compared the combination of atezolizumab (antiPD-L1) and bevacizumab (anti-VEGF) with sorafenib as a first-line treatment. Over 500 patients were randomly assigned to receive atezolizumab (1200mg IV) with bevacizumab (15mg/kg IV) every 3 weeks or sorafenib (400mg twice daily). In the primary analysis, the HR for OS was 0.58 (95% CI, 0.42-0.79, P=0.0006) after a median follow-up period of 8.6 months. After 1 year, OS was higher in the combined treatment group: 67.2% (95% CI, 61.3-73.1) vs 54.6% (95% CI, 45.2 to 64.0) in the sorafenib group. ORR was 27% for the combined treatment group vs 12% (P<0.0001) in the sorafenib group. Median PFS was also increased (6.8 vs 4.3 months; 0.59 95% CI, 0.47-0.76, P < 0.0001). In the sorafenib group, median OS was 13.2 months, while the OS was not reached in the combined treatment group. The safety profile of the combined treatment was comparable to sorafenib, with grade 3-4 adverse events occurring at a rate of 56.5% vs 55.1% respectively (Finn et al., 2020b). Patient reported outcomes of the combined treatment were presented during the 2020 Gastrointestinal Symposium (Galle et al., 2020) and in the final manuscript published in May 2020 (Finn et al., 2020b). Combined treatment improved the OS and PFS, and importantly, the patients' quality of life. ESMO guidelines now recommend combination treatment as a first line therapy for advanced hepatocellular carcinoma, although this may raise further questions regarding the optimal sequence of treatment. There are no current data regarding the effectiveness of previously used drugs following immunotherapy; some second-line treatment molecules are approved for use after sorafenib failure, which may lead to off-label use or use of sorafenib as a second-line treatment.

Eagerly awaited are the HIMALAYA trial results, comparing the combination of tremelimumab and durvalumab to sorafenib as first-line treatments (Clinicaltrials.gov, bl). The phase III RATIONALE 301 trial is comparing Tislelizumab (anti-PD-1, BGB-A317) with sorafenib as a first-line treatment of unresectable HCC, and is estimated to be completed in 2022 (Qin et al., 2019). Other phase I/II trials are also underway: one evaluating the combination of tislelizumab with regorafenib as first-line systemic therapy for patients with advanced HCC (Clinicaltrials.gov., 2020c), and one investigating sitravatinib as a monotherapy and in combination with the monoclonal antibody tislelizumab in patients with unresectable, locally advanced or metastatic HCC (Clinicaltrials.gov., 2020d). Durvalumab and camrelizumab are monoclonal antibodies targeting PD-1/PD-L1, and are currently in phase III trials for treatment of advanced HCC (Clinicaltrials.gov., 2020f). A brief summary of the most interesting trials is presented in Table 4.

Other studies are taking different approaches to HCC treatment. One example is CAR-T cells therapy, although further investigations are needed to confirm its efficacy (Chen et al., 2018). NK-cells based treatment is another therapy in the early phase of development (Hosseinzadeh et al., 2018). One of the greatest current challenges is developing novel predictive factors of response to treatments.

5. Conclusions

HCC remains a challenge for healthcare professionals. Despite medical advances, the prognosis for patients with advanced disease is poor. However, recent trials may revolutionize the treatment paradigm, especially those regarding immunotherapy. New treatment opportunities will also rouse further questions, regarding qualification for treatment, optimal monitoring etc.

Declaration of Competing Interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

The authors acknowledge Proper Medical Writing for editorial assistance.

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Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.16564

REVIEW ARTICLE

The role of tumor-associated macrophages in hepatocellular carcinoma—from bench to bedside: A review

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Accepted for publication 25 March 2024.

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Declaration of conflict of interest: The authors have no relevant financial or non-financial interests to disclose.
Financial support: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Abstract

Hepatocellular carcinoma is one of the most common cancers worldwide. Despite progress in treatment, recurrence after radical treatment is common, and the prognosis remains poor for patients with advanced disease. Therefore, there is a need to identify prognostic and predictive factors for the response to therapy or more intensive surveillance or treatment. Because the tumor microenvironment plays a crucial role in the development of cancer and metastasis, it is a crucial need to understand processes that are involved in carcinogenesis. Within the microenvironment, several immune cells with different roles are present. One of the most important of these is tumor-associated macrophages. These cells may exert either antitumor or protumor roles. Several studies have suggested that tumor-associated macrophages can be used as prognostic markers. Furthermore, they may be involved in resistance to immunotherapy or systemic treatment. As they play an important role in cancer development, tumor-associated macrophages are also a good target for therapy. In this review, we briefly summarize recent progress on knowledge regarding the basic molecular characteristics, impact on prognosis and potential clinical implications of tumor-associated macrophages in hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers diagnosed worldwide.[1] Despite progress in systemic treatment, the prognosis for patients remains poor. According to the Barcelona Clinic Liver Cancer Scale and European Society for Medical Oncology guidelines, different treatment modalities may be applied depending on the disease stage and patient performance.[2] However, even after curative treatment, many patients experience disease recurrence. On the other hand, in advanced disease, the response to treatment varies significantly, and overall survival (OS) remains poor. Therefore, there is a constant need to identify prognostic and predictive factors to better tailor treatment and surveillance to individual tumor biology and patient characteristics. Several factors have already been investigated; however, the findings have been inconsistent.

The tumor microenvironment (TME) plays a vital role in cancer survival and development. Constant injury and hepatocyte regeneration are associated with chronic inflammation. There is a complex interplay among hepatocytes and immune cells that is highly dependent on the TME. The TME consists of the extracellular matrix and tumor cells, fibroblasts, and immune cells with different characteristics. Immune cells include tumor-associated macrophages (TAMs), tumor-infiltrating lymphocytes (TILs), tumor-associated neutrophils (TANs) and bone marrow-derived stem cells. All of these cell types are capable of releasing signaling molecules that exert various effects, such as inducing immune tolerance and angiogenesis, increasing proliferation, or preventing tumor growth and metastasis.[3,4] Several other factors present within the TME, such as growth factors, cytokines, extracellular matrix proteins, and various enzymes, may contribute to or prevent carcinogenesis. Of particular interest are TAMs. TAMs are immune cells that not only are capable of regulating primary tumor growth but also are involved in the antitumor adaptive immune response, tumor angiogenesis, and extracellular matrix remodeling.

This review focuses on TAMs as a major regulator of the TME. We briefly summarize the characteristics of TAMs and their potential use as prognostic and predictive factors in HCC, as well as their potential clinical application as a therapeutic target.

Basic characteristics of TAMs

TAMs are among the most important immune cells within the TME. These terminally differentiated cells are present in all tissues. Kupffer cells—tissue-resident macrophages—are present in the liver, but TAMs may be derived from circulating blood monocytes or bone marrow monocytes as well as from progenitor cells from the yolk sac and fetal liver that are able to proliferate.

TAMs are characterized by the expression of cluster of differentiation (CD)68, a transmembrane glycoprotein that is considered a general macrophage marker. TAMs may exhibit various functional effects and can thus be subdivided into two major classes: M1 and M2 macrophages.

M1 macrophages are vital for proinflammatory and antitumor responses. These cells may be activated by cytokines such as interferon-gamma (IFN-gamma), tumor necrosis factor-alpha (TNF-alpha), and granulocyte-macrophage colony-stimulating factor (GM-CSF) or in response to microbial infection. The production of interleukin (IL)-12 and other proinflammatory cytokines leads to the initiation of a Th-1-dependent immune response. M1 macrophages are also capable of cytotoxic activity against cancer cells.[5]

M2 macrophages are associated with tumor progression and cancer development and are more commonly observed in HCC tissue than M1 macrophages. M2 macrophage markers include CD163, CD204, CD206, and MARCO, which are scavenger receptors.[6] M2 TAMs may be activated by ILs 4, 10, or 13 or by macrophage colony-stimulating factor (M-CSF) and glucocorticoid hormones. M2-like macrophages present different functional phenotypes and are divided into four other types.[7,8] M2a macrophages express CD206, CD209, and Dectin-1 and are involved in tissue repair and cell growth or endocytic processes. Furthermore, it was observed that M2a macrophages may promote tumor progression in human by vascular endothelial growth factor (VEGF) and chemokine (C–C motif) ligand 18 (CCL18) secretion, which results in angiogenesis induction and promotion of tumor cell migration and invasion. M2a cells produce IL-10, CCL17, CCL18, CCL22, and the amino-acid catabolizing enzyme Arg1[9]while M2b macrophages regulate the immune response and release either pro- or anti-inflammatory cytokines, such as TNF-alpha, IL-1-beta, IL-6, and IL-10. M2c macrophages, called inactivated macrophages, are involved not only in the phagocytosis of apoptotic cells but also in immunoregulation through the suppression of M1-dependent inflammation. Several studies suggested that M2c macrophages enhance tumor progression. This subtype presents CD163, Mer tyrosine kinase (MerTK), and Tie2 expression as well as low to medium levels of CD14, CD86, CD16, and CD206. These cells are capable of pro-inflammatory cytokines IL-10 and TGF-beta as well as the chemokines CCL16, CCL18, and CXCL13 secretion.[9] Finally, M2d macrophages, characterized by a CD14-high CD163-high TGF-beta-high CD86-low phenotype, produce high levels of CCL18 and enhance tumor growth and angiogenesis.[10-] TAMs may secrete proangiogenic cytokines such as VEGF, tumor growth factor (TGF)-beta or platelet-derived growth factor, which contribute to tumor progression.[11] Promotion of tumor progression by M2 macrophages is associated with the suppression of effector T cells and increased recruitment of regulatory T cells. This mechanism is driven by the secretion of cytokines such as IL-10 and TGF-beta or chemokines such as CCL17, CCL18, CCL22, and CCL24.[12] A comparison of M1 and M2 TAMs is summarized in Table 1 Of note, CD169 macrophages are distinct population that are present in various tissues, spleen, or lymph nodes. It is suggested that those cells are involved in antitumor immunity as well as other process such as phagocytosis, antigen presentation, and immune tolerance.[13] In vitro studies showed that CD169 cells

Table 1 Basic characteristics of tumor-associated macrophages[5–11]

	Tumor-associated macrophages CD68		
Type	Molecular markers	Role	Examples of mechanism
M1	CD80, CD86, HLA-DR, iNOS	Pro-inflammatory response Antitumoral role Cytotoxic activity against cancer cells	Inducible nitric oxide synthase (iNOS), interleukin (IL)-1beta, tumor necrosis factor (TNF)-alpha secretion.
M2			
A	CD206-high, CD209-high, dectin-1-high, CD163-low–medium, CD86-low, CD14-low–medium, IL-1R	Tissue repairing Metastasis Invasion Tumor cell proliferation	VEGF, CCL18 secretion: angiogenesis, migration. IL-4/STAT6 signaling pathway: involved in tumor cell proliferation.
B	CD163-low, CD86-medium–high, MHCIIhigh, CD14-medium	Tumor progression	CCL1/CCR8 axis: high levels of CCL1 attract CCR8 expressing Th2 and Treg cells, promoting an immunosuppressive microenvironment. IL-10: favors the differentiation of naïve T cells into Treg cells. IL-6: activation of Th2 cells, IL-1.
C	CD163-high, CD14-medium, CD206-low–medium, CD86-medium, MerTK-medium–high, CD16, TLR1, TLR8	Phagocytosis of apoptotic cells	IL-10, TGF-beta, CCL16, CCL18, CXCL13 secretion: pro-inflammatory.
D	CD163-high, CD86-low, CD14-high	Angiogenesis Metastasis	IL-10, TGF-beta secretion. VEGF, MMP9: angiogenesis induction and degradation of the extracellular matrix, facilitating tumor metastasis. Expression of several molecules associated with immune suppression such as the IDO, IL10, Siglec 15, and PD-1 ligands.

significantly enhanced the proliferation, cytotoxicity, and cytokine production capacity of CD8 T cells. It was proven that CD68CD169 are correlated with improved prognosis in HCC patients.[14,15] Characteristics of TAMs is presented in Figure 1.

TAMs as a prognostic factor in HCC

Recently, it was suggested that CD68 macrophages may be used as a prognostic factor in HCC. However, the results of the various studies are inconsistent. In a meta-analysis of 20 observational studies with a total of 4297 patients, TAMs were suggested to be independent predictive indicators and therapeutic targets for HCC, with different effects observed for different subtypes of TAMs. A high density of CD68 TAMs in either the intratumor (IT) (pooled hazard ratio [HR] = 1.417; 95% cofidence interval [CI] = 1.092–1.839; P = 0.009) or peritumor (PT) (pooled HR = 1.393; 95% CI = 1.022–1.899; P = 0.036) region was associated with poor OS. The authors found that a high density of CD68 TAMs in the IT region was also associated with a high AFP level, a large tumor size, the absence of encapsulation, the presence of vascular invasion, and a higher tumor-node-metastasis (TNM) stage. Furthermore, a high density of CD163 macrophages in serum was associated with poor OS (pooled HR = 5.698; 95% CI = 3.062–10.603; $P < 0.001$). Additionally, a high density of CD204 TAMs in the IT region was associated with poor OS (pooled HR = 1.947; 95% CI = 1.387–2.733; $P < 0.001$), and a high density of CD206 TAMs in the IT region was associated with poor OS (pooled HR = 1.723; 95% CI = 1.308–2.270; $P < 0.001$) and disease-free survival (pooled HR = 1.711; 95% CI = 1.214–2.412; P = 0.002). On the other hand, a high density of CD169 TAMs in the IT region was associated with favorable OS (pooled HR = 0.471; 95% CI = 0.343–0.647; P = 0.037).[16]

Of note, the use of TAMs within the TME as a single biomarker may not be sufficient to fully elucidate their role. Furthermore, several other factors should be taken into account when assessing the use of TAMs as a prognostic factor.

First, different results may be observed when CD68 expression is analyzed separately and when the ratio of CD68 expression to that of additional factors is evaluated. Minami *et al*. suggested that there was no significant difference in OS based on a low *versus* a high density of CD8 cells. However, in patients with a median tumor size of ≥3.5 cm and in patients with poorly differentiated HCC, an increase in the density of CD68-positive cells was observed. Furthermore, when survival was analyzed based on the CD163/CD68 ratio with a cutoff value of 1, a decrease in the OS time but not the recurrence-free survival (RFS) time was observed; specifically, for the lower ratio group, the median survival time was significantly shorter compared with the higher ratio group (84.2 *vs* 72.3 months; P = 0.046).[17] It is worth mentioning that CD163 macrophages are considered M2-polarized cells.

The TME also consists of other cells, such as TILs, CD4+ regulatory lymphocytes, or CD8 cytotoxic lymphocytes. Regulatory lymphocytes may promote immune tolerance and inhibit the antitumor roles of other cells, such as CD8 cells. On the other hand, cytotoxic lymphocytes prevent tumor progression by killing cancer cells. Low CD8 cell counts are associated with poorer survival. A higher CD68/CD8 ratio is associated with a poor prognosis.[18] This suggests that not only the presence of particular immune cells but also the imbalance between various cell subsets plays a crucial role. The importance of interactions among immune cells was also shown in another study, which suggested that CD74 could be a marker for favorable prognosis in HCC. Most CD74 was localized on CD68 cells, and blocking CD74 expression attenuated the antitumor activity and proliferation ability of CD8 cytotoxic T lymphocytes. Subsequently, transcriptomic profiling of tumors with CD74 high-expression tumors using the TCGA database showed that multiple immunostimulatory cytokines were upregulated in CD74 high-expression tumors compared with CD74 low-expression tumors.[19]

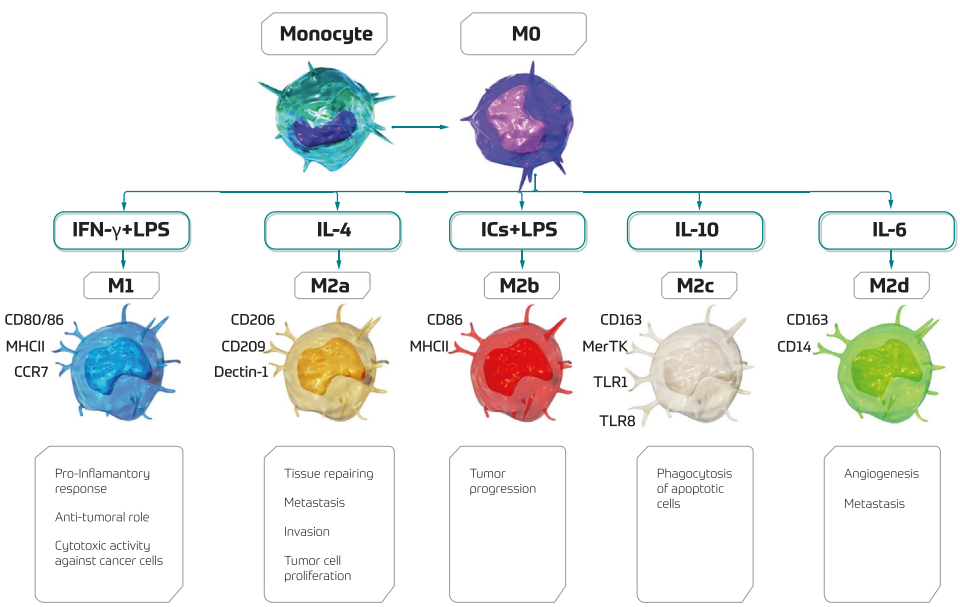

Figure 1 Characteristics of tumor-associated macrophages. IFN, interferon; IL, interleukin.

As already mentioned, the CD68 staining alone may not be sufficient to distinguish the roles of cells. For example, Li *et al*. assessed the densities of CD204CD68 and CD169CD68 cells and observed that the former population was associated with a negative impact but the latter population was associated with a positive impact on OS.[14] CD204 is a phagocytic pattern recognition receptor that is expressed primarily on myeloid lineage cells, may have a protumor function and has been shown to be associated with poor prognosis.[20] In contrast, CD169 may exert an antitumor effect although its role is not fully understood.[13] Similarly, Lam *et al*. observed that CD38 was frequently coexpressed with the macrophage-specific marker CD68. The CD38CD68 macrophage density was associated with improved prognosis after surgery, while the total CD68 macrophage density was associated with poor prognosis,. CD38 is involved in the adenosine pathway, which participates in one of the immunosuppressive mechanisms in cancer. However, CD38 was upregulated on M1 macrophages with pro-inflammatory cytokine profile, which may contribute to the antitumor response. CD38 is associated with IL-6 and TNF-alpha secretion, minimal IL-35 secretion and no secretion of IL-10 and IL-12 cytokines.[21]Furthermore, there is interplay among CD68 TAMs, programmed death-ligand 1 (PD-L1) expression, and the immune response within the TME. Recently, it has been suggested that CD68 M1 TAMs are associated with the induction of PD-L1 in HCC cells, suggesting that they play a protumor role.[22] When PD-L1 expression in HCC was assessed together with CD68 expression, survival analysis showed that the expression of PD-L1 on tumor cells was correlated with tumor progression, whereas the expression of PD-L1 on macrophages had a role in protecting the prognosis of patients with HCC. Moreover, CD68PD-L1 cells were found to be associated with an activated immune microenvironment with high CD8 T-cell infiltration.[23]

Itoh *et al*. analyzed the association between PET/CT activity and outcomes. Patients with a high maximum standardized uptake value (SUVmax) on 18F-FDG PET/CT showed a significantly worse RFS (HR: 1.500; 95% CI: 1.088–2.069; $P = 0.0133$) and OS (HR: 2.259; 95% CI: 1.276–4.000; $P = 0.0052$). Of note, a high SUV was also associated with a PD-L1-positive phenotype (odds ratio: 4.407; 95% CI: 2.265–8.575; $P < 0.0001$). In addition, higher CD68-positive macrophage counts were associated with significantly higher SUVmax values, whereas patients with HCC with high SUVmax values had lower intratumoral CD8-positive T-cell counts.[24]

TAMs combined with blood parameters as a prognostic factor in HCC

A very interesting approach is combining the assessment of CD68 expression with the assessment of soluble factors. In a study conducted in a population of patients undergoing transcatheter arterial chemoembolization (TACE), soluble PD-L1 (sPD-L1) levels were significantly increased in patients with HCC progression ($P = 0.002$) and death ($P < 0.001$). Patients with higher pretreatment sPD-L1 levels had significantly shorter times to progression (10.50 *vs* 18.25 months, $P = 0.001$) and decreased OS times (16.50 *vs* 28.50 months, $P = 0.003$). Of note, high sPD-L1 levels correlated with increased numbers of Treg cells (FOXP3; $P = 0.026$), macrophages (CD68; $P = 0.014$), and M2 macrophages (CD163; $P = 0.026$) and were also associated with IL-10 levels and HBV DNA loads, which suggested immunosuppression when considered in combination.[25]

In another study, the serum c-reactive protein (CRP) level was suggested to be an indicator of an immunosuppressive TME in HCC. Because the importance of serum CRP in the local immune response at the tumor site was not clear, the authors assessed the correlation between CRP and various immune cell populations, including CD11b myeloid cells, CD68 macrophages, CD15 neutrophils, CD8 T cells, and CD206, CD204, CD163 and CD169 macrophages. The analysis revealed that the densities of CD68 TAMs and CD15 TANs were significantly higher in patients with elevated serum CRP levels than in those with low CRP levels (both $P < 0.0001$). Further analysis of TAM subtypes revealed that serum CRP levels were associated with the densities of CD204 and CD163 macrophages ($P < 0.0001$ and $P = 0.0003$, respectively). Moreover, according to transcriptome data analysis, CRP expression is associated with the expression of myeloid cell infiltration-related genes in HCC tumors. These results suggest that the combination of serum CRP with TAMs or TANs in both the nontumor and intratumor regions could be a powerful criterion for predicting patient prognosis.[26]

It was also hypothesized that the preoperative neutrophil–lymphocyte ratio (NLR) could be a predictor of patient prognosis after liver transplantation and reflect the inflammatory TME. Similarly, an elevated perioperative peripheral blood monocyte count may predict a poor prognosis in patients with HCC after resection. These findings supported a plan for a study in which the authors decided to assess the peripheral neutrophil–monocyte/lymphocyte ratio (NMLR) and intratumoral levels of CD4, CD8, CD16, CD68, and CD16/CD8 for correlation with prognosis after curative HCC resection. The authors concluded that the intratumoral CD16 cell-to-CD8 lymphocyte ratio (iMLR) was indicative of immune imbalance in the local microenvironment and strongly predicted the risk of recurrence and/or poor patient survival. Furthermore, there was a positive correlation between the iMLR and systemic NMLR ($r = 0.138$, $P = 0.027$, and $r = 0.182$, $P = 0.037$, respectively). The authors concluded that the combination of the iMLR and NMLR (low *vs* high) could be used as a predictor for OS and RFS and proved to be better than well-established factors, such as tumor size, differentiation status, vascular invasion status, and BCLC/TNM stage. In the same study, no correlation was observed between patient outcomes and CD68 cells.[27]

TAMs as a predictive factor for systemic treatment

In this era of an increasing role for immunotherapy, there is a need to identify predictive factors. In a recently published study, patients with a high CD38CD68 macrophage density had a longer mOS time than patients with a low CD38CD68 macrophage density (34.43 months compared with 9.66 months) when treated with immune checkpoint inhibitors. The authors suggested that CD38 macrophages produce more interferon gamma and other cytokines, which may contribute to a better response to immunotherapy. Of note, the total CD38 cell proportion and CD38CD68 TAM density predicted responsiveness to immunotherapy more accurately than the PD-L1 score or CD8 T-cell density.[28]

Responsiveness to anti-PD-1 immunotherapy according to TME characteristics was also studied in another recently published study.[29] Authors combined spatial trancriptomics with single-cell RNA sequencing and multiplexed immunofluorescence. As a result, a tumor immune barrier (TIB) structure was identified, as a spatial niche composed of SPP1 macrophages and cancer-associated fibroblasts (CAFs) located near the tumor boundary, which was associated with the efficacy of immune checkpoint blockade. It was suggested that hypoxic microenvironment promotes SPP1 expression, and SPP1 macrophages interact with CAFs to stimulate extracellular matrix remodeling and promote TIB structure formation, thereby limiting immune infiltration in the tumor core. Preclinically, the blockade of SPP1 or macrophage-specific deletion of Spp1 in mice led to enhanced efficacy of anti-PD-1 treatment in mouse liver cancer, accompanied by reduced CAF infiltration and increased cytotoxic T-cell infiltration. Furthermore, it is suggested that TAMs are involved in sorafenib resistance through maintenance of tumor growth and metastasis by secreting hepatocyte growth factor (HGF). This, in turn, may activate the HGF/c-Met, extracellular regulated kinase 1/2/mitogen-activated protein kinase (ERK1/2/MAPK), and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathways in tumor cells. Therefore, an interesting strategy would be to combine sorafenib with an HGF inhibitor such as cabozantinib to improve treatment outcomes.[30]

In another study, a higher expression of the M2 TAM marker CD204 and the cancer stem cell (CSC) markers CD44 and CD133 was observed in patients with sorafenib resistance than in those responding to sorafenib. M2 TAMs could increase the level of CSCs but decrease apoptosis induced by sorafenib. Incubation of HCC cells with coculture-conditioned medium increased the formation of spheres that were resistant to sorafenib. Furthermore, chemokine (C-X-C motif) ligand 1 (CXCL1) and CXCL2 were found to be the potential paracrine factors released by M2 TAMs to increase sorafenib resistance in HCC cells. Treatment with CXCL1 and CXCL2 could increase HCC CSC activity but decrease sorafenib-induced apoptosis by affecting BCL-2 family gene expression. Using pharmacological inhibitors, the authors found that CXCR2/ERK signaling was critical to CXCL1- and CXCL2-mediated sorafenib resistance.[31]Therefore, reducing the number of TAMs in the TME or re-educating TAMs for polarization toward the M1 phenotype could be a valuable approach for future research.

TAMs as a treatment target

As most TAMs within the TME are M2 macrophages that promote tumor development, suppress M1 macrophage functions and adaptive immunity, and favor angiogenesis, TAMs have been suggested to be a potential target for novel therapies. M2 TAMs can be converted into M1 TAMs or their recruitment may be reduced. Other options include increasing the population of M1 TAMs.[32] Some of the most interesting concepts are briefly summarized in Table 2. However, a detailed analysis is beyond the scope of this review. Additionally, Table 3 summarizes clinical trials focused on TME modifications in hepatocellular carcinoma.

Because IL-37 exerts potent anti-inflammatory and immunosuppressive effects and accumulating evidence suggests that IL-37 exerts antitumor effects, it was investigated in HCC as a novel treatment strategy. In a recently published study, HCC patient-derived TAMs were infected with lentiviruses expressing IL-37 (LV-IL-37) and IL-37 siRNA, and the conditioned medium from these TAMs was then used to culture HCC cells. A xenograft mouse model was established by subcutaneous injection of a mixture of HepG2 cells and TAMs/LV-IL-37. HCC patient-derived peripheral blood mononuclear cells showed M2 polarization and decreased IL-37 expression. Furthermore, IL-37 promoted TAM polarization from the M2 to the M1 phenotype by inhibiting IL-6/STAT3 signaling. Moreover, IL-6 upregulation by recombinant human IL-6 (rhIL-6) blocked the IL-37 overexpression-mediated inhibition of HCC cell proliferation, migration, and invasion. In addition, IL-37 overexpression in HCC patient-derived TAMs inhibited tumor growth in vivo.[33]

As TAMs may influence tumor progression, several studies have aimed to neutralize their effects. One approach is to re-educate TAMs by inhibiting the M-CSF receptor with pexidartinib (PLX3397), which inhibits tumor growth and increases the population of CD8 lymphocytes. Colony-stimulating factor-1 (CSF-1) and its receptor CSF-1R regulate the differentiation and function of macrophages and play an important role in macrophage infiltration. Treatment with PLX3397, which is a competitive inhibitor with high specificity for the CSF-1R tyrosine kinase, significantly delayed tumor growth in mouse models. PLX3397 inhibited the proliferation of macrophages in vitro, but intratumoral macrophage infiltration was not decreased by PLX3397 treatment in vivo. Gene expression profiling of TAMs showed that TAMs from PLX3397-treated tumors were polarized toward an M1-like phenotype compared with those from vehicle-treated tumors.[34]

Another agent with proven efficacy is baicalin (8-bromo-7-methoxychrysin, BrMC). In the primary study, liver cancer stem cells and conditioned medium were obtained. TAMs and the effects of BrMC on TAMs were validated by immunofluorescence staining, ELISA, and the Griess assay. The expression of cancer stem cell and macrophage markers was analyzed by western blotting. The results showed that BrMC significantly suppressed the expression of the M2 macrophage marker CD163. Furthermore, BrMC influenced the cytokine secretion profile of TAMs. Mechanistically, BrMC reversed the M2 polarization of TAMs due to inhibition of NF-κB activation.[35]

Inhibition of TAM recruitment has also been suggested as a therapeutic option. This strategy has been tested in studies focusing on chemokine receptor type 2 (CCR2) antagonists and showed promising results. The CCL2/CCR2 axis is involved in the recruitment of monocytes/macrophages and is implicated in various liver pathologies, including HCC. CCL2 is overexpressed in human liver cancers and is a prognostic marker for patients with HCC. It was shown that blockade of CCL2/CCR2 signaling by knockout of CCR2 or treatment with a CCR2 antagonist inhibits malignant growth and metastasis, reduces postsurgical recurrence, and improves survival. Furthermore, therapeutic blockade of the CCL2/CCR2 axis inhibits the recruitment of inflammatory monocytes and the infiltration and M2 polarization of TAMs, resulting in reversal of the immunosuppressed status of the TME and activation of an antitumor CD8 T-cell response.[36]

Because the C–C chemokine ligand 5 (CCL5)–C–C chemokine receptor (CCR5) axis facilitates tumor progression, the CCR5 antagonist maraviroc (MVC) was investigated in HCC.

Table 2 Potential treatment strategies involving TAMs in hepatocellular carcinoma[33–43]

Strategy	Target/regulation pathway	Model	Efficacy/consequences	References
Increased M1 TAMs recruitment	SPON2 SPON2 is a matricellular protein involved in recruiting lymphocytes and initiating immune response as well as cell migration and tumor progression.	Mouse model	SPON2-α4β1 integrin signaling activated RhoA and Rac1, increased F-actin reorganization, and promoted M1-like macrophage recruitment.	43
	Blockade of C–C chemokine ligand 5 (CCL5)–C–C chemokine receptor (CCR5) axis. CCL-5/CCR 5 may facilitate tumor progression.	Mouse model	Maraviroc, the CCR5 antagonist - shifted macrophage polarization toward the M1 phenotype which resulted in increased radiosensitivity and apoptosis of hepatoma cells.	37
Decreased M2 TAMs recruitment	Blockade of CCL2/CCR2. The CCL2/CCR2 axis is essential for recruitment of monocytes/macrophages and is implicated in various aspects of liver pathology.	Mouse model	Blockade of the CCL2/CCR2 axis inhibits the recruitment of inflammatory monocytes, infiltration and M2-polarization of TAMs, reversing the immunosuppression status of the TME and activating of an antitumorous CD8 T cell response.	36
Decreased M2 TAMs polarization	8-bromo-7-methoxychrysin (BrMC) induced reversion of M2 polarization due to inhibition of NF-κB activation.	In vitro	BrMC significantly suppressed the expression of the M2 macrophage marker CD163. BrMC influenced the secretion profile of cytokines of TAMs.	34
Conversion of M2 TAMs into M1	Programmed death receptor-1 (PD-1) blockade combined with specific blockade of vascular endothelial receptor 2 (VEGFR-2).	Mouse model	Dual anti-PD-1/VEGFR-2 therapy reprogrammed the TME by increasing CD8 cytotoxic T cell infiltration and activation, shifting the M1/M2 TAMs ratio, reducing T regulatory cell and chemokine (C–C motif) receptor 2-positive monocyte infiltration.	39
Depletion of M2 TAMs	SOCS3-STAT6-PPAR-γ signaling; ferroptosis mediated by xCT. Pathway involved in phenotypic shifting, and activation of intracellular ferroptosis, associated with GPX4/RRM2 signaling regulation.	Mouse model	xCT-specific knockout in macrophages limits tumorigenicity and metastasis, reduces TAM recruitment and infiltration, inhibits M2-type polarization, activates and enhances ferroptosis activity within TAMs. xCT-mediated macrophage ferroptosis significantly increased PD-L1 expression in macrophages and improved the antitumor efficacy of anti-PD-L1 therapy. xCT expression in tumor tissues, especially in CD68 macrophages, can serve as a reliable factor to predict the prognosis of HCC patients.	40
Small molecule drugs	Blockade of Colony-stimulating factor-1 (CSF-1) and its receptor, CSF-1R. CSF-1/CSF-1R regulates the differentiation and function of macrophages. PLX3397 is a competitive inhibitor with high specificity for CSF-1R tyrosine kinase.	Mouse model	Gene expression profiling of TAMs showed that TAMs from the PLX3397-treated tumors were polarized toward an M1-like phenotype compared with those from vehicle-treated tumors. PLX3397 inhibited tumor growth and increased CD8 lymphocytes whereas CD4 T-cell infiltration was decreased.	34
Biologic treatment	Glypican-3 (GPC3) /CD47 bispecific antibody (biAb). Glypican-3 (GPC3) is a HCC-associated antigen. CD47 is an inhibitory innate immune checkpoint protein.	In vitro/mouse model	Bispecific antibodies that bind to GPC3 and CD47 in HCC, exhibited strong antitumor activity. GPC3/CD47 biAb enhanced Fc-mediated effector functions in dual antigen-expressing HCC cells in vitro, and both macrophages and neutrophils were required for its strong efficacy against xenograft HCC tumors. Blockade of the interaction between CD47 and SIRPa with antibodies targeting CD47 represents a promising strategy to enhance the phagocytic clearance of cancer cells.	41
Lentiviruses	TAMs transfection with lentiviruses expressing interleukin (IL) 37 and IL-37 siRNA	Mouse model	IL-37 promoted TAMs polarization from M2 to M1 subtype through inhibiting the IL-6/STAT3 signaling.	33

Table 3 Clinical trials targeting TAMs for HCC treatment

Clinical trial ID	Phase	Agent/Setting	Comment	Status	References
NCT04123379	II	CCR2/CCR5 inhibitor (BMS-813160) + anti-PD1 mAb (Nivolumab). neoadjuvant treatment.	CCR2 and CCR5 are involved in tumor-associated immunosuppression. CCR2 antagonist shows anti-cancer effects by increasing CD8 T cells via blocking tumor-infiltrating macrophage-mediated immunosuppression.	Active, not recruiting	Study details|Neoadjuvant Nivolumab with CCR2/5-inhibitor or anti-IL-8 for non-small cell lung cancer (NSCLC) or hepatocellular carcinoma (HCC)|ClinicalTrials.gov, accessed 11.12.2023.
NCT04050462	II	BMS-986253 or cabiralizumab (anti-CSF1R) combined with Nivolumab or Nivolumab monotherapy in advanced HCC patients.	BMS-986253 is a monoclonal antibody that neutralizes IL-8 and leads to the decreased recruitment of polymorphonuclear myeloid-derived suppressor cells at the tumor site. Cabiralizumab is a macrophage colony-stimulating factor 1 inhibitor. CSF1 is a chemotactic molecule that stimulates monocyte tumor infiltration and differentiation into M2 macrophages and interacts with its receptor CSF1R. Antibodies against CSF1R (αCSF1R) target M2 macrophages	Active, not recruiting	Study details|Phase 1 Study of SF1126 in combination With Nivolumab in patients with advanced hepatocellular carcinoma| ClinicalTrials.gov, accessed 11.12.2023.
NCT03059147	I/II	Pan-PI3K inhibitor (SF1126) Anti-PD1 mAb (Nivolumab) SF1126 in combination with nivolumab in patients with advanced (unresectable or metastatic) HCC and Child-Pugh A or Child-Pugh B7 cirrhosis.	SF 1126 SF1126 (pan PI3K/BRD4 inhibitor) as single agent or in combination with sorafenib inhibited proliferation, cell cycle, apoptosis, and multiple key enzymes in PI3K/AKT/mTOR and Ras/Raf/MAPK pathway in Hep3B, HepG2, SK-Hep1, and Huh7 HCC cell lines.	Lack of recruitment and sponsor's priority changes	Study details|Phase 1 Study of SF1126 in combination with Nivolumab in patients with advanced hepatocellular carcinoma| ClinicalTrials.gov, accessed 11.12.2023.
NCT02716012	Ib	saRNA; MTL-CEBPA ± sorafenib	MTL-CEBPA is a small activating RNA drug which upregulates gene expression of CEBPA. MTL-CEBPA causes radiologic regression of tumors in 26.7% of patients with HCC with an underlying viral etiology.	Unknown	Study details|First-in- human safety, tolerability and antitumour activity study of MTL-CEBPA in patients with advanced liver cancer|ClinicalTrials.gov, accessed 11.12.2023.
NCT04105335	Ia/Ib	saRNA; MTL-CEBPA Anti-PD1 mAb (pembrolizumab)	The transcription factor CEBPA (CCAAT/enhancer-binding protein alpha) is involved in regulation of liver homeostasis, cell cycle control, proliferation and angiogenesis and it activates the myeloid gene expression program. Myeloid lineage specific genetic ablation of CEBPA has been shown to promote tumor growth by increasing the	ACTIVE, NOT RECRUITING	Study details|A study of MTL-CEBPA in combination with a PD-1 inhibitor in patients with advanced solid tumors (TIMEPOINT)| ClinicalTrials.gov, accessed 11.12.2023.

(Continues)

Table 3 (Continued)

Clinical trial ID	Phase	Agent/Setting	Comment	Status	References
			number and tumor infiltration of myeloid-derived suppressor cells (MDSC) leading to a pro-angiogenic, immune suppressive and pro-tumorigenic environment		
NCT04338685	I	TLR7 agonist (RO7119929) RO7119929 is given orally to participants with unresectable advanced or metastatic primary liver cancers and other solid tumors with predominant liver involvement.	TLR 7 is involved in response to infection and participates in the regulation of the host adaptive immunity. Combination therapy with immune check point inhibitors may be needed to enhance its antitumor activity.	COMPLETED	Study details|A study evaluating safety, pharmacokinetics, pharmacodynamics, and clinical activity of RO7119929 (TLR7 agonist) in participants with unresectable advanced or metastatic hepatocellular carcinoma, biliary tract cancer, or solid tumors with hepatic metastases|ClinicalTrials.gov, accessed 11.12.2024.
NCT02868255		Anti-signal regulatory protein α (SIRPα) antibodies (anti-hSIRPα Ab) Collection of human samples. Samples harvested from HCC and ovarian cancer patients will be used in evaluation of the SIRP-CD47 expression and of the effect of the anti-human Signal Regulatory Protein (hSIRP) Ab on various cellular types from patients and healthy volunteers.	Signal regulatory protein-α (SIRPα), is an inhibitory membrane receptor that interacts with ubiquitous receptor CD47 to control macrophage phagocytosis in the tumor microenvironment. Selective SIRPα blockade stimulated tumor nest T cell recruitment by restoring murine and human macrophage chemokine secretion and increased antitumor T cell responses by promoting tumor-antigen cross-presentation by dendritic cells.	Completed	Study details|Myeloid derived suppressor cells control by signal regulatory protein-alpha: investigation in hepatocellular carcinoma|ClinicalTrials.gov, accessed 11.12.2024
NCT04660929	I	Anti-HER2 CAR-M (CT-0508) in patients with HER2 overexpressing solid tumors Anti-PD1 mAb (Pembrolizumab)	CAR macrophages Active CAR-M overcomes the solid tumor immunosuppressive TME by secreting pro-inflammatory cytokines and chemokines and upregulating the antigen presentation machinery for presenting antigens to T cells. CAR-M has phagocytic activity against solid tumors—similar to M1 macrophages in the TME. The U.S. Food and Drug Administration (FDA) has granted Fast Track designation to CT-0508, a human epidermal growth factor receptor 2 (HER2) targeted chimeric antigen receptor macrophage (CAR-M) for the treatment of patients with solid tumors.	Recruiting	Study details|CAR-macrophages for the treatment of HER2 overexpressing solid tumors|ClinicalTrials.gov, accessed 11.12.2023.

The authors of the study showed that after incubation of macrophages with MVC for 24 h, the levels of M1 cytokines significantly increased, whereas those of the M2 phenotype factors ARG1, TGF-beta-1, and IL-10 decreased, accompanied by the activation of signal transducer and activator of transcription 3 (STAT3) and downregulation of suppressor of cytokine signaling 3 (SOCS3). The macrophages reverted to the M2 phenotype after treatment with a STAT3 inhibitor. The shift in macrophage polarization toward the M1 phenotype increased the radiosensitivity and apoptosis of hepatoma cells. Mice administered a combination of X-irradiation and MVC exhibited a better antitumor effect than those administered either MVC or irradiation alone.[37]

Zoledronic acid treatment enhanced the effects of TACE by inhibiting TAM infiltration and tumor angiogenesis in a rat model. Investigation of the underlying mechanisms demonstrated that TACE combined with zoledronic acid treatment inhibited the infiltration of F4/80-positive TAMs and tumor angiogenesis compared with TACE alone at 14 days following the procedure. Additionally, the combination treatment significantly inhibited the secretion of VEGF.[38]

Because activation of the antitumor immune response using programmed death receptor-1 (PD-1) blockade alone showed benefit in only some patients with HCC, combining PD-1 blockade with antiangiogenic therapies has been investigated and been proven to result in better outcomes. In a rat model, combination therapy with VEGF receptor 2 (VEGFR-2) blockade and anti-PD-1 antibody treatment remodeled the immune microenvironment by increasing CD8 + cytotoxic T-cell infiltration and activation, shifting the M1/M2 ratio of TAMs and reducing regulatory T cell (Treg) and chemokine (C–C motif) receptor 2-positive (CCR2) monocyte infiltration in HCC tissue.[39]

In addition to TAMs and PD-L1 expression, osteopontin is another factor associated with tumor immune escape that has been investigated in HCC. Osteopontin increases chemotactic migration and alternative activation of macrophages and promotes PD-L1 expression in HCC via activation of the CSF1-CSF1R pathway in macrophages. In a mouse model, the numbers of TAMs, as well as the expression levels of M2 macrophage markers and PD-L1, were significantly decreased but the levels of cytokines produced by T helper 1 (Th1) cells were increased in tumor tissues from osteopontin-knockout mice compared with those from control mice. Furthermore, positive associations between osteopontin and PD-L1 expression and between osteopontin expression and TAM infiltration in tumor tissues from patients with HCC were observed. Histological analysis, flow cytometric analysis and ELISA revealed increased $CD8^+$ T-cell infiltration, a reduced TAM population and an improved Th1/Th2 cytokine balance in multiple mouse models of HCC.[40]

Another strategy is the use of bispecific antibodies (biAb). In a study evaluating bispecific antibodies that bind to both GPC3 and CD47 in HCC, promising results were reported. Glypican-3 (GPC3) is a well-characterized HCC-associated antigen, while CD47 is an immune checkpoint protein that promotes the evasion of tumors from immune surveillance. Via both in vitro and in vivo assays, it was found that the GPC3/CD47 biAb exhibits strong antitumor activity preferentially against dual antigen-expressing tumor cells. In hCD47/human signal regulatory protein alpha (hCD47/hSIRPα) humanized mice, the GPC3/CD47 biAb had an extended serum half-life without causing systemic toxicity. Importantly, the GPC3/CD47 biAb enhanced Fc-mediated effector functions in dual antigen-expressing HCC cells in vitro, and both macrophages and neutrophils were required for its strong efficacy against xenograft HCC tumors. Notably, the GPC3/CD47 biAb outperformed monotherapies and a combination therapy with anti-CD47 and anti-GPC3 monoclonal antibodies (mAbs) in a xenograft model of HCC.[41]

XCT is upregulated in many cancers and plays an important role in the survival and growth of tumors, mediating the reverse transport of extracellular L-cystine and intracellular L-glutamic acid for intracellular glutathione biosynthesis and antioxidant defense to balance the increased oxidative stress in tumor cells. XCT was recently investigated in HCC, and it was shown that xCT-specific knockout in macrophages inhibits tumorigenesis and metastasis by reducing TAM recruitment and infiltration, inhibiting M2 polarization, and facilitating ferroptosis in TAMs. xCT-mediated macrophage ferroptosis significantly increases PD-L1 expression in macrophages and improves the antitumor efficacy of anti-PD-L1 therapy. XCT expression in tumor tissues, especially in CD68macrophages, can serve as a reliable factor to predict the prognosis of HCC patients.[42]

Another strategy may be based on increased recruitment of M1 macrophages. For example, the extracellular matrix protein SPON2 is involved in recruiting lymphocytes and initiating immune responses. In the TME, SPON2 not only promotes the infiltration of M1-like macrophages but also inhibits tumor metastasis. Moreover, it was shown that in HCC patients, the SPON2 level correlated positively with prognosis.[43]

Conclusions

TAMs are one of the most important immune cells within the TME. They may be subdivided into various classes that are associated with anti-tumoral or protumoral effects (M1 and M2 macrophages, respectively). Because tumor progression is highly dependent on microenvironment and immune status, TAMs play crucial role in the development of host response or tumor. Therefore there are several areas of interest, where TAMs could change the clinical practice. First, TAMs may be used as prognostic factors, such as CD68, CD163, CD204, CD206, although the conflicting results of various studies highlight the need for better understanding of their complex role and interactions within the TME. Furthermore, several studies suggested TAMs as predictive factors for the response to immunotherapy (i.e. CD38CD68 and anti-PD-1/PD-L1 treatment) or other systemic treatments. Finally, the modification of TAM infiltration within the tumor may be a valuable strategy for future research and treatment. TAM-targeted therapies are being extensively investigated, and some of the trials already presented with promising results. The complexity of the network between immune cells, host, and tumor cells makes that there is still a need for better understanding of the processes within the microenvironment.

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Heliyon 10 (2024) e40228

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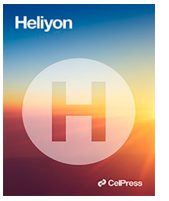

Research article

Prognostic factors for hepatocellular carcinoma recurrence after liver transplantation or resection – single-center experience

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ARTICLE INFO

Keywords:
Liver cancer
Liver transplantation
Liver resection
Tumor microenvironment

ABSTRACT

Introduction: The aim of the study was to assess prognostic factors associated with an increased risk of recurrence of hepatocellular carcinoma (HCC) after radical treatment.
Materials and methods: This is a retrospective, single-center analysis of data on HCC recurrence in patients who underwent radical treatment. Molecular tumor characteristics, baseline laboratory results and hepatic viral status were analyzed.
Results: Data from 111 patients were included in the analysis. The most important prognostic factors for recurrence were vascular microinvasion (HR 4.54; 95 % CI 1.769–11.681; p 0.001), baseline white blood count (HR 2.13; 95 % CI 1.261–3.567; p 0.004) and baseline alpha-fetoprotein (HR 1.00009; 95 % CI 1.000001–1.00002; p 0.034). Microvascular invasion was only prognostic factor which correlate significantly with the overall survival (HR 5.04, 95 % CI 2.352–12.413; $p < 0.001$). PD-L1 expression was confirmed in 4 patients and all of them developed a disease recurrence. However, there was no statistically significant assosciation with prognosis. The presence of CD68 tumor-associated macrophages was confirmed in 62 patients, ranging from 5 % to 40 %. Analysis showed that CD68 was not associated with the risk of recurrence of HCC.
Conclusions: The results confirm that microvascular invasion is the most important factor associated with an increased risk of hepatocellular carcinoma recurrence and death, while PD-L1 and CD68 expression did not have an impact on patient prognosis.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide [1]. Despite several new developments in treatment in recent years, the prognosis for patients remains poor [2,3].

Treatment of hepatocellular carcinoma varies depending on the clinical stage of the disease. According to the European Society for Medical Oncology (ESMO) guidelines [4], patients with BCLC 0-A disease may qualify for liver transplantation, resection, ablation or radiotherapy. Patients with BCLC B disease may also benefit from TACE or systemic treatment when local therapy is not suitable. BCLC

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https://doi.org/10.1016/j.heliyon.2024.e40228
Received 17 March 2024; Received in revised form 1 October 2024; Accepted 6 November 2024
Available online 13 November 2024

C disease is an indication for systemic treatment. Finally, patients with BCLC D should receive the best supportive care.

Although liver transplantation or resection are considered radical options of treatment, some patients develop tumor recurrence. Therefore, there is a need to identify prognostic factors to tailor treatment and oncologic surveillance to patients' needs and tumor characteristics. Furthermore, in the likely upcoming era of adjuvant treatment and immunotherapy, where a good response is observed in some but not all patients, finding predictive factors is crucial.

Recently, it was suggested that the tumor microenvironment plays an essential role in the progression of the disease. One of the most important factors within the microenvironment, are tumor-associated macrophages (TAMs). It is suggested that they could be used as prognostic or predictive factors [5]. Recently published meta-analysis showed that CD68 TAMs are not associated with patients' prognosis [6]. However, several studies have suggested that CD68 expression has negative prognostic value, while some have suggested that it is a positive prognostic factor. Most of the studies analyzing CD68 expression were conducted among patients after curative resection, and only 2 analyzed populations after liver transplantation. The results of one study suggested no impact (n = 206) [7], whereas the second suggested a negative impact (n = 88) [8]. Moreover, it is important to remember, that several other proteins are used as a markers of M1 polarization, such as CD80, CD86 or inducible nitric oxide synthase (iNOS) [9]. Of note, M2 polarized macrophages characterized by CD163 or CD206 expression, are involved tumors progression. M2 macrophages are further subdivided into four subsets (M2a, b,c,d). Each of the types has different characteristics [10]. In the era of immunotherapy, it is important to highlight that a recently published study indicated that CD68 M1 TAMs were associated with the induction of programmed death-ligand 1 (PD-L1) in HCC cells, which suggested their protumor role. When PD-L1 expression in HCC was assessed together with CD68, survival analysis showed that the presence of PD-L1 on tumor cells was correlated with tumor progression, whereas the expression of PD-L1 on macrophages had a protective role in the prognosis of patients with HCC. Moreover, in this study, CD68/PD-L1 cells were associated with an activated immune microenvironment with high CD8 T-cell infiltration [11,12].

PD-L1/PD-1 axis plays an important role in antitumor immunity. The PD-1 receptor may be expressed on immune cells such as T, B or NK (natural killer) lymphocytes, while the PD-L1 ligand may be present on antigen-presenting cells (APCs) or endothelial cells. PD-1/PD-L1 binding leads to the suppression of immune cell activity against the tumor and the development of host tolerance [13]. Overexpression of PD-L1 can be one of the mechanisms used by neoplasms to escape from the host immune system [14]. Several studies have shown that PD-L1 expression may be increased in some cancers and could be associated with worse prognosis [15,16]. According to available data, PD-L1 expression may be associated with an increased risk for a more aggressive disease course, for example, in melanoma or renal cell carcinoma [17,18]. Since anti-PD-1/PD-L1 immunotherapy is currently being investigated in various clinical settings in HCC [19,20] and data regarding the impact of PD-1/PD-L1 expression on patient prognosis are inconsistent, it is valuable to assess the frequency of PD-L1 expression in HCC and to evaluate its prognostic or predictive value [21,22]. According to some results, PD-L1 expression seems not to be associated with patient prognosis in HCC, whereas in other studies, it proved to contribute to worse outcomes [23,24].

The aim of this study was to describe the molecular characteristics of patients with HCC as well as to assess the long-term outcomes of treatment and to search for prognostic factors associated with the recurrence of HCC after liver transplantation, curative resection or qualified systemic treatment.

2. Materials and Methods

We retrospectively screened all medical records of patients with hepatocellular carcinoma treated at a single academic center between 2010 and 2022.

The inclusion criteria were as follows: diagnosis of hepatocellular carcinoma, liver transplantation or curative resection, qualification for systemic therapy, lack of other cancers at the time of diagnosis and availability of cancer tissue for additional pathological testing. Liver transplantation criteria included the Milan criteria (one lesion <5 cm; alternatively, up to three lesions, each <3 cm; no extrahepatic manifestations; no evidence of macrovascular invasion) and University of California San Francisco (UCSF, one tumour ≤ 6.5 cm, three nodules at most with the largest ≤ 4.5 cm and total tumour diameter ≤ 8 cm) criteria or Up-to-7 criteria.

Basic demographic and clinical data, including laboratory blood tests were collected. Laboratory blood test included in the analysis was gathered within one week before the treatment starting date. The focus was on viral infections status, baseline alpha-fetoprotein (AFP), lymphocyte, neutrophil and platelet counts, PALBI score, albumin, bilirubin, ALBI score and grade as well as tumor size and number of lesions. Furthermore, molecular characteristics of tumor was analyzed.

Tissue sampling was performed subsequently after the surgery procedure according to standard protocols. All tissue specimens were reevaluated by an histopathologist who confirmed the diagnosis of HCC and verified histological grading and microvascular invasion. Microvascular invasion was defined as a presence of cancer's cells within the light of vessels. Additionally, CD34 assessment as well as van Gieson or orcein stain were performed. Subsequently, tumor tissue PD-L1 and CD68 expression was assessed.

PD-L1 expression was evaluated in formalin-fixed, paraffin-embedded tissues using a PD-L1 IHC 28-8 pharmDx qualitative immunohistochemical assay (PD-L1 IHC 28-8 pharmDx, Dako Agilent). The EnVision FLEX visualization system on Autostainer Link 48 was used according to the manufacturer's instructions. PD-L1 expression was stratified according to ≥ 1 %, ≥ 5 % or ≥ 10 % tumor cell expression.

CD68 expression was detected using a mouse monoclonal antibody that recognizes human antigen and labels human monocytes and macrophages (IR613 CD68, PG-M1, Unconjugated, FLEX RTU, Agilent Technologies). All procedures were performed according to the manufacturer's instructions.

Data regarding follow-up were gathered during interviews with patients and extracted from the hospital internal system. All incidents of HCC recurrence or new cancer development were reported.

Table 1
Basic characteristics of patient population.

Variable	
Age [median, range]	61,23-84
Biologic sex – male [n]	82
Biologic sex – female [n]	29
Treatment	
Liver transplantation [n]	52
Curative resection [n]	59
Systemic treatment [n]	16
Baseline AFP [median, range]	4467,0.61–251 106
HBV infection/HBsAg	28
HCV infection/aHCV	48
Differentiation grade	
Grade 1	5
Grade 2	81
Grade 3	14
Microvascular invasion	
Yes	36
No	75
Tumor number	
Solitary	62
Multiple	43
Tumor size	
<5 cm	62
>5 cm	44
Tumor PD-L1 expression [n, %]	4, 5–100 %
TILs/TAMs PD-L1 expression [n]	54
CD68 expression [n, %]	62, 5–40 %
Recurrence.[n]	52
Death [n]	45

AFP: alpha-fetoprotein; HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus; aHCV: anti-hepatitis C virus antibodies; PD-L1: programmed death ligand 1; TILs: tumor infiltrating lymphocytes; TAMs: tumor-associated macrophages; CD68: cluster of differentiation 68.

Final analysis assessed the possible prognostic value of the molecular characteristics of the tumor or initial clinical data and their impact on the overall survival and recurrence of HCC.

Statistical analysis was performed using Statistica by StatSoft software. Potential prognostic factors for OS and RFS were evaluated using Cox proportional hazard regression models. After preparing the Cox model, we checked the results using the proportionality hazard test (PH test). P values < 0.05 were considered statistically significant.

All samples were anonymously coded in accordance with local ethical guidelines as requested by the Declaration of Helsinki. The study was acknowledged by the Bioethical Commission of the Medical University of Warsaw under the number AKBE/154/2021 on the September 6, 2021.

3. Results

Over 227 consecutive HCC patients treated in the academic center were screened for tissue available for histopathological assessment. The analysis was performed for the general population and for two subgroups – after liver transplantation or after resection. The third analysis for patients qualifying to systemic treatment was not performed and those patients were excluded also from the general population analysis as the clinical characteristics of those patients is different and the number of patients with available material for histopathologic assessment was too low. Although over 70 patients receiving systemic treatment due to HCC were screened, histopathologic material was available only for 16 patients, which did not allow to perform statistical analysis. Moreover, there was significant heterogeneity between patients in terms of systemic treatment received: 12 patients received sorafenib, 3 received cabozantinib, 1 received gemcitabine with oxaliplatin, and 1 received zoledronic acid; therefore, a separate analysis in the third group was not conducted.

The final analysis included 111 patients meeting inclusion criteria: 52 after liver transplantation, 59 after curative resection.Eighty-two patients were males, and 29 were females, with a median age of 61.7 years. Over 42 % (n = 48) were HCV positive, and 24 % (n = 28) were HBV positive. Most patients had relatively small tumors; lesions of approximately 50 mm or more were detected in 38 % of patients (n = 44).

The median follow-up was 47.95 months, varying from 0.1 month (death due to postsurgical complications) to 138 months. During the time of observation, in the analyzed population 52 cases of recurrence were diagnosed (41/59 patients after resection (69 %) and 11/52 after liver transplantation (21 %) and 45 patients died. The median relapse-free survival (RFS) was 20.5 months, and the time to death after primary treatment in cases of recurrence was 30 months. Fig. 2 presents RFS according to the treatment method.

Analysis was performed in two subgroups: patients after liver transplantation and patients after curative resection. Among patients

Table 2
Tumor recurrence – univariable and multivariable analysis – general population.

	Univariable analysis	
Factor	P value	Hazard ratio
Age	0.819	0.995
Biologic sex	0.462	0.793
HCV infection	0.062	0.581
HBV infection	0.411	0.760
PALBI score	0.065	0.612
PALBI grade	0.015	0.646
AFP baseline	0.001	1.000
Bilirubin baseline	0.032	0.711
Albumin baseline	0.773	0.999
ALBI score	0.185	0.819
ALBI grade	0.135	0.751
NLR	0.075	0.959
WBC	0.115	1.410
PLT	0.013	1.004
PLR	0.890	0.999
Number of tumors	0.383	0.840
Size [mm]	0.001	1.014
Grading	0.002	2.864
Microvascular invasion	0.001	4.865
CD68 expression	0.949	0.870
PD-L1 expression	0.053	6.834
Factor	**Multivariable analysis P value**	**Hazard ratio, (95 % confidence interval)**
HCV	0.469	1.397 (0.564–3.458)
AFP baseline	0.034	1.000 (1.000001–1.00002)
Microvascular invasion	0.001	4.546 (1.769–11.681)
WBC	0.004	2.121 (1.261–3.567)

HBV: hepatitis B virus; HCV: hepatitis C virus; PALBI: platelet–albumin–bilirubin; AFP: alpha-fetoprotein; ALBI: albumin-bilirubin; CD68: cluster of differentiation 68; PD-L1: programmed death ligand 1; NLR: neutrophil-to-lymphocyte ratio; WBC: white blood cells; PLT: platelets; PLR: platelets-to-lymphocytes ratio.

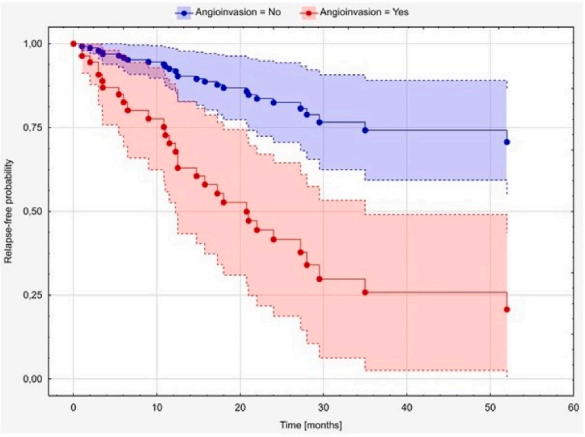

Fig. 1. The impact of microvascular invasion on tumor recurrence.

with disease progression (n = 52), 16 were enrolled in systemic treatment.

The basic characteristics of the patient population are presented in Table 1. After univariable statistical analysis, several factors were defined as prognostic factors for recurrence of hepatocellular carcinoma. The most important negative prognostic factors were tumor size, microvascular invasion and grading, AFP before the treatment, and bilirubin as summarized in Table 2. Other factors were PALBI grade, NLR, WBC and PLT count. PD-L1 expression in tumor tissue was associated with a borderline significant p value of 0.053. In the multivariable analysis, only microvascular invasion (present or absent, Fig. 1), baseline AFP and WBC were strong negative prognostic factors (Table 2).

In the univariable analysis for overall survival in general population, tumor size and microvascular invasion as well as AFP and PLT count were associated with an increased risk for death (Table 3). However, multivariable analysis indicated that only microvascular

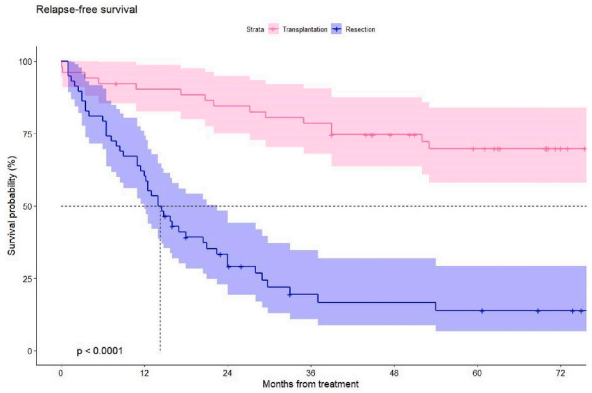

Fig. 2. Relapse free survival according to the treatment method – transplantation vs resection.

Table 3
Overall survival – univariable and multivariable analysis – general population.

Univariable analysis		
Factor	P value	Hazard ratio
Age	0.865	1.003
Biologic sex	0.251	0.678
HCV infection	0.187	0.645
HBV infection	0.861	0.938
PALBI score	0.675	0.886
PALBI grade	0.689	0.922
AFP baseline	<0.001	1.000
Bilirubin baseline	0.625	0.964
Albumin baseline	0.909	0.999
ALBI score	0.266	0.841
ALBI grade	0.208	0.769
NLR	0.075	0.959
WBC	0.115	1.410
PLT	0.013	1.004
PLR	0.890	0.999
Tumor number	0.471	1.162
Tumor size	<0.001	1.014
Grading	0.103	1.832
Microvascular invasion	<0.001	5.234
CD68 expression	0.480	0.120
PD-L1 expression	0.225	3.310
TILs	0.576	0.779
PD-L1 TILs/TAMs	0.713	1.126
Factor	**multivariable analysis P value**	**Hazard ratio (95 % Confidence interval)**
AFP baseline	0.074	1.000 (0.999–1.000)
Microvascular invasion	<0.001	5.404 (2.352–12.413)

HBV: hepatitis B virus; HCV: hepatitis C virus; PALBI: platelet–albumin–bilirubin; AFP: alpha-fetoprotein; ALBI: albumin-bilirubin; CD68: cluster of differentiation 68; PD-L1: programmed death ligand 1; TILs: tumor infiltrating lymphocytes; TAMs: tumor-associated macrophages; NLR: neutrophil-to-lymphocyte ratio; WBC: white blood cells; PLT: platelets; PLR: platelet-lymphocyte ratio.

invasion was a negative prognostic factor (Table 3).

Subgroup analysis showed that among patients after liver transplantation, age, tumor size, grade, microvascular invasion and CD68 expression were negative factors in the univariable analysis. In the multivariable analysis, only microvascular invasion and grading proved statistically significant. Similarly, in the univariable analysis for overall survival, AFP, tumor size, grading and microvascular invasion, PD-L1 expression and NLR were suggested as prognostic factors, while in the multivariable analysis, only microvascular invasion and NLR were associated with statistical significance. Detailed results of subgroup analysis are presented in Tables 4–7.

Among patients after liver resection, only age, AFP and WBC were shown to be prognostic factors for recurrence in the univariable analysis. However, multivariable analysis did not confirm statistical significance. Overall survival analysis suggested AFP, bilirubin and microvascular invasion as prognostic factors. The results of the multivariable analysis also confirmed this finding.

PD-L1 expression was confirmed in 4 samples in the total population. Among patients after liver transplantation, it was observed in 2 patients, and both of them had recurrence. In one case, progression was diagnosed after 87 months, and PD-L1 expression was

Table 4
Tumor recurrence – univariable and multivariable analysis – after tumor resection.

Univariable analysis		
Factor	P value	Hazard ratio
Age	0.076	0.969
Biologic sex	0.918	0.963
HCV infection	0.457	1.305
HBV infection	0.584	0.792
PALBI score	0.309	0.740
PALBI grade	0.108	0.685
Baseline AFP	0.056	1.000
Baseline bilirubin	0.836	0.943
Baseline albumin	0.246	0.999
ALBI score	0.316	0.878
ALBI grade	0.500	0.867
NLR	0.152	0.959
WBC	0.074	1.584
PLT	0.564	1.001
PLR	0.147	0.997
Tumor number	0.139	1.456
Tumor size	0.974	1.000
Grading	0.800	0.873
Vascular microinvasion	0.224	1.598
CD68 expression	0.214	0.033
PD-L1 expression	0.973	1.336
TILs	0.194	0.499
PD-L1 TILs/TAMs	0.720	0.888
Multivariable analysis		
Factor	**P value**	**Hazard ratio (95 % CI)**
Age	0.185	0.968 (0.924–1.015)
Baseline AFP	0.089	1.000 (0.999–1.000)
WBC	0.112	1.566 (0.900–2.726)

HBV: hepatitis B virus; HCV: hepatitis C virus; PALBI: platelet–albumin–bilirubin; AFP: alpha-fetoprotein; ALBI: albumin-bilirubin; CD68: cluster of differentiation 68; PD-L1: programmed death ligand 1; NLR: neutrophil-to-lymphocyte ratio; WBC: white blood cells; PLT: platelets; PLR: platelets-to-lymphocytes ratio.

present in 10 % of cells, while in the second case, recurrence was diagnosed after 10.8 months, and PD-L1 was expressed in 100 % of cells. Furthermore, 2 other cases with PD-L1 expression were reported among patients after resection: the first patient had confirmed expression of PD-L1 in 5 % of tumor cells and died due to recurrent disease 14.75 months after surgery; the other patient had 10 % expression, and 24 months after surgery, recurrence was diagnosed. The patient died 3 months after the recurrence diagnosis. The low number of expressors makes the finding should be interpreted with caution, although in a statistical analysis, it was not associated with a significantly increased risk for recurrence ($p = 0.053$) or death. Only in a univariate analysis was it suggested as a negative prognostic factor in patients after liver transplantation.

PD-L1 expression was also detected on tumor-associated macrophages or tumor-infiltrating lymphocytes in 55 patients: 17 patients after liver transplantation and 31 after resection, including 8 treated with systemic therapy. However, it was not associated with prognosis.

CD68 staining was performed in all tissue samples, and the presence of CD68 tumor-associated macrophages was confirmed in 62 cases, ranging from 5 % to 40 %. Analysis showed that CD68 was not associated with the risk of recurrence of HCC.

Tumor-infiltrating lymphocytes (TILS) were observed in 19 cases. The presence of TILs was not associated with prognosis in either of the subgroups or in the general population.

4. Discussion

To our knowledge, this is the first analysis of PD-L1 and CD68 expression in combination with the clinical characterization of hepatocellular carcinoma patients in a central European population that included patients after liver transplantation. Data analyzed in the study were gathered from unselected, consecutive patients, which may be considered an advantage. However, there are several limitations of the study. First, it was a retrospective, single-center analysis. Furthermore, the follow-up period varied between patients. Notably, recurrence was observed even after a long time after transplantation – 87 months; thus, it cannot be excluded that after longer follow-up, more cases of recurrence could be diagnosed. The sample size was limited, which may be considered another drawback.

This analysis confirmed that well-established factors such as microvascular invasion, grading and baseline AFP level are crucial for prognosis. It also showed that molecular assessment is often impossible and that patients with advanced disease may need other prognostic and predictive factors.

It is estimated that recurrence of hepatocellular carcinoma after liver transplantation affects up to 16–18 % of patients [25,26].

Table 5
Overall survival – univariable and multivariable analysis – after liver resection.

Univariable analysis		
Factor	P value	Hazard ratio
Age	0.800	0.994
Biologic sex	0.818	1.102
HCV infection	0.396	0.653
HBV infection	0.588	1.288
PALBI score	0.989	0.995
PALBI grade	0.675	1.122
Baseline AFP	0.028	1.000
Baseline bilirubin	0.034	1.725
Baseline albumin	0.929	0.999
ALBI score	0.444	0.888
ALBI grade	0.489	0.835
NLR	0.908	0.997
WBC	0.626	0.867
PLT	0.307	1.003
PLR	0.795	1.000
Tumor number	0.281	1.352
Tumor size	0.102	1.009
Grading	0.960	0.973
Vascular microinvasion	0.025	3.171
CD68 expression	0.269	0.007
PD-L1 expression	0.201	69558.428
TILs	0.922	0.947
PD-L1 TILs/TAMs	0.273	1.616
Multivariable analysis		
Factor	**P value**	**Hazard ratio (95 % CI)**
Baseline AFP	0.022	1.000045 (1.000006–1.00008)
Baseline bilirubin	0.0009	3.323 (1.630–6.773)
Vascular microinvasion	0.0107	3.972 (1.374–11.483)

HBV: hepatitis B virus; HCV: hepatitis C virus; PALBI: platelet–albumin–bilirubin; AFP: alpha-fetoprotein; ALBI: albumin-bilirubin; CD68: cluster of differentiation 68; PD-L1: programmed death ligand 1; NLR: neutrophil-to-lymphocyte ratio; WBC: white blood cells; PLT: platelets; PLR: platelets-to-lymphocytes ratio.

After curative resection, the recurrence rate is even higher, ranging from 10 % in the first year to 70 % after 5 years. In this study, recurrence after curative resection was observed in 41 of 59 patients (69 %) within a median time to recurrence of 15 months. On the other hand, in our study, the number of patients with recurrence was 11/52 (21 %) after LT. Most patients experience extrahepatic recurrence with metastases in the lungs, bones or suprarenal glands. Early recurrence could be associated with micrometastases or circulating tumor cells present at the time of hepatectomy and transplantation [27]. It is suggested that late recurrence could be related to engraftment of latent or indolent cancer cells or with the de novo development process, possibly with underlying viral hepatitis [28]. It is worth to highlight significant difference between the recurrence rate after resection and liver transplantation. This finding emphasizes the need for optimal treatment and early diagnosis of HCC.

Among patients after resection, the results of several analyses reported a different outcomes on prognosis associated with CD68 expression [29–34]. Most of them were conducted among patients after curative resection. Two studies were identified that involved the post-transplant population. The first analysis revealed that no association was found between CD68 cells and overall survival or disease recurrence [7]. Conversely, in the second study, Atanasov et al. [8] concluded that CD68 TAMs in the central tumor area were associated with worse survival. In our analysis CD68 expression was not proven to impact the risk for recurrence ($p = 0.94$) in the general analysis. However, in a univariate analysis among patients after liver transplantation, it was a negative factor ($p = 0.002$).

The present analysis included an evaluation of basic laboratory tests. It was shown in a large retrospective analysis that patients with pretransplant AFP $\geq$500 ng/mL had a 1.6-fold higher risk of death than those with AFP $\leq$20 ng/mL ($P < 0.001$). Another analysis suggested that the AFP level may predict patient prognosis, showing that patients with a tumor burden exceeding the Milan criteria had excellent post-transplant survival if their serum AFP level was 0–15 ng/mL (AHR = 0.97, 95 % CI = 0.66–1.43), while patients within the Milan criteria had poor survival if their serum AFP level was substantially elevated (for a serum AFP level $\geq$66 ng/mL, AHR = 1.93, 95 % CI = 1.74–2.15) [35,36]. In our model, it seemed to impact the time to recurrence significantly.

It has been suggested that several factors may have an impact on the prognosis of patients with HCC [37], particularly viral infection. In this analysis, neither HBV nor HCV were associated with RFS or OS.

Another easy-to-apply prognostic marker could be the pretransplant or preoperative neutrophil-to-lymphocyte ratio (NLR). However, available data showed mixed results; in a recent review, elevated NLR was associated with worse OS following LT for HCC in 8 studies out of 13, with reported 5-year OS rates ranging from 20 % to 62 % in the high-NLR group versus 62 %–84 % in the low NLR group. On the other hand, in the same analysis, pretransplant NLR levels seemed strongly associated with RFS; scholars in 11 out of the 13 studies concluded that a high preoperative NLR was predictive of a shorter RFS post-LT, with an HR and 95 % CI ranging from 1.088

Table 6
Tumor recurrence after liver transplantation – univariable and multivariable analysis.

Univariable analysis		
Factor	P value	Hazard ratio
Age	0.026	0.921744
Biologic sex	0.978	1.017951
HCV infection	0.583	0.726728
HBV infection	0.756	1.197612
PALBI score	0.551	1.412263
PALBI grade	0.716	1.154122
Baseline AFP	0.529	1.000060
Baseline bilirubin	0.802	0.973027
Baseline albumin	0.374	0.652554
ALBI score	0.523	1.321183
ALBI grade	0.523	1.330010
NLR	0.161	0.944869
WBC	0.059	2.329669
PLT	0.193	1.004743
PLR	0.925	0.999816
Tumor number	0.772	0.899157
Tumor size	0.001	1.022982
Grading	0.000434	10.649136
Vascular microinvasion	0.000166	11.638938
CD68 expression	0.029	16995.642133
PD-L1 tumor	0.246	42988.853032
TILs	0.667	1.333544
PDL1 TILs/TAMs	0.765	1.190692
Multivariable analysis		
Factor	**P value**	**Hazard ratio (95 % CI)**
Age	0.971	0.998 (0.928–1.073)
Grading	0.002	12.183 (2.434–60.965)
Vascular microinvasion	0.004	9.373 (2.006–43.785)

HBV: hepatitis B virus; HCV: hepatitis C virus; PALBI: platelet–albumin–bilirubin; AFP: alpha-fetoprotein; ALBI: albumin-bilirubin; CD68: cluster of differentiation 68; PD-L1: programmed death ligand 1; NLR: neutrophil-to-lymphocyte ratio; WBC: white blood cells; PLT: platelets; PLR: platelets-to-lymphocytes ratio.

CI: 1.029–1.151 to 67 CI: 11–413 ($p < 0.05$) [38]. According to the presented results, NLR proved to be a prognostic factor associated with the tumor recurrence in the univariable analysis only.

Another important issue after liver transplantation may be carcinogenesis associated with immunosuppression. In our analysis, six cases of new cancers were detected. This included two basal cell carcinomas, one melanoma, lung and metastatic ovarian cancer and one case of Mantel cell lymphoma. This highlights the need for appropriate surveillance and increased awareness of the potential risks for patients. Currently, there are scarce data regarding the risk of cancer development in patients after LT due to HCC. Of note, in a large Scandinavian study, 461 cancers were observed in 424 individuals of the 4246 LT patients during a mean 6.6-year follow-up [39].

In the era of the increasing role of immunotherapy in hepatocellular carcinoma and the rising number of systemic treatments, there is a need to identify reliable prognostic and predictive factors that could be used in clinical practice. The planned analysis for molecular prognostic and predictive factors among patients receiving systemic treatment was not performed as the amount of available histopathologic material was too small. This highlights the need to identify factors that may be obtained without pathomorphological examination. Because histologic confirmation is not always needed in HCC diagnosis, most patients in this cohort had the disease recognized with radiological criteria only. Moreover, tissue material required for molecular testing is often derived from curative resection material. This group of patients requires special attention as it was observed in the ImBrave 150 trial that not all patients respond well to combined targeted treatment, although general results are better than with sorafenib [40].

5. Conclusions

The results of this analysis suggest that microvascular invasion is the most important factor associated with an increased risk of HCC recurrence and overall survival for patients after liver transplantation or curative resection. Such patients may benefit from more intensive surveillance, independent of the initial treatment method. PD-L1 expression seems to be infrequently present in HCC samples and thus should not be used as a prognostic or predictive factor. Further studies on tumor microenvironments are needed to better characterize tumor biology and to predict which patients may benefit more from various treatment methods. Since the pathological material for molecular analysis is often unavailable, analysis based on the laboratory findings could only be of interest among patients qualified for systemic treatment.

Table 7
Overall survival after liver transplantation – univariable and multivariable analysis.

Univariable analysis		
Factor	P value	Hazard ratio
Age	0.314	0.962
Biologic sex	0.103	0.401
HCV infection	0.917	1.059
HBV infection	0.833	0.882
PALBI score	0.644	1.294
PALBI grade	0.911	1.040
Baseline AFP	0.004	1.000027
Baseline bilirubin	0.844	0.983
Baseline albumin	0.972	0.986
ALBI score	0.997	1.001
ALBI grade	0.884	0.948
NLR	0.078	0.930
WBC	0.091	1.973
PLT	0.520	1.002
PLR	0.650	0.999
Tumor number	0.480	1.260
Tumor size	0.012	1.014
Grading	0.046	3.505
Vascular microinvasion	0.000183	8.866
CD68 expression	0.607	10.898
PD-L1 expression	0.091	6.278
TILs	0.574	0.651
PD-L1 TILs/TAMs	0.343	0.571
Multivariable analysis		
Factor	**P value**	**Hazard ratio (95 % CI)**
Baseline AFP	0.149	1.000 (0.999–1.000)
Vascular microinvasion	0.0004	18.517 (3.667–93.502)
PD-L1 expression	0.059	9.96840 (0.911–108.967)
NLR	0.039	0.915 (0.841–0.995)

HBV: hepatitis B virus; HCV: hepatitis C virus; PALBI: platelet–albumin–bilirubin; AFP: alpha-fetoprotein; ALBI: albumin-bilirubin; CD68: cluster of differentiation 68; PD-L1: programmed death ligand 1; NLR: neutrophil-to-lymphocyte ratio; WBC: white blood cells; PLT: platelets; PLR: platelets-to-lymphocytes ratio.

CRediT authorship contribution statement

Maciej Gryziak: Writing – original draft, Methodology, Conceptualization. **Rafał Stec:** Validation, Supervision, Software, Resources. **Krzysztof Woźniak:** Methodology, Formal analysis, Data curation. **Benedykt Szczepankiewicz:** Validation, Resources, Methodology. **Maciej Krasnodębski:** Writing – review & editing, Formal analysis, Data curation. **Michał Grąt:** Resources, Methodology, Investigation. **Leszek Kraj:** Writing – review & editing, Visualization, Conceptualization.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by Ethics Committee of Medical University of Warsaw (AKBE/154/2021).

Data availability statement

The data that has been used is confidential.

Funding

The research leading to these results received funding from the National Science Centre, Poland (NCN) under Grant Agreement No

2020/04/x/nz5/00718; Medical University of Warsaw under Grant Agreement No 2/W11/1/M/MBS/N/21. The sponsors had no in role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Declaration of competing interest

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

Acknowledgements

none.

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Systematic Review

Impact of Thrombocytopenia on Survival in Patients with Hepatocellular Carcinoma: Updated Meta-Analysis and Systematic Review

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Simple Summary: Currently, hepatocellular carcinoma (HCC) is one of the most prevalent oncological diagnoses worldwide. Despite intensive research into its pathogenesis and clinical course, numerous issues remain unresolved. In the presented meta-analysis and systematic review, we investigate the potential of blood platelet levels in patients with HCC for prognostic assessment. Blood platelets may play a significant role as an easily measurable laboratory parameter in assessing the prognosis of patients with HCC.

Abstract: Background: Platelets (PLT) have a role in the pathogenesis, progression, and prognosis of hepatocellular carcinoma (HCC) and could represent a readily measurable laboratory parameter to enhance the comprehensive evaluation of HCC patients. Methods: The PubMed, Web of Science, and Scopus databases were searched with a focus on survival as well as patient and tumor-specific characteristics in correlation to reported PLT counts. Survival outcomes were analyzed with both common-effect and random-effects models. The hazard ratio (HR) and its 95% confidence interval (CI) from analyzed trials were incorporated. Studies that did not provide survival data but focused on platelet count correlation with HCC characteristics were reviewed. Results: In total, 26 studies, including a total of 9403 patients, met our criteria. The results showed that thrombocytopenia in HCC patients was associated with poor overall survival (common-effect HR = 1.15, 95% CI: 1.06–1.25; random-effect HR = 1.30, 95% CI: 1.05–1.63). Moreover, three studies reveal significant correlations between PLT indices and tumor characteristics such as size, foci number, and etiology of HCC development. Conclusion: Our meta-analysis confirmed that PLT count could act as a prognostic marker in HCC, especially with a PLT count cut off $<100 \times 10^3/mm^3$. Further prospective studies focusing on the role of PLT in clearly defined subgroups are necessary.

Keywords: hepatocellular carcinoma; HCC; thrombocytopenia; platelets; outcome; tumor features

Citation: Kraj, L.; Chmiel, P.; Gryziak, M.; Grabowska-Derlatka, L.; Szymański, Ł.; Wysokińska, E. Impact of Thrombocytopenia on Survival in Patients with Hepatocellular Carcinoma: Updated Meta-Analysis and Systematic Review. *Cancers* **2024**, *16*, 1293. https://doi.org/10.3390/cancers16071293

Academic Editor: Hiromitsu Hayashi

Received: 27 February 2024
Revised: 24 March 2024
Accepted: 25 March 2024
Published: 27 March 2024

1. Introduction

Primary hepatocellular carcinoma remains one of the most commonly diagnosed malignancies with a male predilection, occupying sixth place in incidence and third in mortality worldwide [1,2]. It is predicted to maintain a steady growth, increasing by 55.0% between 2020 and 2040, with an estimated 1.4 million new diagnoses in 2040 [3]. Multiple risk factors have been established for HCC including chronic hepatitis B (HBV) and C viral (HCV) infections, environmental carcinogen exposure, chronic alcohol consumption,

metabolic disorders, and rare genetic alterations. Irrespective of the underlying etiology, liver cirrhosis remains the predominant risk factor for HCC with risk rates of approximately 8.3% at 5 years and 12.2% at 10 years [4]. HCC can be understood as a heterogeneous malignancy with diverse etiologies, risk factors, and clinical course, which makes the management of patients challenging.

The current therapeutic approach in HCC is driven by patient performance status, tumor stage, and liver function, which are incorporated into the Barcelona Clinic Liver Cancer Classification System (BCLC) [5]. The updated version stratifies patients treated with curative intent into those eligible for local-regional therapies as well as those considered to be candidates for liver transplant (LT) [6]. For the purpose of transplantation, qualifying liver function and tumor-specific parameters are referred to as the "extended criteria for liver transplantation" [7]. Patients with advanced or metastatic tumors, as well as those excluded from procedures due to severe liver dysfunction, are eligible for palliative systemic treatment. Currently, this involves a combination of immune checkpoint inhibitors (ICIs) and tyrosine kinase inhibitors (TKIs) [8]. Despite a better understanding of this disease and improvement in diagnostics and treatment, the prognosis for HCC is still poor with an overall 5-year survival rate estimated at approximately 20%, with some studies utilizing mainly TKI reporting rates as low as 9.72% for advanced HCC [2,9].

Cancer research has been focused on establishing effective prognostic and predictive markers to guide therapy over the last several decades [10,11]. Ideally, those markers are minimally invasive, cost-efficient, and highly specific to enable their use in clinical management [12]. Platelets play an important role in both cancer growth and spread [13]; with their significance documented for pathogenesis and prognosis in HCC [14,15]. In a healthy organism, platelets are physiologically part of the inflammatory response, transporting and releasing cytokines such as serotonin, platelet-derived growth factors, and transforming growth factor-β in addition to their role in primary hemostasis [16]. In disease states, platelet quantitative and functional indices show a strong correlation with hepatic fibrosis and cirrhosis, precursor states to HCC development [17–19]. Consequently, the platelet count can be used with other inflammatory markers in scoring indexes for HCC, such as the AST to Platelet Ratio Index (APRI) or Platelet-Albumin-Bilirubin Index (PALBI) [20–22], with studies showing worse overall survival in HCC patients with an index representing a more advanced stage [15,23,24]. Recent analyses of PLT's role in HCC demonstrated substantial variations depending on the patient cohort characteristics and the geographical context of the research. In contrast to those of preceding investigations, the results of Scheiner et al. showed that thrombocytopenia and platelet activation parameters distinctly correlated with a more favorable prognosis [25]. In a cohort of 378 patients with thrombocytopenia undergoing palliative treatment, defined as <150 g/L, there was an association between thrombocytopenia and advantageous baseline tumor characteristics, including a diminished diameter of the largest nodule, constrained extrahepatic spread, diminished macrovascular invasion, and lower BCLC stages. Moreover, the composite variable of thrombocytopenia and elevated mean platelet volume (MPV), a platelet activation parameter, independently correlated with prolonged overall survival (HR 0.80, 95% CI—0.65–0.98; $p = 0.029$) [25]. However, a subsequent follow up of this work, undertaken on a distinct Taiwanese population, reported markedly dissimilar findings [26]. Despite the thrombocytopenia group manifesting characteristics suggestive of lower tumor aggressiveness in the cohort of over three thousand patients, no observable difference in terms of OS emerged between the groups. Regrettably, data detailing the treatment methodology for these patients were not disclosed [26].

Motivated by these observed differences and taking into account existing meta-analyses [27], our current study endeavored to enhance the understanding of the role of platelet count in HCC prognosis. To achieve this objective, we conducted a comprehensive systematic review and meta-analysis with a subgroup analysis of patients treated with curative and palliative intent. Our working hypothesis was that thrombocytopenia is associated with a worse patient survival independently of the intended treatment modality.

Consequently, we reviewed and summarized additional clinicopathological data pertaining to PLT, presenting a consolidated overview herein.

2. Materials and Methods

2.1. Search Strategy

The aim of the study was to answer the following questions: (1) Does thrombocytopenia affect the survival of patients with HCC? (2) Is there a correlation between thrombocytopenia and the clinicopathological features of the tumor? (3) How does thrombocytopenia correlate with HCC etiology? In order to answer these questions, we followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement guidelines to design the study, analyze the results, and report our findings. A systematic literature review of PubMed, Web of Science, and Scopus databases was performed by two identifications in November 2023. The core search included the following terms: platelets OR PLT OR thrombocytopenia OR thrombopenia AND HCC AND hepatic cancer AND hepatocellular carcinoma AND liver cancer AND prognosis. After the initial search, 4105 articles were found. Eventually, after removing duplicate papers and excluding articles that didn't meet the inclusion criteria, 26 articles were included in a qualitative synthesis. As one of the included studies developed three separate datasets [28], the rest of our reporting in this meta-analysis will refer to 28 separate studies. Three articles with clinicopathological findings regarding the HCC and PLT count correlation were included in the systematic review to answer our second query but were not included in the meta-analysis results. The literature search included only human studies with no restrictions regarding the year of publication. The authors chose only articles in English. A detailed search strategy is presented in Figure 1. Additional papers were identified by a manual search of the references from the eligible review articles.

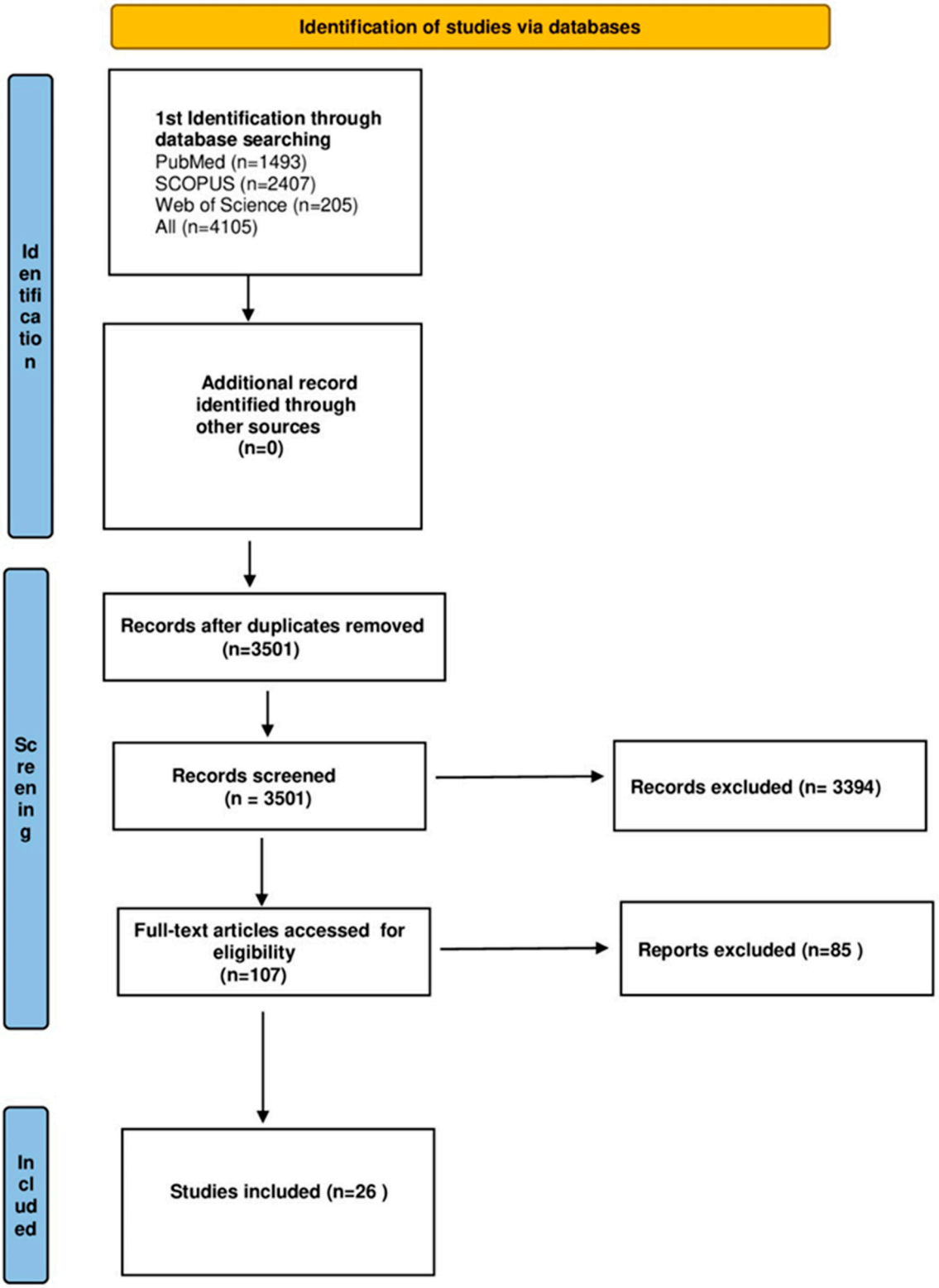

Figure 1. Flowchart presenting the process of article selection, according to Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.

2.2. Eligibility Criteria

The inclusion criteria were as follows: (1) HCC confirmed in histopathology or by imaging, (2) only original studies, (3) studies cover the topic of HCC patient's survival or tumor features in relation to platelet count, (4) reported hazard ratios (HRs) and 95%

confidence intervals (CIs) for overall survival (OS) or provided sufficient data to calculate these values, and (5) the subjects were divided into low and high PLT level groups or provided data was sufficient to estimate these groups.

Exclusion criteria were: (1) pathological type was not HCC; (2) not relevant to the prognosis or features of HCC; (3) the risk effects and corresponding 95% CI were not provided and available data were not sufficient to calculate them; (4) studies without original clinical data, such as reviews, systematic reviews, meta-analysis, expert opinions, editorial, or comment; (5) clinical trials exclusively evaluating drugs/medical interventions among patients with HCC.

The search results were reviewed by 2 independent researchers (P.C./L.K.) for potentially eligible studies. Disagreements over the eligibility of an article were resolved by consensus.

2.3. Data Extraction and Quality Assessment

Relevant information was extracted including author name, publication year, country, study design, sample size, platelet count and cut-off values, data regarding patient survival such as OS, DFS, and postoperative complications, data regarding tumor features such as tumor diameter, and HCC etiology. Where possible we extracted HR with 95% CI; where it was not stated explicitly, we used the parameters from the study to calculate it ourselves. The quality assessment was conducted using the Newcastle–Ottawa scale (NOS) method.

2.4. Data Analysis

The pooled HR value for overall survival was calculated using a fixed- and random-effects model. The heterogeneity among the studies was assessed using the Q value and the I^2 statistic value. A random-effect model was used for data showing statistically significant heterogeneity if $p < 0.1$ as determined by the Q statistic or $I^2 > 50\%$; otherwise, we considered that there was no obvious heterogeneity and used a fixed-effect model. In cases of high heterogeneity, we conducted a subgroup analysis and meta-regression to explore potential sources of the effect. Furthermore, an influence analysis was performed to assess whether any individual study significantly affected the result. We analyzed those covariates that may have contributed to the potential heterogeneity, for which the PLT cut-off value, treatment intent, presence of HCV infection, number of recruited patients, and Child–Pugh grade were assessed. Eventually, publication bias was examined with Egger's tests. Statistical analysis was performed and visualized using R version 4.3.2 with the 'meta' package [29].

3. Results

3.1. Characteristics of the Included Studies

The baseline characteristics of the included studies are summarized in Table 1. We were able to identify a cohort of 9403 patients, predominantly male (>50%), with a follow-up duration ranging from 5 months to 5.8 years. PLT cut-off values varied between 75 and 150 ($\times 10^9$/L), with most studies adopting the threshold based on the definition of thrombocytopenia (<100 $\times$ 10^9/L). The studies exhibited moderate to high quality, as reflected by Newcastle–Ottawa Scale (NOS) scores ranging from 4 to 9, with a mean score of 6.54. The studies included predominantly Asian populations. The assessed interventions included curative and palliative therapies; all were analyzed collectively and within subgroups. The hazard ratios (HR) for overall survival (OS) were available in all included studies, providing a comprehensive evaluation of the outcomes. The characteristics of the patient groups from the studies included in the analysis are summarized in Table 2.

Table 1. Baseline characteristics of the studies included in the meta-analysis.

Study	Reference	Country	Number of Patients (M/F)	Treatment	Child–Pugh (A/B/C)	PLT Cut-Off Value (10^9/L)	Follow-Up (Years)	NOS
Amano et al.	[30]	Japan	127/24	Surgery	129/22	100	4.1	6
Arimura et al.	[31]	Japan	95/45	PEIT/TACE	77/48/15	80	NA	4
Bräu et al.	[32]	America	287/2	Surgery /RFA	113/134/42	100	NA	6
Cammá et al.	[33]	Italy	131/71	RFA	165/37	100	1.25	8
Hashimoto et al.	[34]	Japan	120/29	Surgery	NA	120	3.5	9
Ikeda et al.	[35]	Japan	122/46	TACE	86/82	75	2.8	5
Kao et al.	[36]	Taiwan	162/69	RFA	226/32	100	2.375	6
Kim et al.	[37]	Korea	39/13	TACE	16/23/13	150	0.4	6
Kobayashi et al.	[38]	Japan	146/53	Surgery	199	100	3.25	7
Lee et al.	[39]	Taiwan	1245/415	Surgery /RFA/TACE	830/349/149	118	5	7
Miyatake et al.	[40]	Japan	260/135	Surgery	317/74/4	100	3.5	7
Meinhardt et al.	[28]	America	221 95 318	Systemic treatment	A major	150	NA	4
Niizeki et al.	[41]	Japan	56/15	TACE	43/28	120	NA	5
Nishikawa et al.	[42]	Japan	217/151	RFA	70/162/100	100	3	8
Nouso et al.	[43]	Japan	116/41	RFA/TACE	157(C)	80	NA	7
Ochiai et al.	[44]	Japan	208/76	Surgery	273/11	110	3	6
Roayaie et al.	[45]	America	95/37	Surgery	132	150	3.2	7
Santi et al.	[46]	Italy	457/192	Surgery/ RFA/TACE	477/172	100	3.3	9
Schrecker et al.	[47]	Germany	96/32	Surgery	126/2	100	4.6	8
Su et al.	[48]	Taiwan	152/36	Surgery	A major	100	5.8	8
Taketomi et al.	[49]	Japan	167/43	Surgery	158/52	150	2.4	7
Tseng et al.	[50]	Taiwan	48/34	Surgery /RFA/TACE	NA	100	NA	6
Wu et al.	[51]	Taiwan	104/57	RFA	A major	100	3.2	4
Wu et al.	[52]	China	79/7	Surgery	A major	100	NA	7
Venkant et al.	[53]	America	1411/686	Surgery	NA	150	NA	7
Xie et al.	[54]	China	408/79	RFA/TACE	NA	97	NA	6

Abbreviations: PEIT—Percutaneous ethanol injection therapy; RFA—Radiofrequency ablation; TACE—Transcatheter arterial chemoembolization; NOS—Newcastle–Ottawa scale.

Table 2. Characteristics of patient groups in included studies.

Study	Age (Years)	Race	Follow-Up	Disease Stage (BCLC)	Viral Infection	Alcohol Intake
Meinhardt et al. [28]	NA	NA	NA	NA	NA	NA
Amano et al. [30]	>18	NA	4.1 ± 3.1 years	NA	HCV	NA
Arimura et al. [31]	63.3 ± 8.54	NA	NA	NA	NA	NA
Bräu et al. [32]	52.2 (±8.0)–63.9 (±11.0)	White, Latino, Black, Asian	NA	A-D	HIV, HCV, HBV	+
Cammà et al. [33]	66.8 (±8.2) 67.4 (±6.9)	NA	15 months	A-B	HCV, HBV	+
Hashimoto et al. [34]	61.7	N/A	42.1 months	N/A	HCV, HBV	N/A
Ikeda et al. [35]	63 (45–80)	N/A	2.8 years	N/A	HCV, HBV	+
Kao et al. [36]	>18	N/A	28.5 ± 18.7 months	N/A	HCV, HBV	N/A
Kim et al. [37]	57 (35–80)	N/A	5 months	N/A	HCV, HBV	+
Kobayashi et al. [38]	62 (29–80)–67 (38–87)	N/A	3.3 years	N/A	HCV, HBV	+
Lee et al. [39]	N/A	N/A	5 years	A	HCV, HBV	N/A
Miyatake et al. [40]	58	N/A	1.3	N/A	HCV	N/A
Niizeki et al. [41]	65	N/A	N/A	N/A	HCV, HBV	N/A
Nishikawa et al. [42]	69.9 ± 9.0	N/A	N/A	N/A	HCV, HBV	N/A
Nouso et al. [43]	63	N/A	N/A	N/A	HCV, HBV	N/A

Table 2. *Cont.*

Study	Age (Years)	Race	Follow-Up	Disease Stage (BCLC)	Viral Infection	Alcohol Intake
Ochiai et al. [44]	63.9	N/A	36 months	N/A	HCV, HBV	+
Roayaie et al. [45]	63.1 ± 10.5	N/A	37.5 months	0	HCV, HBV	+
Santi et al. [46]	67	N/A	38.6 ± 32.8 months	N/A	HCV, HBV	+
Schrecker et al. [47]	65 (34–81)	N/A	55.1 months	0-B	N/A	N/A
Su et al. [48]	61.5 (52.0–70.75)	N/A	69.8 months	N/A	HCV, HBV	N/A
Taketomi et al. [49]	60.7 ± 7.9	N/A	26.6 ± 22.0 months	N/A	N/A	N/A
Tseng et al. [50]	65.8 ± 9.6	N/A	4 years	0-B	HCV, HBV	N/A
Wu et al. [51]	67.5 ± 11.4	N/A	38.1 ± 20.8 months	N/A	HCV, HBV	N/A
Wu et al. [52]	>18	N/A	7 years	I–IV *	HBV	N/A
Venkat et al. [53]	64	Caucasian, African–American	N/A	N/A	N/A	N/A
Xie et al. [54]	52 ± 7.3–69 ± 3.7	N/A	N/A	N/A	HCV, HBV	N/A

*—Study accessed stage based on TNM classification. +—Included in the analysis.

As mentioned before, three additional studies were selectively incorporated into the systematic review due to their intriguing contextual relevance to the clinical-pathological characteristics of HCC based on the patient's blood platelet count and activation. These supplementary investigations are elucidated in the paragraph following the meta-analysis.

3.2. Pooled HR Values for All of the Studies

By accessing general effects, we estimated that the low PLT count in HCC patients was associated with worse OS (HR = 1.30, 95% CI: 1.05–1.63). The forest plot for this estimation is shown in Figure 2. A significant degree of heterogeneity was noted between studies; however, due to consistencies reported in results, all were included in the analysis.

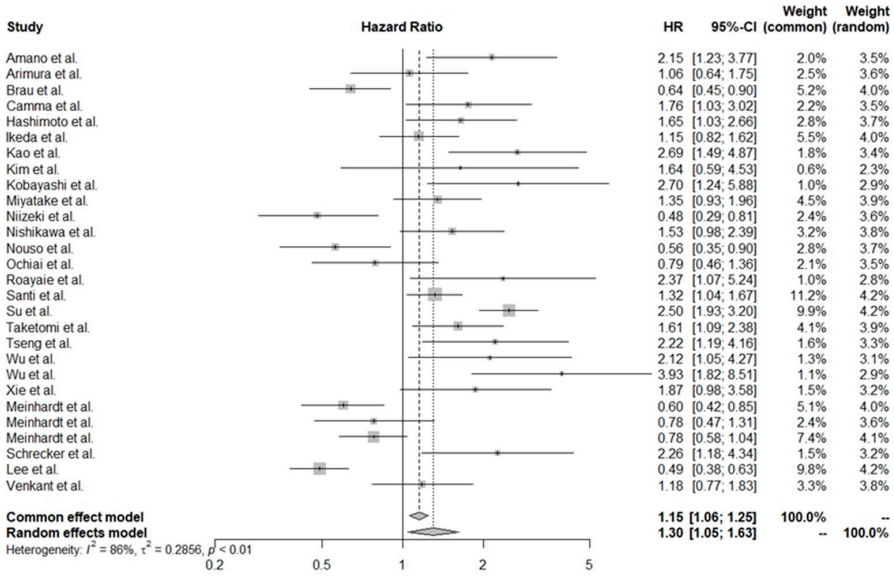

Figure 2. Effect of thrombocytopenia on overall survival in patients with hepatocellular carcinoma [28,30–54].

We observed a significant degree of heterogeneity between studies; however, based on our analysis we believe that all of the abovementioned studies evaluated the same methodological approach and decided to interpret them further (I^2 = 86.0%, p < 0.01).

3.3. Adjusted Significance of PLTs in HCC

For more accurate results, a separate analysis including an adjusted HR for OS, using Cox multivariate analysis, was performed. Nineteen studies involving 5885 patients were included in this analysis, yielding results that confirmed an unfavorable prognosis (HR: 1.47, 95% CI 1.15–1.86). The forest plot for this estimation is shown in Figure 3.

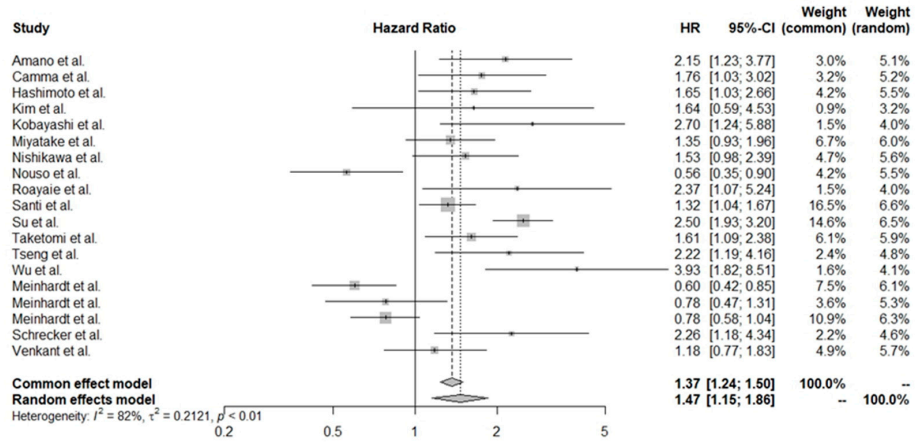

Figure 3. Effect of thrombocytopenia on overall survival in patients with HCC (adjusted) [28,30,33,34,37,38,40,42,43,45–51,53].

3.4. Pooled HR Values for Various Treatment Groups

Due to significant prognostic heterogeneity in HCC, the studies included in the overall analysis were stratified based on treatment intent. Curative treatment was defined as resection or RFA, while palliative (non-curative) treatment involved TACE and systemic therapy. However, it is important to note that segmental TACE can currently be used with curative intent [55]. We conducted separate estimations for patients treated with curative intent for those receiving palliative interventions. In the cohort undergoing radical treatment, a decreased platelet level indicated a worse prognosis (HR 1.62, 95% CI 1.25–2.11). A different trend was also observed for the palliative care group, which allowed for a contradictory conclusion, where lower PLT levels correlated with better OS (HR 0.81, 95% CI 0.62–1.05). The forest plots for these estimations are shown in Figures 4 and 5.

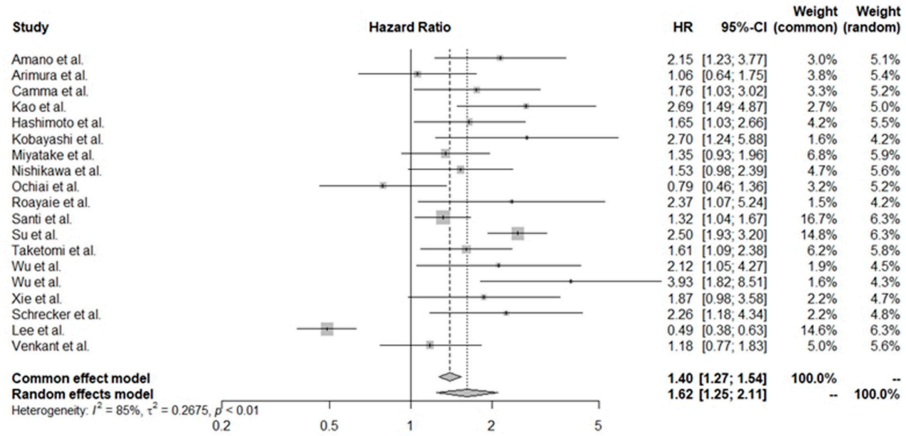

Figure 4. Pooled HR values for patients treated with curative intent [30,31,33,34,36,38–40,42,44–49,51–54].

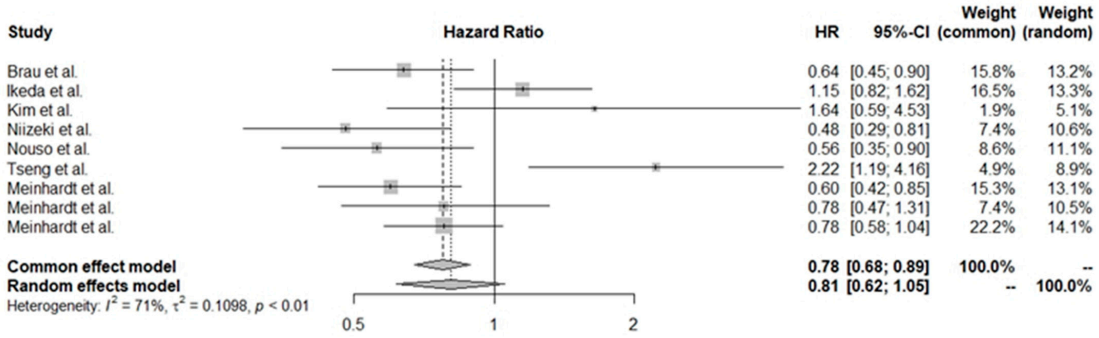

Figure 5. Pooled HR values for patients treated with non-curative intent [28,32,35,37,41,43,50].

3.5. Exploration of Heterogeneity

As previously mentioned, the estimated HR for OS exhibited considerable heterogeneity despite the inclusion of numerous studies. To elucidate the origins of this heterogeneity, we conducted subgroup heterogeneity analyses. The analyzed covariates encompassed platelet (PLT) cut-off values (150, 101–149, or ≤100), treatment modality (curative vs. palliative), hepatitis C virus (HCV) presence (<50% vs. >50% of patients), total patient enrollment (≥200 vs. <200), and Child–Pugh grade (studies with >50% patients classified as grade A vs. <50%). The findings of this analysis are detailed in Table 3. Our assessment suggests that the treatment intent and the PLT cut-off value may have influenced the pooled effect size ($p < 0.05$ in the subgroup). However, it is important to acknowledge that there are additional unexplored factors that could have impacted our results, especially in a setting of high heterogeneity of the studies. Notably, the PLT level emerged as a significant prognostic factor for patients undergoing curative therapy, with an HR value of (HR 1.62, 95% CI 1.25–2.11). Conversely, for patients undergoing palliative treatment, an association between PLT levels and survival was noted, although a discernible trend towards better prognosis was observed. Also, subgroups with studies that included less than 50% of patients with Child–Pugh A showed no correlation with platelet levels.

Table 3. Subgroup Analysis (Random-Effects Model).

Covariates	Subgroup	No	HR	Heterogeneity		
				Ps	I^2	Pa
Curative	No	9	0.7754 [0.6760; 0.8894]	0.115	70.5%	<0.001
	Yes	19	1.4006 [1.2717; 1.5426]	<0.001	84.7%	
Child–Pugh	≤50%	4	0.9099 [0.5312; 1.5587]	0.731	78.5%	0.349
	>50%	23	1.3921 [1.0847; 1.7865]	0.009	87%	
	No Data	1	1.1800 [0.7656; 1.8187]	0.453	0%	
HCV	≤50%	10	1.2986 [0.8032; 2.0995]	0.286	92.4%	0.883
	>50%	14	1.2797 [0.9778; 1.6748]	0.019	79.7%	
	No Data	4	1.4110 [1.0576; 1.8825]	0.072	32%	
PLT	≤100	17	1.6004 [1.2515; 2.0465]	<0.001	80.1%	0.028
	101–149	5	0.8207 [0.4778; 1.4099]	0.474	84.0%	
	150	6	1.0251 [0.7137; 1.4724]	0.893	77.4%	
Number of patients	≤200	15	1.5324 [1.1127; 2.1105]	0.009	82.2%	0.127
	>200	13	1.1016 [0.8341; 1.4548]	0.495	85.8%	

Ps—*p*-value for subgroups, Pa—*p*-value for all included.

3.6. Sensitivity Analysis and Test of Publication Bias

A sensitivity analysis was performed to evaluate the reliability of the aforementioned findings. We examined the application of both random-effects and (common) fixed-effects models for the analysis of the studies and observed no discernible disparities between them (comparisons are shown in the corresponding figures). Furthermore, we performed an influence analysis and found that no single study affected the summary estimation (Figure 6). To assess publication bias, Egger's test was conducted with a funnel plot, shown in Figure 7 (estimated *p*-value of 0.0925).

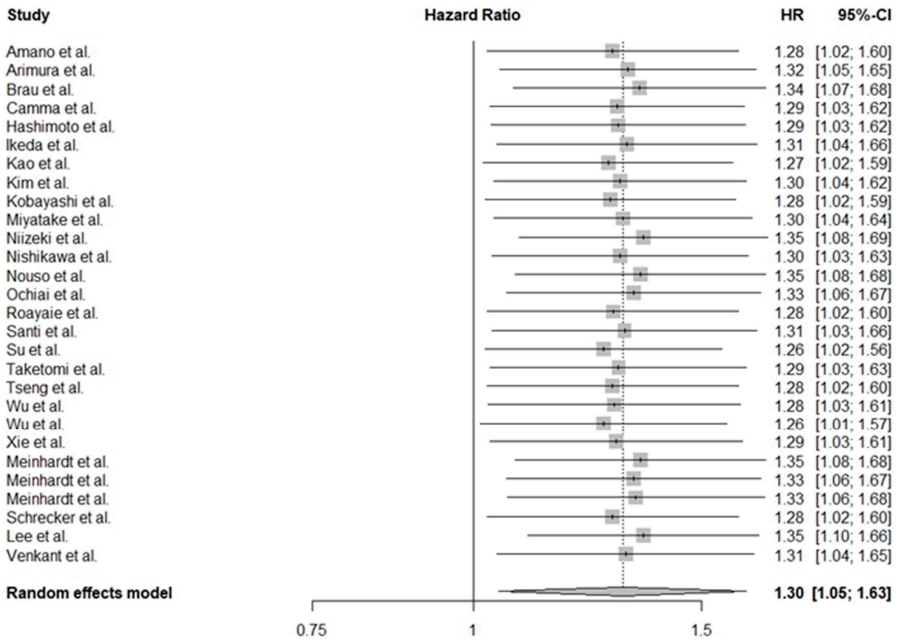

Figure 6. Influence analysis of included studies [28,30–54].

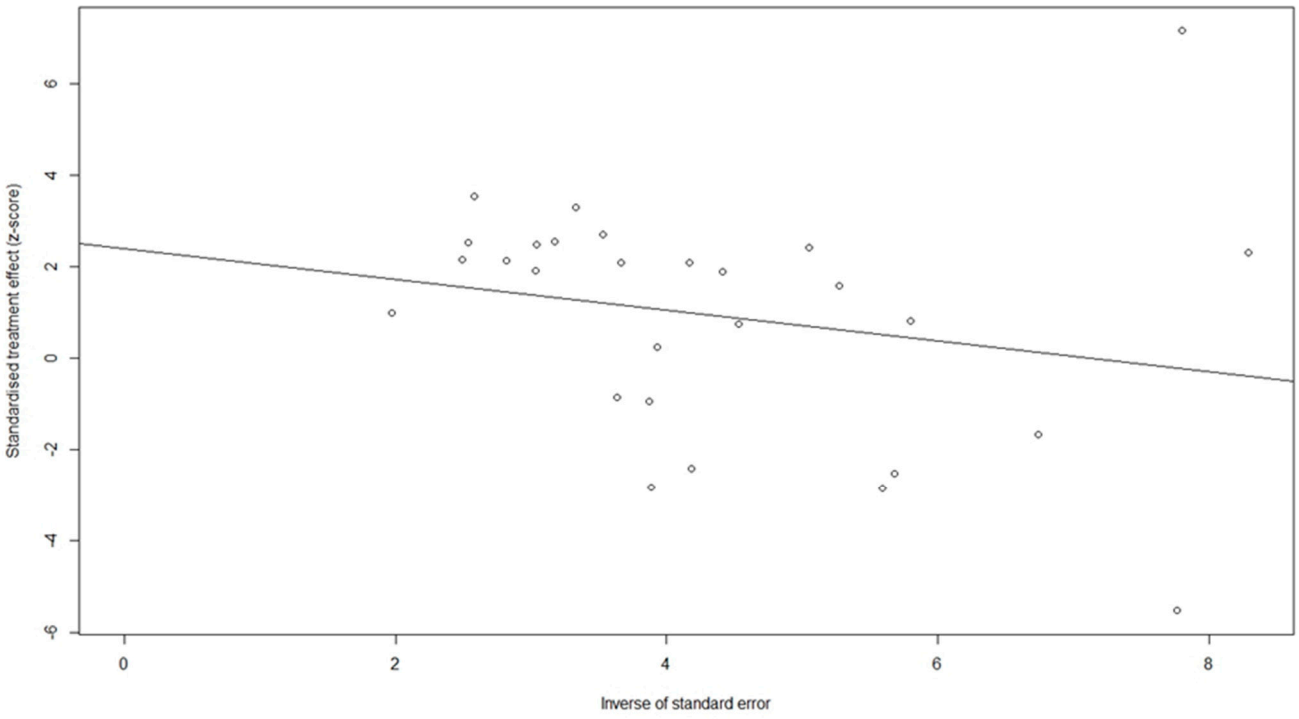

Figure 7. Egger funnel plot for the included studies.

3.7. Additional Clinicopathological Findings

During the literature search, three studies closely approached our inclusion criteria; however, due to the absence of prognostic analyses, they were not included in the meta-analysis [56–58]. Nevertheless, their significance in exploring the clinical-pathological features and disease progression as they relate to patient's platelet counts was noteworthy, leading to their inclusion in this review section. All three studies aimed to establish correlations between commonly assessed parameters in patients with HCC, such as gamma-glutamyl transferase (GGTP), PLT, alpha-fetoprotein (AFP), or bilirubin levels, and characteristics of the tumors, including the size and number of lesions. A lower PLT count was observed primarily in small tumors ($\leq$3 cm) with concurrent higher bilirubin values. This observation seemed contradictory to the potential process of parenchymal destruction in the setting of tumor growth, suggesting compelling hypotheses about new pathways in HCC carcinogenesis [57]. Small tumors were also observed in cirrhotic livers, where their development is constrained by both liver function and parenchymal collagen remodeling. A discernible association was observed between increasing tumor size and elevated platelet levels, perhaps due to the presence of paraneoplastic phenomena. A subsequent cohort analysis revealed that the mean platelet levels were 142, 158, and 239 $\times$ 10^9/L, respectively, corresponding to the progressive increase in tumor size (p = 0.0001). Additionally, patients in the cohort with the largest tumors exhibited a significantly lower incidence of cirrhosis. This led to speculation that platelet-releasing mediators and growth factors might in fact contribute to the more aggressive tumor growth and larger sizes, partly addressing the question of interactions between the tumor microenvironment and HCC characteristics [56]. However, after adjusting these parameters for patient survival, no statistically significant correlations were found [58].

4. Discussion

Despite the association of platelet count with HCC outcomes, it is not currently directly incorporated into any of the widely used predictive and prognostic tools, with only the indirect inclusion of platelet count as a predictor of portal hypertension [59,60].

Our analysis underscores role of thrombocytopenia in the prognosis of patients with HCC and may offer impetus for the further refinement of prognostic models. Platelet counts below 100 $\times$ 10^9/L increased the overall risk of death by 30% across all patient groups. In individuals undergoing treatment with curative intent, this risk was amplified by 62%. Additionally, an individual analysis revealed an independent association between thrombocytopenia and OS in patients with HCC. Notably, there were no significant differences between the results obtained using random- and fixed-effects models, and the pooled HR value remained largely unaffected by any single study. These outcomes underscore the robustness of our data. Through subgroup analysis, potential sources of heterogeneity were identified, particularly in the context of PLT level cut-off values, warranting further evaluation alongside other covariates. Our analysis may serve as a rationale for further research on the more comprehensive inclusion of PLT in the overall assessment of HCC patients. Additionally, future analyses may be further enriched by incorporating more tumor-specific characteristics in relation to platelet values, providing an additional patient stratification tool.

Somewhat unexpectedly, our analysis revealed that low platelet counts did not influence outcomes in patients treated in a palliative setting. We expected patients with low PLT to be at risk of receiving less systemic therapy as in clinical trials of the newest immunotherapy, targeted therapy, and their combinations, the cut-off value for platelet counts for inclusion oscillated at around $\geq$60–75 $\times$ 10^9/L [61–63]. As the studies we scrutinized utilized a higher cut-off value for the platelet levels compared to the recommended thresholds for patient inclusion in a clinical trial, it is very difficult to gauge whether the reception of therapy would have influenced those results and if results would have been different with lower PLT thresholds.

There were several limitations to our study. First, some included studies did not report important data (such as Child–Pugh grade and HCV infection rates) essential for a comprehensive analysis. Furthermore, there are major obstacles to obtaining a homogenous group of HCC patients. Clinical trial results may be difficult to apply to real-world situations due to incomplete or unclear reporting of data. However, it can also be a starting point for in-depth analysis for the future. Second, most of the included studies investigated the impact of platelet levels among patients undergoing diverse surgical procedures, with a minority focusing on systemic therapy in the form of chemotherapy, immunotherapy, or targeted therapy. Consequently, the number of patients receiving systemic therapy in our analysis was limited. Third, the quality of some studies included in the analysis was moderate, as reflected by a Newcastle–Ottawa Scale (NOS) score of 4. Fourth, the PLT value cut-off points in most studies were set to 100×10^9/L; only four studies considered a lower cut-off point. Consequently, establishing the precise PLT level indicating a worse prognosis remains elusive at present. However, the primary challenge encountered in our analysis pertains to the pronounced heterogeneity, surpassing that observed in previous studies of a similar nature. While it might be attributed to the inclusion of a novel patient group in recent years and the expansion of clinical indications for surgical treatments, including transplantation and hepatectomy, the exact source of this heterogeneity is yet to be determined.

Despite extensive in vitro and in vivo investigations into the relationship between platelets and HCC, certain questions remain unresolved. The evident influence of both thrombocytopenia and thrombocytosis on the pathogenesis of HCC is primarily attributed to the interaction of platelet mediators with a cirrhotic liver and tumor microenvironment [64,65]. It has been established those metabolites released from platelets, including platelet-derived growth factor (PDGF), actively promote tumorigenesis. Within HCC cells, the level of PDGF receptor alpha (PDGFRα) is elevated in comparison to normal hepatocytes [64,66], resulting in heightened chemosensitivity under normo- or hypoxic conditions when PDGF is suppressed [64]. Additionally, tumors exhibiting an overexpression of PDGFRα are significantly associated with increased micro-vessel density, macroscopic vascular invasion, shorter overall survival, and a higher rate of HCC recurrence [67]. Moreover, serotonin, a crucial mediator released from platelets, may impact disease progression. Circulating serotonin levels were notably higher in cirrhotic patients with HCC than in those without HCC [68]. Furthermore, in a cohort of 40 HCC patients undergoing partial hepatectomy, intra-platelet serotonin levels were predictive of HCC recurrence (HR 0.1, 95% CI—0.01–0.89) [69]. Hence, the PLT count, and consequently, levels of mediators released from its granules may exhibit correlations with both cirrhosis and the characteristics and aggressiveness of tumors. The discussed studies exploring the relationship of platelet count and activity with HCC tumorigenesis regrettably could not be incorporated into our meta-analysis owing to discrepancies in the OS assessment criteria employed by the researchers [25]. Nonetheless, our findings exhibit concordance with the aforementioned work, as well as with the divergent commentary concerning that analysis [26]. Consequently, we combined the conclusions from both studies, which showed different prognostic outcomes depending on the patient cohort and type of therapy employed [25,26].

Individual analyses of the impact of PLT on a patient's prognosis provide valuable information about the prognosis in a specific group of patients. For instance, an analysis that considers only surgically treated patients. However, we have not observed a direct comparison of palliatively and radically treated patients in a trial. We decided to investigate whether the thresholds for inclusion were appropriate, given the strict guidelines for patient inclusion in different treatments. For instance, based on our findings, it may soon be feasible to incorporate individuals with significantly lower PLT levels, who are currently ineligible for treatment, into both clinical trials and routine care. The primary benefit of our research is its clinical relevance, which could offer physicians more choices for patients who experience ongoing platelet abnormalities and related symptoms. The results of the individual analyses showed some inconsistencies. For instance, some analyses indicated that patients with

reduced platelets had a better prognosis, while others suggested a worse prognosis. Our work suggests that the type of treatment may be a possible source of this discrepancy.

5. Conclusions

There is a strong association between platelet counts and survival outcomes in HCC patients, although a comprehensive incorporation of platelet assessment in the evaluation of HCC patients requires further refinement. This will require the identification of specific subgroups of patients with this heterogeneous cancer where platelet count and other PLT indices may influence pathogenesis and prognosis. Moreover, further prospective trials, especially on patients treated with systemic therapy, are much needed with clear inclusion criteria and comprehensive reporting.

Author Contributions: Conceptualization, L.K., P.C. and E.W.; methodology, P.C. and L.K.; formal analysis, all; writing—original draft preparation, P.C. and L.K.; writing—review and editing, all; visualization, P.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data can be shared up on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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Podsumowanie i wnioski

Cele postawione przed rozpoczęciem pracy naukowej zostały osiągnięte.

1. Opisano molekularną charakterystykę pacjentów leczonych z powodu HCC w UCK WUM, stwierdzając relatywnie rzadko obserwowaną ekspresję PD-L1; zbadano wpływ ekspresji PD-L1 oraz CD68 i TIls na rokowanie pacjentów.
2. Przeanalizowano dane dotyczące przeżycia całkowitego oraz czasu do nawrotu choroby w populacji pacjentów z HCC po przeszczepie oraz po radykalnej resekcji. Stwierdzono istotnie wyższą częstość nawrotów po resekcji w porównaniu do transplantacji (69% vs 21%), co podkreśla potrzebę optymalnego leczenia i wczesnej diagnostyki HCC.
3. Analiza danych klinicznych pacjentów chorych na HCC wykazała, że dla rokowania pacjentów chorych na HCC (OS, RFS) kluczowe znaczenie mają dobrze znane czynniki, takie jak inwazja mikronaczyniowa, stopień zaawansowania i wyjściowy poziom AFP. Natomiast ekspresja PD-L1 oraz CD68 nie wpływają na rokowanie pacjentów. Nie wykazano również, aby parametry jak ALBI, PALBI, NLR, PLR wiązały się z OS i RFS pacjentów w analizowanej populacji.

   Przeprowadzone analizy wieloczynnikowe zasugerowały kilka możliwych do zastosowania w praktyce klinicznej czynników prognostycznych:
   - nawrót choroby, populacja ogólna:
     - wyjściowy poziom AFP, *p=0.034,* HR=1.000 (1.000001–1.00002)
     - inwazja mikronaczyniowa, *p=0.001,* HR=4.546 (1.769–11.681)
     - WBC, *p=0.004*, HR=2.121 (1.261–3.567)
   - nawrót choroby po transplantacji:
     - stopień zróżnicowania, *p=0.002*, HR=12.183 (2.434–60.965)
     - inwazja mikronaczyniowa, *p=0.004*, HR=9.373 (2.006–43.785)
   - przeżycie całkowite, populacja ogólna:
     - inwazja mikronaczyniowa, *p <0,001,* HR=5.404 (2.352–12.413)
   - przeżycie całkowite, po resekcji:
     - wyjściowy poziom AFP, *p=0.022*, HR=1.000045 (1.000006–1.00008)
     - wyjściowy poziom bilirubiny, *p=0.0009*, HR=3.323 (1.630–6.773)
     - inwazja mikronaczyniowa, *p=0.0107,* HR=3.972 (1.374–11.483)
   - przeżycie całkowite, po transplantacji:

- inwazja mikronaczyniowa, *p=0.0004*, HR=18.517 (3.667–93.502)
- NLR, *p= 0.039*, HR= 0.915 (0.841–0.995)

Dodatkowo przeprowadzona meta-analiza wykazała, że liczba płytek krwi może być ważnym markerem prognostycznym u pacjentów chorych na HCC, zwłaszcza w przypadku leczenia radykalnego.

Podsumowując, leczenie chorych na HCC w istotny sposób zmieniło się w ostatnich latach. Dostępne coraz liczniejsze opcje terapeutyczne sprawiają, że istotne jest znalezienie nowych czynników predykcyjnych oraz prognostycznych w różnych grupach pacjentów.
Spośród znanych parametrów, które mogą być wykorzystywane jako czynniki prognostyczne w przypadku chorych na raka wątrobowkomórkowego największą rolę odgrywa inwazja mikronaczyniowa oraz stopień zróżnicowania nowotworu. Wydaje się, że ocena ekspresji PD-L1 ma ograniczone zastosowanie ze względu na niską częstość występowania. Dodatkowo, wielu pacjentów chorych na HCC może nie dysponować materiałem histologicznym do badań molekularnych. Podobnie, ocena ekspresji CD68 wymaga dalszych, pogłębionych badań w różnych subpopulacjach TAMs.

Ocena wpływu parametrów laboratoryjnych różnie się istotnie w różnych subpopulacjach pacjentów chorych na HCC. Stąd wydaje się, że potrzeba dalszych, prospektywnych badań, być może z zastosowaniem technik płynnej biopsji lub innych parametrów oznaczanych w płynach ustrojowych a niekoniecznie na podstawie materiału tkankowego.

Opinia Komisji Bioetycznej lub Etycznej

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Warszawa, dnia 06 września 2021r.

AKBE/ 154 / 2021

Dr hab. n .med. Rafał Stec
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02-097 Warszawa

OŚWIADCZENIE

Niniejszym oświadczam, że Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym w dniu 06 września 2021 r. przyjęła do wiadomości informację na temat badania pt „ Wpływ mikrośrodowiska guza oraz jego charakterystyki molekularnej na wyniki leczenia pacjentów chorych na raka wątrobowokomórkowego."
Przedstawione badanie nie stanowi eksperymentu medycznego w rozumieniu art. 21 ust. 1 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty(Dz.U. z 2018 r. poz. 617) i nie wymaga uzyskania opinii Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym, o której mowa w art. 29 ust.1 ww. ustawy.

Przewodnicząca Komisji Bioetycznej

Prof. dr hab. n. med. Magdalena Kuźma –Kozakiewicz

Oświadczenia współautorów publikacji

Warszawa, 29.05.2025

prof. dr hab. n. med. Rafał Stec

OŚWIADCZENIE

Jako współautor pracy pt. Milestones in the treatment of hepatocellular carcinoma: A systematic review. Crit Rev Oncol Hematol. 2021 Jan;157:103179. doi: 10.1016/j.critrevonc.2020.103179 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%, tj. nadzór oraz edycję manuskryptu.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, analizę oraz opracowanie metodyki oraz manuskryptu, wizualizację (75%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

KIEROWNIK
Kliniki Onkologii
prof. zw. dr hab. n. med. Rafał Wiesław Stec

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

dr n. med. Leszek Kraj

OŚWIADCZENIE

Jako współautor pracy pt. Milestones in the treatment of hepatocellular carcinoma: A systematic review. Crit Rev Oncol Hematol. 2021 Jan;157:103179. doi: 10.1016/j.critrevonc.2020.103179 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%, tj. wizualizację oraz edycję manuskryptu.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, analizę oraz opracowanie metodyki oraz manuskryptu, wizualizację (75%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

Krzysztof Woźniak

OŚWIADCZENIE

Jako współautor pracy pt. Milestones in the treatment of hepatocellular carcinoma: A systematic review. Crit Rev Oncol Hematol. 2021 Jan;157:103179. doi: 10.1016/j.critrevonc.2020.103179 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 5%, tj. zebranie danych oraz edycję manuskryptu.

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konceptualizację, analizę oraz opracowanie metodyki oraz manuskryptu, wizualizację (75%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

prof. dr hab. n. med. Rafał Stec

OŚWIADCZENIE

Jako współautor pracy pt. The role of tumor-associated macrophages in hepatocellular carcinoma-from bench to bedside: A review. J Gastroenterol Hepatol. 2024 Aug;39(8):1489-1499. doi: 10.1111/jgh.16564 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%, tj. nadzór oraz edycję manuskryptu.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, analizę oraz opracowanie metodyki oraz manuskryptu, wizualizację (80%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

KIEROWNIK
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.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

dr n. med. Leszek Kraj

OŚWIADCZENIE

Jako współautor pracy pt. The role of tumor-associated macrophages in hepatocellular carcinoma-from bench to bedside: A review. J Gastroenterol Hepatol. 2024 Aug;39(8):1489-1499. doi: 10.1111/jgh.16564 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%, tj. wizualizację oraz edycję manuskryptu.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, analizę oraz opracowanie metodyki oraz manuskryptu, wizualizację (80%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025
(miejscowość, data)

Prof. dr hab. n. med. Rafał Wiesław Stec

.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. .Prognostic factors for hepatocellular carcinoma recurrence after liver transplantation or resection - single-center experience. Heliyon. 2024 Nov 13;10(22):e40228. doi: 10.1016/j.heliyon.2024.e40228. oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%, tj. nadzór, dostęp do oprogramowania, korekty.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, opracowanie metodologii oraz przygotowanie manuskryptu (60%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

KIEROWNIK
Kliniki Onkologii
prof. zw. dr hab. n. med. Rafał Wiesław Stec

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji. metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

dr n. med. Leszek Kraj

OŚWIADCZENIE

Jako współautor pracy pt. .Prognostic factors for hepatocellular carcinoma recurrence after liver transplantation or resection - single-center experience. Heliyon. 2024 Nov 13;10(22):e40228. doi: 10.1016/j.heliyon.2024.e40228. oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%, tj. konceptualizację, wizualizację oraz edycję manuskryptu.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

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(podpis oświadczającego)

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Warszawa, 29.05.2025
(miejscowość, data)

Krzysztof Woźniak

.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. .Prognostic factors for hepatocellular carcinoma recurrence after liver transplantation or resection - single-center experience. Heliyon. 2024 Nov 13;10(22):e40228. doi: 10.1016/j.heliyon.2024.e40228. oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 5%, tj. analiza i zebranie danych.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, opracowanie metodologii oraz przygotowanie manuskryptu (60%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

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(imię i nazwisko kandydata do stopnia)

Krzysztof Woźniak
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(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

30.05.2025
(miejscowość, data)

Benedykt Szczepankiewicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Prognostic factors for hepatocellular carcinoma recurrence after liver transplantation or resection - single-center experience. Heliyon. 2024 Nov 13;10(22):e40228. doi: 10.1016/j.heliyon.2024.e40228, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 5%, tj, opracowanie materiału patomorfologicznego.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

Konceptualizację, opracowanie metodologii oraz przygotowanie manuskryptu (60%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

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(imię i nazwisko kandydata do stopnia)

Benedykt Szczepankiewicz

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

Dr hab. n. med. Maciej Krasnodębski

OŚWIADCZENIE

Jako współautor pracy pt. .Prognostic factors for hepatocellular carcinoma recurrence after liver transplantation or resection - single-center experience. Heliyon. 2024 Nov 13;10(22):e40228. doi: 10.1016/j.heliyon.2024.e40228. oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 5%, tj. analizę oraz zebranie danych.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, opracowanie metodologii oraz przygotowanie manuskryptu (60%)

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Warszawa, 29.05.2025

prof. dr hab. n. med. Michał Grąt

OŚWIADCZENIE

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(imię i nazwisko kandydata do stopnia)

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(imię i nazwisko kandydata do stopnia)

KIEROWNIK
Kliniki Chirurgii Transplantacyjnej i Wątroby
Centralny Szpital Kliniczny UCK WUM

prof. dr hab. n. med. Michał Grąt

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

dr n. med. Leszek Kraj

OŚWIADCZENIE

Jako współautor pracy pt. Impact of Thrombocytopenia on Survival in Patients with Hepatocellular Carcinoma: Updated Meta-Analysis and Systematic Review. Cancers (Basel). 2024 Mar 27;16(7):1293. doi: 10.3390/cancers16071293 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 55%, tj. konceptualizację, wizualizację oraz edycję manuskryptu.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, analizę oraz opracowanie manuskryptu, wizualizację (15%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

Paulina Chmiel

OŚWIADCZENIE

Jako współautor pracy pt. Impact of Thrombocytopenia on Survival in Patients with Hepatocellular Carcinoma: Updated Meta-Analysis and Systematic Review. Cancers (Basel). 2024 Mar 27;16(7):1293. doi: 10.3390/cancers16071293 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%, tj. wizualizację oraz edycję manuskryptu.

Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, analizę oraz opracowanie manuskryptu, wizualizację (15%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

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Warszawa, 29.05.2025
(miejscowość, data)

dr hab. n. med. Laretta Grabowska-Derlatka

OŚWIADCZENIE

Jako współautor pracy ptImpact of Thrombocytopenia on Survival in Patients with Hepatocellular Carcinoma: Updated Meta-Analysis and Systematic Review. Cancers (Basel). 2024 Mar 27;16(7):1293. doi: 10.3390/cancers16071293 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 5%, tj. wizualizacja oraz edycja manuskryptu.

Wkład Macieja Gryziak w powstawanie publikacji obejmował konceptualizację, analizę oraz opracowanie manuskryptu, wizualizację (15%).

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

L. Grabowska

.....

(podpis oświadczającego)

Warszawa, 29.05.2025

Łukasz Szymański

OŚWIADCZENIE

Jako współautor pracy pt. Impact of Thrombocytopenia on Survival in Patients with Hepatocellular Carcinoma: Updated Meta-Analysis and Systematic Review. Cancers (Basel). 2024 Mar 27;16(7):1293. doi: 10.3390/cancers16071293 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 5%, tj. wizualizację oraz edycję manuskryptu.

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(imię i nazwisko kandydata do stopnia)

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(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 29.05.2025

Ewa Wysokińska

OŚWIADCZENIE

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Wkład Macieja Gryziaka w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację, analizę oraz opracowanie manuskryptu, wizualizację (15%)

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Macieja Gryziaka.

(imię i nazwisko kandydata do stopnia)

Wysokińska

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

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[2] Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–E386. https://doi.org/10.1002/ijc.29210.

[3] Pons F, Varela M, Llovet JM. Staging systems in hepatocellular carcinoma. HPB (Oxford). 2005;7(1):35–41. https://doi.org/10.1080/13651820410024058.

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Limfocyty naciekające guz (TIL) zaobserwowano w 19 przypadkach. Obecność TIL nie była związana z rokowaniem w żadnej z podgrup ani w populacji ogólnej.

Zgodnie z wynikami analizy jednoczynnikowej w populacji pacjentów chorych na raka wątrobowokomórkowego, poziom PLT należał również do czynników prognostycznych. Z tego względu przeprowadzono meta-analizę i przegląd systematyczny, które stały się podstawą czwartej publikacji w cyklu. Celem pracy było zbadanie wpływu trombocytopenii na przeżycie pacjentów z rozpoznaniem raka wątrobowokomórkowego oraz ocena związku liczby płytek krwi z cechami kliniczno-patologicznymi nowotworu. W ramach pracy przeprowadzono systematyczny przegląd literatury i metaanalizę zgodnie z wytycznymi PRISMA, przeszukano bazy: PubMed, Web of Science i Scopus. Na podstawie wyszukiwania zidentyfikowano 26 badań (łącznie 9403 pacjentów), które spełniały określone kryteria wyszukiwania. Analizowano współczynniki hazardu (HR) dla przeżycia całkowitego (OS) w zależności od liczby płytek krwi. Przeprowadzone analizy wskazują, że trombocytopenia ($<100 \times 10^9$/L) była związana z gorszym przeżyciem całkowitym: HR (model efektów losowych) =1.30 (95% CI: 1.05–1.63), HR (model wieloczynnikowy)= 1.47 (95% CI: 1.15–1.86). W grupie leczonej z zamiarem radykalnym (np. resekcja, RFA) HR=1.62 (95% CI: 1.25–2.11), natomiast w grupie leczonej paliatywnie (np. TACE, leczenie systemowe) HR=0.81 (95% CI: 0.62–1.05) – co sugeruje możliwy odwrotny trend. Ponadto niska liczba płytek była częstsza u pacjentów z mniejszymi guzami i marskością wątroby, natomiast wyższe wartości PLT korelowały z większymi guzami i mniejszą częstością marskości. Wskazuje to na możliwy udział płytek krwi i ich mediatorów (np. czynnik wzrostu płytek krwi, platelet-derived growth factor, PDGF, serotoniny) w progresji HCC. Liczba płytek krwi może być użytecznym, łatwo dostępnym markerem prognostycznym u pacjentów chorych na HCC, szczególnie w leczeniu radykalnym. Niemniej, potrzebne są dalsze badania prospektywne, szczególnie w grupach leczonych systemowo, z uwzględnieniem dokładnych kryteriów włączenia.

Bibliografia

[1] Globoscan. 2020 Available online: http://globocan.iarc.fr/old/FactSheets/cancers/live r-new.asp

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