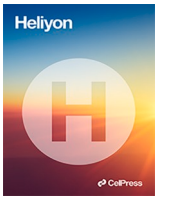
Heliyon 10 (2024) e40228

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

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

Maciej Gryziak [a,*], Rafał Stec [a], Krzysztof Woźniak [a], Benedykt Szczepankiewicz [a], Maciej Krasnodębski [a], Michał Grąt [a], Leszek Kraj [a,b]

[a] Medical University of Warsaw, ul. Żwirki i Wigury 61, 02-091, Warsaw, Poland
[b] Department of Molecular Biology, Institute of Genetics and Animal Biotechnology, Polish Academy of Sci-ences, 05-552, Garbatka, Poland

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

* Corresponding author.
E-mail addresses: maciej.gryziak@wum.edu.pl (M. Gryziak), rafal.stec@wum.edu.pl (R. Stec), krzysztof.wozniak@wum.edu.pl (K. Woźniak), benedykt.szczepankiewicz@wum.edu.pl (B. Szczepankiewicz), maciej.krasnodebski@wum.edu.pl (M. Krasnodębski), michal.grat@wum.edu.pl (M. Grąt), leszek.kraj@wum.edu.pl (L. Kraj).

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.

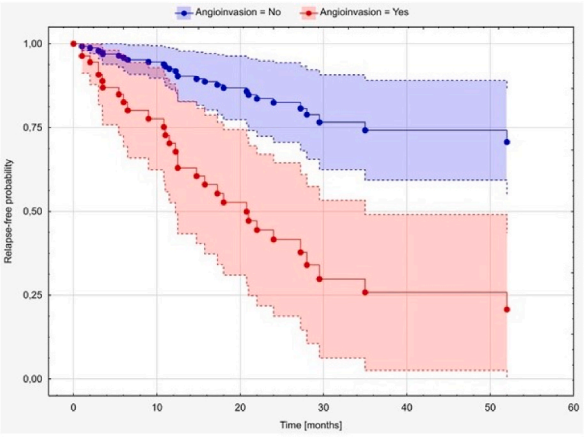


Fig. 1. The impact of microvascular invasion on tumor recurrence.

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

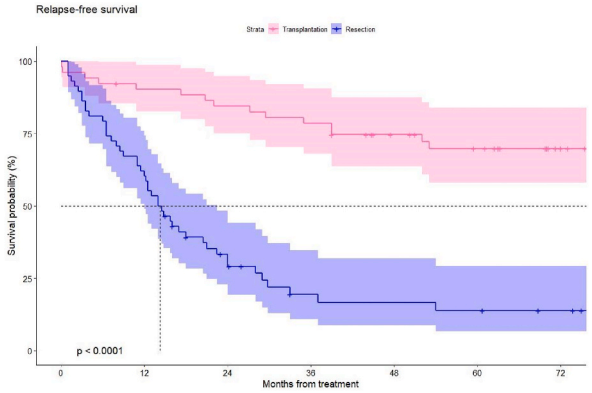


Fig. 2. Relapse free survival according to the treatment method – transplantation vs resection.

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.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 68 (2018) 394–424.
[2] P. Golabi, S. Fazel, M. Otgonsuren, et al., Mortality assessment of patients with hepatocellular carcinoma according to underlying disease and treatment modalities, Medicine (Baltim.) 96 (2017) e5904.
[3] K.A. McGlynn, J.L. Petrick, H.B. El-Serag, Epidemiology of hepatocellular carcinoma, Hepatology 73 (Suppl 1) (2021) 4–13.
[4] A. Vogel, A. Cervantes, I. Chau, et al., ESMO guidelines committee. Hepatocellular carcinoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 29 (Suppl 4) (2018) iv238–iv255. Erratum in: Ann Oncol. 2019 May 1;30(5):871-873. Erratum in: Ann Oncol. 2022;33(6):666.
[5] L. Yang, Y. Zhang, Tumor-associated macrophages: from basic research to clinical application, J. Hematol. Oncol. 10 (1) (2017) 58.
[6] J. Zhang, L. Chang, X. Zhang, et al., Meta-analysis of the prognostic and clinical value of tumor-associated macrophages in hepatocellular carcinoma, J. Invest. Surg. 34 (3) (2021 Mar) 297–306.
[7] Q. Gao, J. Zhou, X.Y. Wang, et al., Infiltrating memory/senescent T cell ratio predicts extrahepatic metastasis of hepatocellular carcinoma, Ann. Surg Oncol. 19 (2) (2012) 455–466.
[8] G. Atanasov, K. Dino, K. Schierle, et al., Recipient hepatic tumor-associated immunologic infiltrates predict outcomes after liver transplantation for hepatocellular carcinoma, Ann. Transplant. 13 (2020) e919414, 25.
[9] I. Larionova, G. Tuguzbaeva, A. Ponomaryova, et al., Tumor-associated macrophages in human breast, colorectal, lung, ovarian and prostate cancers, Front. Oncol. 10 (2020) 566511.
[10] Q. Zhang, M. Sioud, Tumor-associated macrophage subsets: shaping polarization and targeting, Int. J. Mol. Sci. 24 (8) (2023) 7493.
[11] C.Q. Liu, J. Xu, Z.G. Zhou, et al., Expression patterns of programmed death ligand 1 correlate with different microenvironments and patient prognosis in hepatocellular carcinoma, Br. J. Cancer 119 (1) (2018) 80–88.
[12] Z. Zong, J. Zou, R. Mao, et al., M1 macrophages induce PD-L1 expression in hepatocellular carcinoma cells through IL-1β signaling, Front. Immunol. 10 (2019) 1643.
[13] M.E. Keir, M.J. Butte, G.J. Freeman, A.H. Sharpe, PD-1 and its ligands in tolerance and immunity, Annu. Rev. Immunol. 26 (2008) 677–704.
[14] M. Kudo, Immune checkpoint inhibition in hepatocellular carcinoma: basics and ongoing clinical trials, Oncology 92 (Suppl 1) (2017) 50.
[15] T. Mocan, Z. Sparchez, R. Craciun, et al., Programmed cell death protein-1 (PD-1)/programmed death-ligand-1 (PD-L1) axis in hepatocellular carcinoma: prognostic and therapeutic perspectives, Clin. Transl. Oncol. 21 (6) (2019) 702–712.
[16] X.S. Li, J.W. Li, H. Li, et al., Prognostic value of programmed cell death ligand 1 (PD-L1) for hepatocellular carcinoma: a meta-analysis, Biosci. Rep. 40 (4) (2020) BSR20200459.
[17] K.S. Kim, R.R. Sekar, D. Patil, et al., Evaluation of programmed cell death protein 1 (PD-1) expression as a prognostic biomarker in patients with clear cell renal cell carcinoma, OncoImmunology 7 (4) (2018) e1413519.
[18] S. Lassalle, S. Nahon-Esteve, E. Frouin, et al., PD-L1 expression in 65 conjunctival melanomas and its association with clinical outcome, Int. J. Mol. Sci. 21 (23) (2020) 9147.
[19] A.B. El-Khoueiry, B. Sangro, T. Yau, T.S. Crocenzi, et al., Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial, Lancet 389 (10088) (2017) 2492–2502.
[20] H. Chang, W. Jung, A. Kim, et al., Expression and prognostic signifcance of programmed death protein 1 and programmed death ligand-1, and cytotoxic T lymphocyte-associated molecule-4 in hepatocellular carcinoma, APMIS 125 (8) (2017) 690–698.
[21] G. Kan, W. Dong, The expression of PD-L1 APE1 and P53 in hepatocellular carcinoma and its relationship to clinical pathology, Eur. Rev. Med. Pharmacol. Sci. 19 (16) (2015) 3063–3071.
[22] K. Sideras, K. Biermann, J. Verheij, et al., PD-L1, Galectin-9 and $CD8^+$ tumor-infiltrating lymphocytes are associated with survival in hepatocellular carcinoma, OncoImmunology 6 (2) (2017) e1273309.
[23] R. Pei, W. Zhang, S. Wang, et al., Prognostic value of PD-L1 in patients with hepatocellular carcinoma, Clin. Lab. 65 (5) (2019).
[24] H.I. Jung, D. Jeong, S. Ji, et al., Overexpression of PD-L1 and PD-L2 is associated with poor prognosis in patients with hepatocellular carcinoma, Cancer Res Treat 49 (1) (2017) 246–254.
[25] N. de'Angelis, F. Landi, M.C. Carra, et al., Managements of recurrent hepatocellular carcinoma after liver transplantation: a systematic review, World J. Gastroenterol. 21 (39) (2015) 11185–11198.
[26] M. Berenguer, P. Burra, M. Ghobrial, et al., Posttransplant management of recipients undergoing liver transplantation for hepatocellular carcinoma. Working group report from the ILTS transplant Oncology consensus conference, Transplantation 104 (6) (2020) 1143–1149.
[27] Z.Q. Hu, H.Y. Xin, C.B. Luo, et al., Associations among the mutational landscape, immune microenvironment, and prognosis in Chinese patients with hepatocellular carcinoma, Cancer Immunol. Immunother. 70 (2) (2021) 377–389.
[28] M.D. Wang, H. Xiang, L. Zhang, et al., Integration of OV6 expression and CD68+ tumor-associated macrophages with clinical features better predicts the prognosis of patients with hepatocellular carcinoma, Transl Oncol 25 (2022) 101509.
[29] L.Q. Kong, X.D. Zhu, H.X. Xu, et al., The clinical significance of the CD163+ and CD68+ macrophages in patients with hepatocellular carcinoma, PLoS One 8 (3) (2013) e59771.
[30] J.Q. Li, X.J. Yu, Y.C. Wang, et al., Distinct patterns and prognostic values of tumor-infiltrating macrophages in hepatocellular carcinoma and gastric cancer, J. Transl. Med. 15 (1) (2017) 37.
[31] K. Minami, K. Hiwatashi, S. Ueno, et al., Prognostic significance of CD68, CD163 and Folate receptor-β positive macrophages in hepatocellular carcinoma, Exp. Ther. Med. 15 (5) (2018) 4465–4476.
[32] P. Dong, L. Ma, L. Liu, G. Zhao, S. Zhang, L. Dong, R. Xue, S. Chen, $CD86^+/CD206^+$, diametrically polarized tumor-associated macrophages, predict hepatocellular carcinoma patient prognosis, Int. J. Mol. Sci. 17 (3) (2016) 320.
[33] Q.H. Shu, Y.S. Ge, H.X. Ma, et al., Prognostic value of polarized macrophages in patients with hepatocellular carcinoma after curative resection, J. Cell Mol. Med. 6 (2016) 1024–1035.

[34] P.D. Agarwal, M.R. Lucey, Management of hepatocellular carcinoma recurrence after liver transplantation, Ann. Hepatol. 27 (1) (2022) 100654.
[35] N. Mahmud, B. John, T.H. Taddei, et al., Pre-transplant alpha-fetoprotein is associated with post-transplant hepatocellular carcinoma recurrence mortality, Clin. Transplant. 33 (7) (2019) e13634.
[36] K. Berry, G.N. Ioannou, Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma, Liver Transpl 19 (6) (2013) 634–645.
[37] N.A. Filgueira, Hepatocellular carcinoma recurrence after liver transplantation: risk factors, screening and clinical presenta-tion, World J. Hepatol. 11 (3) (2019) 261–272.
[38] M. Najjar, S. Agrawal, JCet al Emond, Pretreatment neutrophil-lymphocyte ratio: useful prognostic biomarker in hepatocellular carcinoma, J. Hepatocell. Carcinoma 5 (2018) 17–28.
[39] A. Nordin, F. Åberg, E. Pukkala, et al., Decreasing incidence of cancer after liver transplantation-A Nordic population-based study over 3 decades, Am. J. Transplant. 18 (4) (2018) 952–963.
[40] Y.E. Chon, J. Cheon, H. Kim, et al., Predictive biomarkers of survival in patients with advanced hepatocellular carcinoma receiving atezolizumab plus bevacizumab treatment, Cancer Med. 12 (3) (2023) 2731–2738.