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Identifying Subgroup at High Risk of Transarterial Chemoembolization Failure Among Patients with Hepatocellular Carcinoma Awaiting Liver Transplantation

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Abstract: Background/Objectives: Transarterial chemoembolization (TACE) is the most widely used bridging treatment for hepatocellular carcinoma (HCC) before liver transplantation (LT) but may be associated with dropout and post-LT HCC recurrence. We aimed to identify a subgroup of HCC LT candidates at high risk of TACE-to-LT strategy failure (TLSF). Methods: All consecutive HCC LT candidates with French AFP-scores ≤ 2 who underwent at least one bridging TACE at Paul Brousse Hospital in 2013–2018 were included (n = 173). Dropout for HCC progression during waiting list and post-LT HCC recurrence was defined TLSF. Results: The one-year TLSF cumulative incidence was 15%. According to univariate analysis, pre-TACE AFP > 15 ng/mL was the only factor associated with decreased overall survival (OS) and TLSF-free survival (TLSF-FS) after the first TACE. The absence of complete radiological response (CRR) or pre-TACE AFP > 15 ng/mL were associated with reduced OS and TLSF-FS after a second TACE (n = 118). The cumulative incidence of TLSF reached 41% one year after the second TACE in patients with both AFP > 15 ng/mL and no CRR, while it was 7% for others ($p < 0.001$). Conclusions: HCC patients receiving bridging TACE, with pre-TACE AFP > 15 ng/mL and no CRR after two TACEs, are at high risk of delisting for HCC progression or of post-LT recurrence. Alternative therapeutic strategies should be proposed early for this better-defined population.

Keywords: hepatocellular carcinoma; transarterial chemoembolization; liver transplantation; TACE failure; liver cancer

1. Introduction

Liver transplantation (LT) is the best option for the curative treatment of hepatocellular carcinoma (HCC) in cirrhotic patients because it simultaneously treats all tumors with maximal margins and the underlying disease that represents a high-risk factor for the development of new tumors. Organ shortage and the increasing number of patients on the waiting list for the many other indications for LT severely limit immediate access to LT for HCC patients, and a "waiting period" of several months to years is common [1]. Tumor control during the LT waiting time is essential to avoid delisting due to tumor progression and to reduce the risk of tumor recurrence after transplantation [2].

Transarterial chemoembolization (TACE) remains the most widely used treatment for patients with intermediate-stage HCC [3], and, in the LT setting, it may be used as a bridging [4] or a downstaging treatment [5]. It involves the catheter-based selective infusion of cytotoxic chemotherapy agents directly into the tumor's blood supply via the hepatic artery, together with the embolization of tumor-feeding vessels with embolic agents. This dual approach aims to deliver high concentrations of chemotherapeutic agents locally while inducing ischemia and necrosis in the tumor through vascular occlusion [6]. TACE is recognized as a standard of care in European [3] and American guidelines [7]. Multiple sessions of TACE are often performed for persistent tumor activity or in cases of new tumors [8,9]. Overall, the TACE-to-LT approach can offer good results, despite a dropout rate of 15% at one year [10] and a post-LT recurrence rate of about 20% [11–13].

While the indications for TACE are approved worldwide, there are no validated stopping rules to avoid harmful TACE without adequate therapeutic efficacy or validated scores for defining refractoriness to TACE. Furthermore, the proposed scores are not used in clinical practice or adaptable to different clinical settings. The definition of TACE failure/refractoriness is an issue for experts worldwide [14–16]. Indeed, the benefit of repeated TACE in the absence of complete imaging response in patients awaiting LT remains debated.

This study aimed to identify more accurate clinical, biological, and/or radiological criteria predicting TACE-to-LT strategy failure (TLSF) defined by dropout for tumor progression or HCC recurrence after LT in a large cohort of patients enlisted for LT and supposed to be at low risk of dropout and post-LT recurrence (i.e., within AFP score $\leq$2) and treated by TACE as a bridging treatment for HCC.

2. Materials and Methods

2.1. Study Population

We assessed all consecutive adult patients (n = 173) with HCC initially eligible for LT who received at least one TACE as a bridging treatment between January 2013 and December 2018 at Paul Brousse Hospital, Villejuif, France. Eligibility for LT was defined by the combination of (i) a French AFP score $\leq$2, a validated predictor of HCC recurrence after LT, currently used in France, and (ii) no age- or comorbidity-related contraindications to LT. Patients with HCC initially beyond the transplantation criteria (French AFP score > 2) and patients who could not be treated with TACE as a bridging therapy before LT because of poor liver function (Child–Pugh score > B7 or MELD score > 10) or technical/vascular contraindication were not included.

The diagnosis and evaluation of HCC were based on MRI and CT scan imaging, re-read by an expert radiologist according to validated international criteria [7,17], and were confirmed after LT on pathological examination of the native liver.

Information regarding follow-up and outcomes after LT was obtained from our prospectively maintained LT database. Follow-up data included clinical (i.e., recipient survival, observance of immunosuppressive treatment, and short- and long-term complica-

tions), biological (i.e., graft function, tumor markers, and renal function) and radiological (i.e., complete response, partial response, or progressive disease) data. Outcome data included grafts and recipients' survival and HCC recurrence.

This study was undertaken in accordance with French legislation.

2.2. Choice of HCC Treatment and TACE Procedure

In patients with well-preserved liver function, therapeutic options were discussed at a multidisciplinary board involving hepatologists, oncologists, hepatobiliary surgeons, radiotherapists, and interventional radiologists. Our strategy was to favor liver resection or thermal ablation in patients with a single peripheral HCC and good liver function (MELD < 10). In contrast, upfront LT was proposed for patients with either multiple nodules, deeply localized tumors, indirect evidence of portal hypertension, or impaired liver function (MELD 10–20). LT was also the treatment of choice for recurrent HCC after liver resection, as previously reported [18].

In the study cohort, TACE was the only feasible treatment option for each patient with active and evolutive HCC, not eligible for resection or RFA, and with a MELD score <10 and an expected waiting list time of at least six months. This strategy remained unchanged throughout the study period.

Hyperselective TACE was performed in patients with an HCC with a tumor-feeding artery, mainly in Child–Pugh score B <7 patients, whereas lobar TACE was chosen in the other cases. In the presence of bilobar nodules, sequential TACE (right then left or conversely) was usually performed at 6-week intervals. Intra-arterial injection of a mixture of antitumoral agent (doxorubicin) and lipiodol followed by resorbable microembolic particles was performed as previously described [6,19], through femoral access under radioscopic guidance. Lipiodol (Guerbet, Paris, France) was used both as a vehicle to carry and localize the chemotherapeutic agent within the tumor and as a microembolic agent.

2.3. Evaluation of Response After TACE

Thirty days after the first TACE procedure, all patients' response to treatment was assessed using an abdominal CT scan and MRI, serum AFP levels, and liver function tests. Expert radiologists assessed the radiological response according to modified RECIST criteria as reported by the American Association for the Study of Liver Diseases [17] and evaluated by the local multidisciplinary board.

Patients presenting with a complete radiological response (CRR) were followed up every three months by a CT scan. An additional TACE session was discussed for patients without CR or in cases of new tumor development, and the same strategy was validated after each TACE session.

Post-TACE LT was performed in patients with a French AFP score <2, according to national guidelines [11].

TLSF was defined by the occurrence of delisting for tumor progression beyond LT criteria (French AFP score > 2) or of post-LT HCC recurrence.

2.4. Histological and Immunohistochemical Analysis of Native Livers

Native livers from all transplanted patients with post-LT HCC recurrence and 18 matched control patients without post-LT HCC recurrence were analyzed. Surgical specimens were fixed with 4% neutral formaldehyde. All tumors and non-tumoral livers were sampled. For each tumor, two to 12 blocks were sampled from tumoral and peritumoral tissue depending on the tumor size, with the rule of at least one block per 1 cm of the largest diameter. Blocks were embedded in paraffin and cut at 3 µm thickness. Slides were stained with hemalun–eosin and saffron and examined by experts at the pathology department of Bicêtre Hospital according to the latest knowledge on HCC histology [20,21].

Immunohistochemistry was performed on tissue sections deparaffinized in Bond Leica immunostainer (Bond III-Leica Biosystems) with anti-EpCAM (cloneVU-1D8-OZYME) [22] and anti-beta-catenin (clone 14/Beta-catenin-CliniScience) [23] antibodies.

Native livers were analyzed for the following features: number of HCC nodules, nodule size, WHO grade, presence of macro or microvascular invasion, and satellite nodules. The presence of peritumoral ductular reaction (PDR) was assessed semi-quantitatively.

EpCAM expression in tumor cells was assessed as % of positive tumor cells. Beta-catenin immunostaining was described as membranous, cytoplasmic, or nuclear with a semi-quantitative evaluation of nuclear staining.

2.5. Post-Transplant Management

No adjuvant therapy was administered after transplantation, even in recipients with poor prognostic features on liver explant. Post-LT follow-up was performed with a CT scan and serum AFP every 3 months.

2.6. Statistics

Continuous variables were expressed as median (range) and compared with the Wilcoxon test. The chi-square test and Fisher test were used to compare categorical variables.

To assess the distribution of the data, a normality test was conducted using the Shapiro–Wilk test, with a *p*-value threshold of 0.05 to determine if the data deviated significantly from a normal distribution.

The purpose of our analysis was to assess the response to TACE. Thus, time for survival analysis was calculated from the date of the 1st and 2nd TACE response evaluation to limit immortality bias.

The event of interest for TLSF-free survival (TLSF-FS) was dropout for tumor progression, HCC recurrence after LT, or death. After the first TACE, patients were exposed to a variety of potential outcomes, such as transplantation, delisting for tumor progression, and death for non-tumor-related causes, which subsequently affected the likelihood of undergoing a second TACE. Therefore, we performed a second analysis on a subgroup of patients who underwent a 2nd TACE, using the date of the 2nd TACE. The Kaplan–Meier method was used to calculate the probability of survival and plot survival curves. Cox proportional hazard models were used after the univariate analysis. The final multivariate model was decided on the basis of the lowest Akaike information criterion. The proportional hazard assumption was tested using Schoenfeld residuals.

In the last step, the cumulative incidence of TLSF was assessed using the competing risk model proposed by Fine and Gray [24], in which death unrelated to HCC (occurring either during the waiting period or after LT) was considered as a potentially competing event.

3. Results

3.1. Characteristics of the Study Population

Supplementary Figure S1 shows the flow chart over the entire study period. Table 1 shows the characteristics of the study population. A total of 173 cirrhotic patients were included. Most patients were men (85.5%), with a median age of 61 years and a majority with cirrhosis of viral and alcoholic origin. NASH degree stratification was not performed and NASH was only reported as part of the etiology of chronic liver disease. The median number of HCC nodules was two per patient, and the median tumor diameter was 23 mm, confirming the relevance of the indication for TACE. On explanted livers, only thirteen non-HCC liver cancers were observed: one cholangiocarcinoma and twelve hepatocholangiocarcinomas. All patients recorded a French AFP score <2, and 66.9% were within the Milan criteria. It should be noted that patients' inclusion in this study started in 2013,

after the introduction of direct antiviral agents for HCV treatment, meaning that, as LT candidates, most of our patients obtained HCV eradication.

Table 1. Characteristics of the study population.

Variables	Study Population N = 173
Age	61 (33–74)
Male/Female	148 (85.5)/25 (14.5)
Ethnicity	
White	144 (83.2)
African-American	28 (16.2)
Others	1 (0.6)
Cause of liver disease	
Virus	66 (38.2)
Alcohol	70 (40.5)
NASH	22 (12.7)
Others	15 (8.7)
Child-Pugh score A/B	127 (73.4)/46 (26.6)
Previous hepatic resection	44 (25.4)
Before TACE 1	
Initial MELD score	8.00 (6.00–20.00)
Initial AFP, ng/mL	9 (2–780)
Number of tumors	2 (1–6)
Maximum tumor diameter, mm	23 (7–60)
Milan criteria inside/outside	133 (66.9)/40 (23.1)
AFP score 0/1/2	87 (51)/50 (28.9)/36 (20.1)
After TACE 1	
Residual tumor activity on imaging after TACE 1	129 (74.6)
Number of patients with a second TACE 2	118 (68.2)
Residual tumor activity on imaging after TACE 2	83 (70%)
Outcomes at last follow-up	
Transplanted	128 (74.0)
Still on waiting list	6 (3.5)
Delisted	39 (22.5)
Delisted for tumor progression	32 (18.5)
Transplanted group–explanted livers	N = 128
Interval between TACE 1 - LT, months	12 (1–67)
Complete pathological response	22 (17.2)
Other than HCC (IHCCA or HCC-IHCCA)	13 (10.1)
Microvascular invasion	47 (36.7)
Maximun tumor size, mm	20 (8–75)
Number of tumors	2 (0–37)
Differentiation grade	
Unknown	1 (1.0)
Well	22 (22.7)
Moderate	48 (49.5)
Poor	26 (26.8)

Categorical variables are expressed as number of patients (percentage) and quantitative variables are expressed as median (inter-quartile range).

After the first TACE, CRR was observed in 44 (25.4%) patients. A second TACE was performed in 118 patients after a median interval of 3 months between the first and second TACE.

HCC progression leading to dropout was observed in 32 patients out of 173 (18.4%). With a median follow-up of 65 months since the first TACE, 128 patients (74.6%) were transplanted after a median time of 12 months. On histology, a complete pathological response was observed in only 22 (17.2%) patients. Among the transplanted patients, 18 (14%) developed post-LT HCC recurrence.

3.2. Survival Analysis After First and Second TACE Sessions

The overall survival (OS) rate of the cohort was 63% at 5 years. The probability of being alive without progression beyond the transplantation criteria or post-LT recurrence was 56% at 5 years. Table 2 shows the results of univariate analysis for OS and TLSF-FS in patients treated by at least one TACE. Initial AFP > 15 ng/mL was the only factor associated with both decreased OS and TLSF-FS.

Table 2. Univariate analysis among patients treated with at least one TACE.

		Overall Survival				**TLSF-Free Survival**			
	n	**Events (n)**	**HR**	**95% CI**	***p***	**Events (n)**	**HR**	**95% CI**	***p***
Sex									
Male	148	57	1.000			63	1.000		
Female	25	9	0.877	0.434–1.772	0.714	10	0.861	0.442–1.679	0.661
Age, yrs	173		1.004	0.969–1.041	0.812		0.996	0.964–1.03	0.821
Underlying disease									
Virus	66	20	1.000			24	1.000		
Alcohol	70	32	1.816	1.038–3.177	0.036	33	1.415	0.836–2.395	0.196
NASH	22	7	1.139	0.481–2.697	0.767	7	0.867	0.374–2.014	0.741
Others	15	7	2.047	0.863–4.852	0.104	9	1.814	0.842–3.911	0.128
MELD score before TACE1	173		1.008	0.93–1.092	0.853		1.005	0.932–1.084	0.893
Child-Pugh									
Child A	127	47	1.000			51	1.000		
Child B	46	19	1.192	0.699–2.033	0.518	22	1.259	0.764–2.077	0.366
Previous liver resection									
No	129	50	1.000			54	1.000		
Yes	44	16	0.989	0.563–1.739	0.970	19	1.189	0.704–2.009	0.518
AFP score									
0	104	39	1.000			43	1.000		
1	35	16	1.285	0.718–2.301	0.398	18	1.335	0.77–2.315	0.304
2	34	11	0.945	0.483–1.85	0.870	12	0.884	0.465–1.677	0.705

Table 2. *Cont.*

	Overall Survival					TLSF-Free Survival			
	n	**Events (n)**	**HR**	**95% CI**	***p***	**Events (n)**	**HR**	**95% CI**	***p***
AFP > 15 ng/mL									
No	117	38	1.000			43	1.000		
Yes	54	28	1.782	1.091–2.911	0.021	30	1.954	1.224–3.12	0.005
Number of Tumors on imaging	173		0.933	0.769–1.131	0.479		0.937	0.78–1.125	0.486
Max tumor size on imaging, mm	173		1.009	0.987–1.031	0.445		1.010	0.989–1.032	0.343
Milan criteria									
Inside	133	52	1.000			57	1.000		
Outside	40	14	0.914	0.506–1.652	0.766	16	0.909	0.521–1.583	0.735
Tumor activity on imaging after TACE1									
No	44	14	1.000			15	1.000		
Yes	129	52	1.316	0.729–2.376	0.362	58	1.440	0.816–2.541	0.208

Table 3 shows the results of univariate analysis for OS and TLSF-FS in patients who received at least two TACEs (n = 118). Incomplete response after the second TACE and AFP > 15 g/mL were associated with decreased OS and TLSF-FS. Kaplan–Meier survival curves according to response after the second TACE are shown in Figure 1. Both characteristics remained significant in the multivariate Cox model for OS and TLSF-FS (Table 4). The cumulative incidence of TLSF was 4% one year after a complete response to the 2nd TACE versus 20% in patients with an incomplete response ($p < 0.001$; Figure 2A). Patients with initial AFP > 15 ng/mL and incomplete response to the second TACE had a 41% incidence of failure versus 7% for others ($p < 0.001$; Figure 2B). Neither the radiological response nor the initial AFP level influenced the incidence of death from non-tumoral causes.

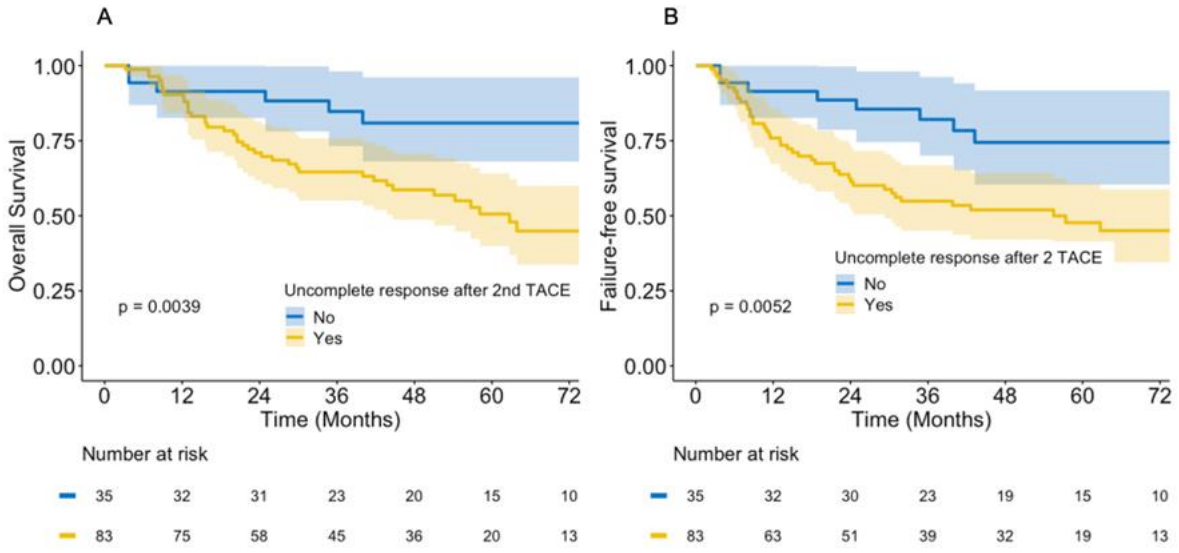

Figure 1. Kaplan–Meier survival curves after second TACE. (**A**) overall survival after 2nd TACE; (**B**) failure-free survival after 2nd TACE.

Table 3. Univariate analysis among patients treated with at least two TACEs.

	Overall Survival					TLSF-Free Survival			
	n	**Events (n)**	**HR**	**95% CI**	***p***	**Events (n)**	**HR**	**95% CI**	***p***
Sex									
Male	102	40	1.000			44	1.000		
Female	16	6	0.857	0.363–2.022	0.724	7	0.934	0.420–2.076	0.867
Age, yrs	118		0.994	0.952–1.038	0.793		0.993	0.952–1.035	0.725
Underlying Disease									
Virus	43	15	1.000			17	1.000		
Alcohol	47	21	1.504	0.775–2.921	0.228	22	1.344	0.713–2.534	0.361
NASH	16	5	0.948	0.344–2.613	0.917	5	0.796	0.291–2.148	0.645
Others	12	5	1,360	0.493–3.753	0.553	7	1.690	0.698–4.091	0.245
MELD score before TACE1	118		1.012	0.923–1.109	0.803		1.012	0.928–1.103	0.793
Child_Pugh									
Child A	89	34	1.000			37	1.000		
Child B	29	12	1.182	0.610–2.290	0.620	14	1.329	0.716–2.467	0.368
Previous liver resection									
No	88	34	1.000			37	1.000		
Yes	30	12	1.150	0.594–2.227	0.678	14	1.236	0.667–2.290	0.500
AFP score									
0	66	26	1.000			29	1.000		
1	26	13	1.357	0.697–2.642	0.370	14	1.329	0.702–2.517	0.383
2	26	7	0.718	0.311–1.659	0.438	8	0.710	0.324–1.557	0.393
AFP > 15 ng/mL									
No	82	26	1.000			30	1.000		
Yes	34	20	2.477	1.371–4.476	0.003	21	2.409	1.369–4.238	0.002
Number of Tumor on imaging	118		0.847	0.671–1.068	0.160		0.857	0.687–1.069	0.171
Max tumor size on imaging, mm	118		1.002	0.975–1.030	0.891		1.003	0.977–1.029	0.826
Milan criteria									
Inside	83	35	1.000			38	1.000		
Outside	35	11	0.714	0.362–1.410	0.332	13	0.757	0.403–1.425	0.389
Tumor activity on imaging after TACE2									
No	35	6	1.000			8	1.000		
Yes	83	40	3.306	1.399–7.810	0.006	43	2.806	1.318–5.976	0.007
Multivariate analysis									
			Overall survival			TLSF -free survival			
			HR	95% CI	*p*	HR		95% CI	*p*
AFP > 15 ng/mL			2.262	1.241–4.113	0.007	2.208		1.250–3.902	0.006
Uncomplete response after 2nd TACE			3.213	1.353–7.628	0.008	2.719		1.271–5.815	0.009

Table 4. Multivariate analysis from second TACE.

	Overall Survival			TLSF -Free Survival		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
AFP > 15 ng/mL	2.2621	1.241–4.113	0.007	2.208	1.250–3.902	0.006
Uncomplete response after 2nd TACE	3.2130	1.353–7.628	0.008	2.719	1.271 - 5.815	0.009

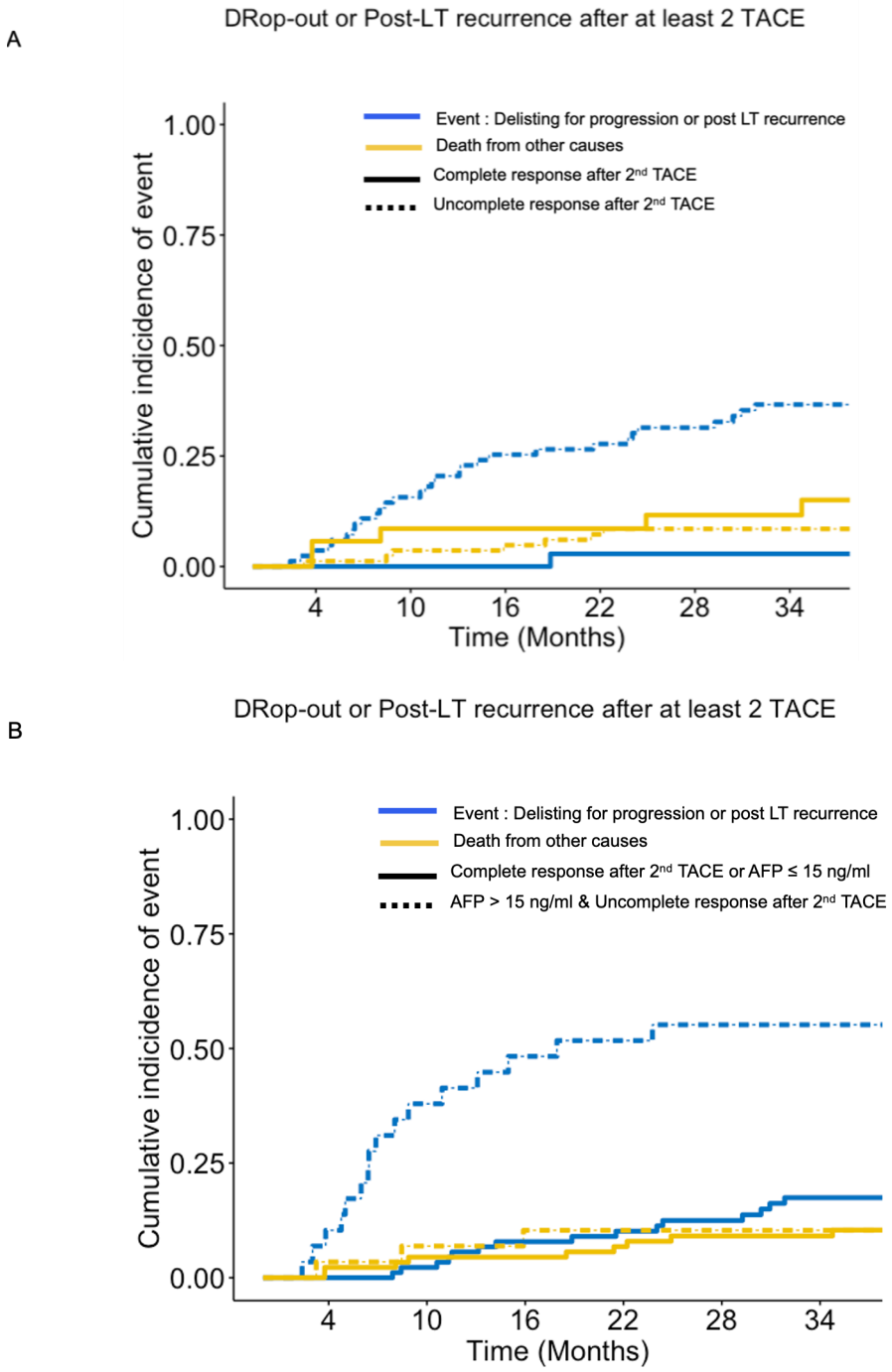

Figure 2. Cumulative incidence of failure after second TACE. (**A**) Cumulative incidence of failure after second TACE according to the radiological response (complete or incomplete radiological response after second TACE); (**B**) Cumulative incidence of failure after second TACE according to the presence of both AFP > 15 ng/mL and uncomplete response after second TACE or the presence of just one of these unfavorable characteristics.

3.3. Scoring System for Prediction of Poor Outcome After Second TACE

We derived a simple scoring system, attributing one point for each present factor (AFP > 15 ng/mL before the first TACE and lack of complete response on imaging after the 2nd TACE), to better understand the clinical significance of the Cox model. The observed survival according to each patient group (0 points, n = 32; 1 point, n = 57; 2 points, n = 26), shown in Figure 3, indicates that patients in the 2-point group have poor outcomes after the second TACE and provide early identification of a high-risk subgroup on the waiting list.

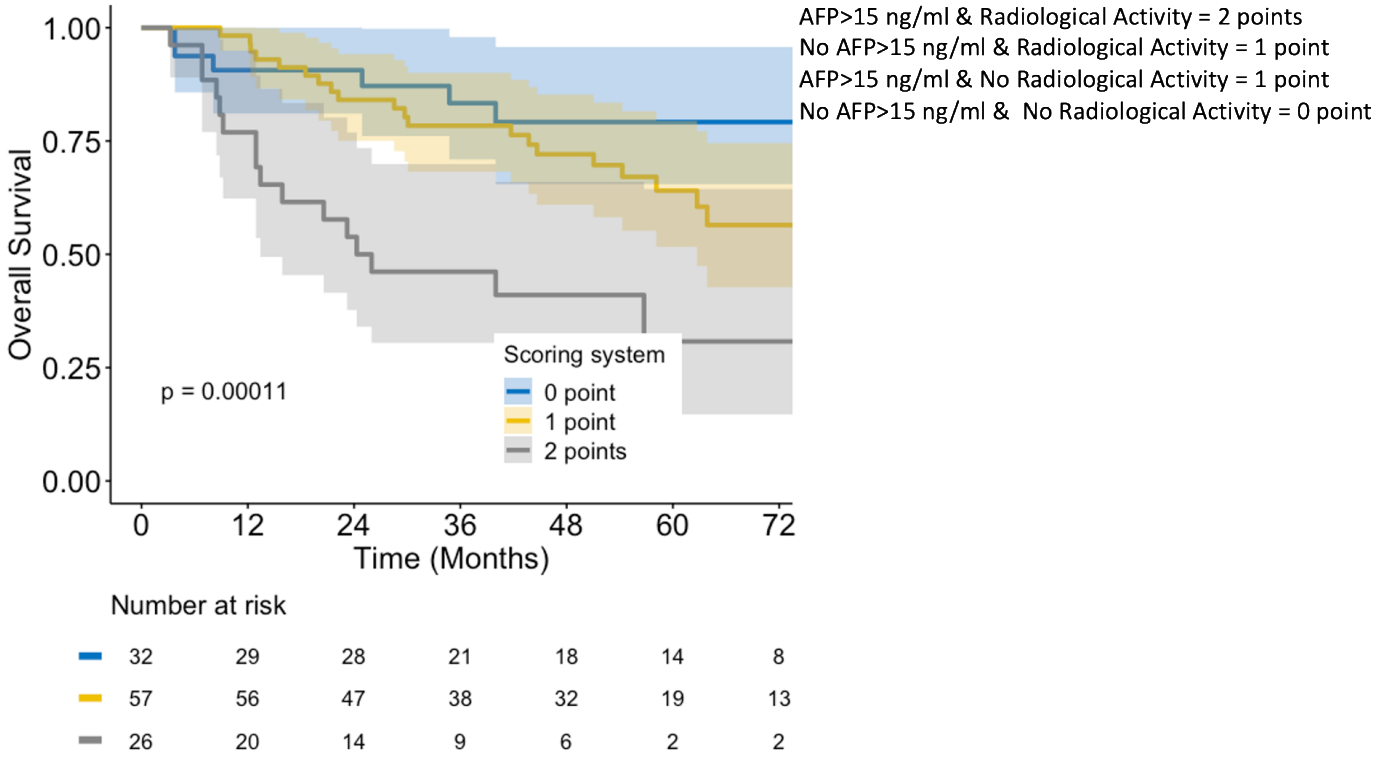

Figure 3. Scoring system for prediction of poor outcome after second TACE.

3.4. Pathological Examination of Native Livers

The native livers from the 18 transplanted patients who developed post-LT HCC recurrence during follow-up were retrospectively assessed. A control group of 18 matched native livers of patients who did not develop post-LT HCC recurrence was formed for comparison. These patients were matched for age, sex, cirrhosis etiology, liver disease severity by MELD and Child–Pugh scores, time of LT, French AFP score, and serum AFP levels.

The main histological prognostic factors were not significantly different between the two groups of patients. In contrast, we found that PDR was significantly more frequent in the native livers of patients with post-LT HCC recurrence than the native livers of those without tumor recurrence (27.5% vs. 9%, $p < 0.001$). Six of the 36 patients underwent liver resection at the initial diagnosis of HCC before TACE and LT. No PDR was identified at the time of liver resection. In addition, 21 patients (75%) among the 28 treated with at least two TACEs presented PDR in the explanted liver compared with three (37.5%) patients among the eight treated with only one TACE session ($p = 0.047$). EpCAM expression by tumor cells was observed in six (33%) of eighteen patients who developed post-LT HCC recurrence during follow-up, whereas only one of eighteen patients without post-LT HCC recurrence had HCC with significant EpCAM expression.

In contrast, no differences in the histological expression profiles of beta-catenin were found between tumoral and peritumoral areas. In the post-LT HCC recurrence group,

twelve (66.6%) patients had a membrane, six (33.3%) cytoplasmic, and four (22.2%) nuclear beta-catenin expression patterns. In the group without post-LT HCC recurrence, twelve (66.6%) patients had a membrane expression pattern, six (33.3%) cytoplasmic, and five (27.8%) nuclear beta-catenin expression patterns. Altogether, these data suggest a possible association between the number of TACE sessions and liver stem/progenitor cell emergence or activation.

4. Discussion

Patients enlisted for LT for HCC who are under the criterion French AFP score are theoretically considered a population at low risk of dropout or recurrence. Bridging treatments are usually proposed for these patients due to an expected waiting time longer than six months due to organ shortage [25]. Most of the literature on LT for HCC targets the dropout risk and post-LT HCC recurrence in a population treated for HCC downstaging, considering that patients treated for bridging to LT have a better outcome. Several scoring systems for TACE failure have also been described in the literature, and in particular Lai et al. from EurHeCaLT have nicely demonstrated in a large European cohort that MELD, AFP, initial, tumor burden and radiological response to locoregional treatments impact waitlist outcomes and survival benefit [26]. Nevertheless, in the present study, we found that in this homogeneous cohort of patients fulfilling HCC transplant criteria who received TACE only as a bridging therapy, two simple clinically appliable sources of data, namely a high pretreatment serum AFP level (>15 ng/mL) and lack of CRR after a second TACE are major risk factors for TLSF, leading to higher dropout rates before LT or tumor recurrence after LT. The expected LT waiting time of longer than six months is the main and substantial difference from the previously published paper on this topic by Affonso et al. [4]. While the criteria for enlisting HCC patients in our center were very restrictive (i.e., French AFP $\leq$ 2 [11]), the cumulative incidence of dropout before LT or of recurrence after LT in the subgroup with the above characteristics reached 41%. This result by itself suggests that there is a high-risk subgroup of HCC patients even in this overall low-risk population. Our findings show that this subgroup can be identified while on the waiting list using simple and robust criteria, namely serum AFP and radiological response to TACE, and could thereby benefit from an early change in bridging therapeutic strategy.

Complete histological response of the tumor in the native liver of patients previously treated with bridging treatments is a highly positive prognostic factor indicating a low risk of HCC recurrence after LT, [27] but the pathological analysis is by definition available only after LT. However, it is crucial for the LT community to have reliable and early markers predictive of outcomes in this setting (i.e., the dropout risk or tumor recurrence) identified.

Elevated serum AFP has been recognized as a marker of biological aggressiveness for decades [28,29] and AFP is consequently incorporated into the majority of current LT selection criteria. [11,12,30] Here, we show that even slightly elevated pretreatment (pre-first TACE) AFP (>15 ng/mL) is associated with lower overall and failure-free survival, not only after the first TACE but also after the second TACE. The predictive value of AFP for dropout has been established [9,31,32] and our results are in accordance with the literature. However, they further show that even in highly selected patients with low tumor burden and within the well-defined French LT AFP score, [11] a slight increase in AFP remains by itself a major prognostic factor both before and after LT.

Among other prognostic criteria available before LT, the relationship between tumor burden and the number of TACE sessions required to control the tumor with the outcome after LT has been reported [2,29,30,33]. Indeed, recent studies have shown that patients treated with TACE as a bridging therapy before LT required fewer TACE sessions, had smaller tumors, and had a lower incidence of post-LT recurrence. In contrast, patients

treated with TACE as a downstaging treatment required more TACE sessions, had larger tumors, longer waiting times, and higher post-LT HCC recurrence rates [4,33,34].

The prognostic value of morphologic response to TACE as shown by imaging has also been highlighted previously, including in patients enlisted for LT [35]. The study by Otto et al. showed that imaging after pre-LT TACE is the most reliable dynamic criterion for the correct classification of pre-LT HCC and is associated with subsequent oncologic outcomes. Furthermore, Kim et al. reported that HCC patients with CRR after a first TACE session outside the context of LT had the most prolonged OS, followed by those who achieved CRR after two or more TACE sessions, suggesting that achieving an early complete response is a major predictor of favorable oncologic outcomes [36]. These findings are in agreement with our results, showing that persistent radiological tumor activity combined with pretreatment AFP level has a strong value in predicting oncologic outcomes in the context of LT for HCC. The simple compound score we describe here thus offered an early signal to change the therapeutic strategy in the face of a high risk of TACE failure.

Whether patients with these two unfavorable characteristics but still within LT criteria should be delisted remains uncertain and cannot be definitively addressed here. However, once a high risk of strategy failure in these patients is known, other therapeutic alternatives should be discussed to reduce the risk of HCC progression and post-LT recurrence, especially in this time of organ shortage. The number of therapeutic options in intermediate–advanced HCC has considerably increased in recent years, and many trials are ongoing, with results urgently awaited. It is premature to address the question of the best therapy, but we can hypothesize that the high-risk subgroup identified here should benefit from other therapeutic approaches, namely systemic and combined treatments [37–39].

Finally, we report here that HCC nodules from the explanted livers of patients with HCC recurrence after LT frequently exhibited a specific histologic phenotype characterized by EpCAM expression and the presence of PDR, both of which have been previously associated with a worse prognosis of HCC [22,40,41]. In addition, a significant proportion of these patients had a mixed hepatocholangiocarcinoma that could not be identified by imaging alone before LT. Interestingly, this particular phenotype was not detected in the treatment-naive liver tumors resected before TACE and LT. These tumor phenotypic changes may be associated, at least in part, with the number of TACEs by inducing repeated hypoxia by selecting more aggressive tumor cell clones and reprogramming to a biliary and/or liver progenitor phenotype [42]. Unfortunately, we did not have information on PDR presence and EpCAM expression in the patients who dropped out because of tumor progression, so their role in HCC progression beyond LT criteria cannot be definitively demonstrated here.

The strength of this study was that patient selection criteria for LT, repeat-TACE policy, and graft allocation rules for HCC patients remained the same throughout the study period and are still applied in all French centers. This is warranted by the homogeneity of the population and the pertinence of the strategy.

However, this study had several limitations: it included a relatively limited number of patients, an external validation cohort was not available, it is a retrospective and monocentric study, and no histological information on PDR presence and EpCAM expression in patients who dropped out because of tumor progression was available.

5. Conclusions

In conclusion, we confirm that TACE may be an excellent bridging therapeutic option for patients with an expected waiting time longer than 6 months. Furthermore, we have shown that the peculiar sub-group of HCC patients with an initial AFP > 15 ng/mL and no CRR after a second TACE represents a high-risk subgroup for TACE-based strategy

failure, even in a selected population considered at low risk of dropout or recurrence. These patients should be considered for an early change in therapeutic approach towards other locoregional and/or systemic therapies. External validation and additional studies in larger populations, including patients with different ethnicities, should be able to confirm these data before using them in new recommendations for therapeutic options before LT.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/livers5010009/s1, Figure S1: Flow chart of patient inclusion during the study period.

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Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author, E.P.

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