Partial Necrosis on Hepatocellular Carcinoma Nodules Facilitates Tumor Recurrence after Liver Transplantation

Matteo Ravaioli,[1] Gian Luca Grazi,[1] Giorgio Ercolani,[1] Michelangelo Fiorentino,[2] Matteo Cescon,[1] Rita Golfieri,[3] Franco Trevisani,[4] Walter Franco Grigioni,[2] Luigi Bolondi,[5] and Antonio Daniele Pinna[1,6]

Background. The presence of partial necrosis in hepatocellular carcinoma (HCC) nodules is a common histologic finding after liver transplantation, but its correlation with tumor recurrence has never been investigated.
Methods. we retrospectively reviewed the outcome of 54 patients with a single histologically proven HCC after liver transplantation. All cases had a survival of more than 6 months, and patients treated preoperatively had a transarterial chemoembolization (TACE) procedure. Since 1996, our center has applied the Milan criteria. Correlations between tumor recurrences and clinicopathologic variables, including the presence of partial necrosis, were performed. Etiologic factors for HCC partial necrosis were also investigated.
Results. Sixteen of 54 (29.6%) HCC nodules presented partial necrosis, and 4 (25%) of them developed HCC recurrence compared with 1 of 38 (2.6%) cases without this histologic finding ($P<0.05$). Partial necrosis was related to TACE procedure ($P<0.05$), patient age less than 50 years ($P<0.05$), and tumor diameter greater than 2 cm ($P<0.05$). Multivariate analysis showed only TACE as an independent variable. The other variables related to the five (9.3%) tumor recurrences were HCC diameter greater than 2 cm ($P<0.05$), year of liver transplantation before 1996 ($P<0.05$), and the presence of satellite nodules ($P<0.05$). The Cox regression analysis showed the presence of partial necrosis as an independent variable related to tumor recurrence. The analysis of the recurrence-free survival confirmed the results of the recurrence rate.
Conclusion. Partial necrosis was a risk factor for tumor recurrence after liver transplantation. Patients and procedures should be selected while also bearing in mind the side-effect of incomplete necrosis of the nodules.

Keywords: Selection criteria, Transarterial chemoembolization, Survival, Recurrence-free survival, Hepatocellular carcinoma.

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Liver transplantation (LT) is an established effective treatment for small hepatocellular carcinomas (HCCs) on cirrhosis (*1*), but many issues continue to be debated: the ideal tumor criteria of selection (*2*, *3*), the clinicopathologic variables related to recurrence (*4–7*), the best scoring system to allocate organs for these patients (*8*, *9*), and the most appropriate preoperative treatments to prevent the progression of the tumor and consequent patient dropout of the waiting list (*10–12*). We reviewed our series of LT for HCC on cirrhosis to add clinical evidence to preoperative treatments and to their relation with partial necrosis and tumor recurrence.

Although transarterial chemoembolization (TACE) therapies have been extensively investigated to improve patient survival before LTs and liver resections (*2*, *13–15*), standard protocols well accepted by all centers are still lacking, particularly currently with the diffusion of percutaneous treatments such as radiofrequency ablation (*16*).

The efficacy of TACE and percutaneous therapies has been evaluated by analyzing patient survival and the percentage of necrosis in the nodules, which may range from 0% to 100% (*17*). Extensive necrosis is supposed to reduce the cancer growth rate and to have a beneficial effect on patient survival (*14*, *18*).

No reports have investigated the relationship between necrosis and HCC recurrence after LT, although previous studies on liver resections have suggested a correlation between this histologic feature and tumor recurrence (*17*). Partial necrosis may favor the angiogenic process, tumor cell dislodgment, and consequently HCC recurrence (*19*). We therefore analyzed the role of partial necrosis in relation to TACE and to HCC recurrence after LT in our series.

MATERIALS AND METHODS

Patients

The study population was selected according to the following criteria: single HCCs proven on histologic samples and patient survival longer than 6 months. Patients receiving preoperative treatments except for TACE were excluded. These features were considered essential for a more accurate evaluation of the presence of partial necrosis on HCC nodules (*17*).

[1] Department of Liver and Multi-organ Transplantation, Sant'Orsola-Malpighi Hospital, University of Bologna, Italy.
[2] Department of Onco-hematology, Pathology Division of the "F. Addarii" Institute, Sant'Orsola-Malpighi Hospital, University of Bologna, Italy.
[3] Department of Radiology, Sant'Orsola-Malpighi Hospital, University of Bologna, Italy.
[4] Department of Internal Medicine Cardioangiology Hepatology, Sant'Orsola-Malpighi Hospital, University of Bologna, Italy.
[5] Department of Internal Medicine and Gastroenterology, Sant'Orsola-Malpighi Hospital, University of Bologna, Italy.
[6] Address correspondence to: Dr. Antonio Daniele Pinna, Department of Liver and Multi-organ Transplantation, Sant'Orsola-Malpighi Hospital, University of Bologna, Via Massarenti 9, 40138 Bologna, Italy. E-mail: tonyirc@yahoo.com.
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After these exclusions, the study included 54 (8%) cases from a total of 674 LTs performed between November 1986 and November 2001 at the Department of Surgery and Transplantation of the University of Bologna. The mean follow up was 3.4±3 years (range 6 months–15 years). There were 43 (79.6%) males, and the median recipient age was 51.3±7.7 (range 29–63) years. According to Child status (*20*), there were 4 (7.4%) Child A, 26 (48.1%) B, and 24 (44.5%) C cases. The causes of liver cirrhosis were 26 (46.3%) cases of hepatitis C virus-induced cirrhosis, 16 (29.6%) cases of hepatitis B virus-induced cirrhosis, 2 (3.7%) cases of cirrhosis secondary to ethanol abuse, and other causes in 10 (18.5%) cases.

The HCC histologic features were evaluated in the postoperative samples, as previously reported (*21*). Nodules with complete necrosis on the samples with a preoperative diameter less than 2 cm and without a preoperative biopsy confirming the HCC were not considered tumors, as according to the Barcelona criteria (*22*).

TACE was applied in 19 (35.2%) patients without any prospective randomized protocol. We tend to apply this therapy when one or both of the following patient features are present: liver function not severely compromised (*23*) and expected waiting time (according to the number of patients on the waiting list) longer than 3 months.

TACE was not repeated when liver function deteriorated, and 14 patients therefore had one procedure, 4 had two procedures, and 1 had three procedures. The mean interval between the end of the procedure and the LT was 140±112 (range 10–360) days. The comparison with the explanted livers showed a decrease in tumor size or number in 14 (74%) cases, whereas 5 (26%) had an increase in tumor size.

The standard procedure was TACE performed with the Seldinger technique (*24*), which combined vascular occlusion and regional intra-arterial chemotherapy: an emulsion of Lipiodol and anticancer drugs (usually doxorubicin or cisplatin) were injected into the proper hepatic artery (bilobar TACE), into the left or right hepatic artery, or into segmental branches (segmental TACE), depending on the site and the number of tumors. At the end of the injection, 1 to 5 mm Gelfoam particles, which had been soaked in contrast medium, were embolized into the artery until a markedly diminished flow was observed (*14*, *25*).

In October 1999 we started to apply percutaneous treatments in selected patients; these were excluded from this analysis because of the small number of cases to avoid statistical bias. After LT, no patients received adjuvant chemotherapy; chemotherapies were given only to patients with HCC recurrence and with individual protocols. Starting from 1996, our evaluation protocol included the Milan criteria, which indicated a maximum diameter of the tumor up to 5 cm if the tumor appeared to be single and up to 3 cm in the case of two or three nodules (*2*).

The liver allocation system was mainly based on the patient's Child score, and therefore recipients without severely compromised liver function, which was necessary to perform preoperative treatments, had a longer waiting time. Consequently, the mean time between HCC diagnosis and LT was 170±125 days in the TACE group versus 130±86 days in the group without treatment.

Tumor Necrosis, Tumor Vascular Invasion, and E-Cadherin Expression

Explanted livers were carefully sampled to evaluate all macroscopically suspect nodules, and neoplastic masses were thoroughly examined. All tumors were histologically diagnosed and graded according to Edmondson scale. The presence or absence of microscopic neoplastic vascular invasion was also assessed for each tumor. The presence of partial necrosis was assessed in a semiquantitative manner as the percentage of necrotic tissue divided by total tumor tissue.

Immunohistochemistry (IHC) for E-cadherin was accomplished using the anti–E-cadherin monoclonal antibody (Dako, Carpinteria, CA) diluted 1:50. IHC results were evaluated by a pathologist (MF) blinded to clinical data. The E-cadherin membrane signal was semiquantitatively assessed by comparing the expression in neoplastic and adjacent nonneoplastic liver. HCCs were then categorized as high E-cadherin expressors or low-equal expressors at the tumor-normal interface, as previously described (*26*).

Statistical Analysis and Criteria of Analysis

The results were expressed as mean±standard deviation. Chi-square analysis was performed to evaluate categorical variables in relation to tumor recurrence. The recurrence rate was computed from the day of surgery to the first follow-up visit at which recurrence was detected. Multiple logistic regression analysis with the maximum likelihood estimation was performed with the risk factors significant at the univariate analysis. Continuous variables were transformed into binary variables and the cutoffs were chosen according to previous studies.

The survival rates and the recurrence-free survival were obtained by the Kaplan-Meier method, and the differences were compared by the log-rank test. Survival was considered from the day of surgery to the day of death or to the most recent follow-up visit. Recurrence-free survival and cumulative incidence of recurrence was assessed in the following ways: patients who died of cancer-unrelated cause were considered free of recurrence at the time of death; patients who developed recurrence were considered affected by the event at the time of diagnosis (*27*). After univariate analysis, only variables that emerged as significant were used in the multivariate analysis using Cox's proportional hazard model.

A P value less than 0.05 was considered statistically significant. Statistical analysis was carried out with SPSS (Chicago, IL) (*28*).

RESULTS

Tumor Recurrence: Univariate and Multivariate Analysis

In this series of LT for single HCCs, there were five (9.3%) tumor recurrences, all but one of which developing within the 2 years postLT. The variables significantly related to higher HCC recurrence were HCC diameter greater than 2 cm ($P<0.05$), year of LT before 1996 ($P<0.05$), the presence of satellite nodules ($P<0.05$), and of partial necrosis ($P<0.05$) (Table 1).

Tumor-vascular invasion was always present in the cases of HCC recurrence, although this variable was not significantly related to the recurrence. The Cox regression anal-

TABLE 1. Univariate and multivariate analysis of the variables related to tumor recurrence

Variables		No. patients	No. recurrences (percent)	Chi-square
Sex	Male	43	5 (11.6)	P=NS
	Female	11	0	
Age	0–50 years	25	3 (12)	P=NS
	>50 years	29	2 (6.9)	
Preoperative treatment	TACE	19	3 (15.8)	P=NS
	No TACE	35	2 (5.7)	
Etiology of cirrhosis	HCV positive	26	2 (7.7)	P=NS
	HCV negative	28	3 (10.7)	
Waiting time	0–6 months	35	3 (8.6)	P=NS
	>6 months	19	2 (10.5)	
Alfa-feto protein level	0–30 ng/dL	36	2 (5.6)	P=NS
	>30 ng/dL	18	3 (16.7)	
Tumor diameter	0–2 cm	28	0	$P<0.05$
	>2 cm	26	5 (19.2)	
Peritumoral capsule	Presence	15	2 (13.3)	P=NS
	Absence	39	3 (7.7)	
Tumor vascular invasion	Presence	34	5 (14.7)	P=NS
	Absence	20	0	
Satellite nodules	Presence	4	2 (50)	$P<0.05$
	Absence	50	3 (6)	
Edmonson differentiation degree	I°–II°	38	2 (5.3)	P=NS
	III°–IV°	16	2 (12.5)	
Histological partial necrosis	Yes	16	4 (25)	$P<0.05$[a]
	No	38	1 (2.6)	
Year of LT	Before 1996	13	4 (24.1)	$P<0.05$[a]
	After 1996	41	1 (2.4)	

[a] Independently related to tumor recurrence on the multivariate analysis (partial necrosis RR=29.5 CI=1.6–539; year of LT RR=40.5 CI=2.3–729).
TACE, transarterial chemoembolization; HCV, hepatitis C virus; LT, liver transplantation; RR, relative rate; CI, confidence interval.

ysis showed that only the presence of partial necrosis and year before 1996 were related to higher tumor recurrence: $P<0.05$ (relative risk [RR] 29.5, confidence interval [CI] 1.6–539) and $P<0.05$ (RR 40.5, CI 2.3–729), respectively.

Patient Survival and Recurrence-Free Survival

There were 12 (22.2%) deaths overall in the study population, which was selected with at least 6 months survival after LT. Deaths were caused by tumor recurrence in five cases (41.7%), recurrence of liver disease in one (8.3%), infections in three (25%), and neurological complications in three (25%).

The overall 3- and 5-year actuarial survival rates were 76.2% and 71.6%, respectively, whereas the cumulative incidence of recurrence at 3 and 5 years was 8.1% and 11%, respectively. The variables related to a lower recurrence-free survival were the same as those related to the recurrence rate: tumor diameter greater than 2 cm ($P<0.05$), presence of satellite nodules ($P<0.005$), year of LT before 1996 ($P<0.05$), and presence of partial necrosis on the HCC nodules ($P<0.05$).

The Cox regression analysis confirmed that only the presence of partial necrosis (Fig. 1) and year before 1996 were significantly related to a lower recurrence-free survival: $P<0.05$ (RR 28.3 CI 2–399) and $P<0.005$ (RR 40.7 CI 3.1–529).

Partial Necrosis, TACE, and Biologic Evaluation

These data confirmed the hypothesis concerning the relationship between partial necrosis and HCC recurrence after LT. We thus tried to investigate the variables related to this histologic finding and in particular the biologic basis of this correlation.

According to past reports, the angiogenic process and tumor-cell dislodgment are the most accredited mechanisms (*17*, *19*). To investigate tumor-cell dislodgment, we evaluated the immunohistochemical expression of E-cadherin (*29–31*), a cell-adhesion protein that plays an important role in tumor invasion (*32*).

The univariate analysis showed the following variables to be related to the presence of partial necrosis: recipient age lower than 50 years ($P<0.05$), tumor diameter greater than 2 cm ($P<0.05$), preoperative TACE procedure ($P<0.05$), and low E-cadherin expression ($P<0.05$). Most cases, 11 of 16 (69%), with partial necrosis presented tumor vascular invasion and low E-cadherin expression, but because of the false-positive cases, the first variable did not reach any statistical

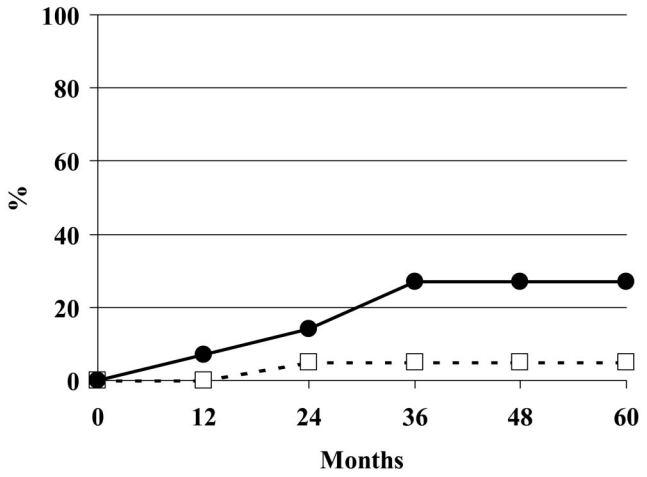

FIGURE 1. Cumulative incidence of recurrence according to the presence of partial necrosis (partial necrosis ●; no partial necrosis □).

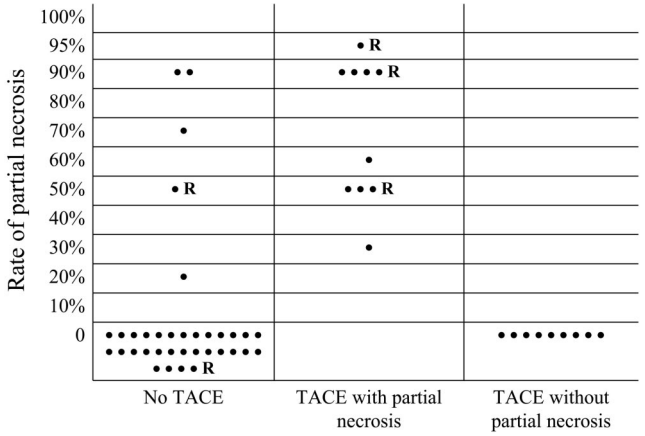

FIGURE 2. Rate of partial necrosis and Hepatocellular Carcinoma recurrences (R) distributed according to the transarterial chemoembolization (TACE) procedure (No. cases ●).

correlation (Table 2). In the Cox regression analysis, only the preoperative treatment with TACE and the low E-cadherin expression were related to the presence of partial necrosis: $P<0.05$ (RR 21.9 CI 2.4–204) and $P<0.05$ (RR 9.1 CI 1–84).

Concerning the TACE group, there were nine patients without necrosis after the procedure, and none experienced HCC recurrence (Fig. 2). They had a higher waiting time (192 ± 210 days vs. 98 ± 78, $P<0.05$) and a different rate of low E-cadherin expression (0% vs. 50%, $P<0.05$) compared with the TACE group with partial necrosis, as reported in Table 3. No patients treated with TACE before LT had complete tumor necrosis, and 6 of 35 (17.1%) cases without preoperative treatment presented partial necrosis on the sample.

DISCUSSION

The results of our study confirmed the hypothesis that partial necrosis increases the risk of tumor recurrence after LT, favoring tumor-cell dislodgment into the bloodstream. This tumor feature was mainly secondary to TACE, which should therefore be applied and selected bearing in mind the side effects of incomplete nodule necrosis.

Most of the studies that investigated the effects of preoperative treatments for HCC patients who were candidates for LT analyzed the rate of dropout from the waiting list, the effectiveness of tumor down-staging, and the overall patient survival (*10*, *13*, *14*). These end points have a fundamental clinical relevance in an intention-to-treat analysis, but they do not consider the tumor behavior. No reports have extensively analyzed the relationship between HCC recurrence after LT and the presence of partial necrosis, which is the main parameter for evaluating the efficacy of the preoperative treatment on the tumor growth.

Although the aim of all neoadjuvant procedures before LT is to achieve 100% necrosis of the nodules, this result was reported in less than 30% of cases when the histologic evaluation of removed livers was performed (*10*, *14*, *15*, *33*). A better knowledge of the consequences on tumor behavior after preoperative treatment is therefore mandatory, and it may help to select the patients and the procedures with the highest probability of achieving 100% HCC necrosis. In a series of liver resections, Adachi et al. (*17*) showed that TACE improved patient survival when it produced complete necrosis, whereas in the cases of partial necrosis, it increased the risk of HCC recurrence. Zhang et al. (*34*) subsequently contradicted these results, leading us to investigate this topic after LT.

To avoid any statistical bias related to the presence of multiple nodules, where partial necrosis could be present in one but not all nodules and related to cases treated preoperatively with different procedures, we selected only single HCCs, which in the event of preoperative treatment had a TACE procedure. In this series of LT for HCCs, we confirmed the results found after liver resections (*17*): the presence of partial necrosis increased the tumor recurrence rate and reduced the recurrence free-survival in the univariate and multivariate analyses. To the best of our knowledge, this is the first report that investigates the prognostic role of partial necrosis after LT. The multivariate analysis also showed that the most important variable related to this histologic finding was the preoperative treatment with TACE.

In regard to the correlations with tumor recurrence of the satellite nodules and of the tumor diameter, these data confirmed the results of the previous studies (*4*, *27*). The year 1996 was instead related to the recurrence rate because that is the year we started to apply the Milan criteria, as reported in a recent report from our center (*21*).

The evidence of a higher tumor recurrence in the presence of partial necrosis stimulated us to investigate the clinical causes and the biologic basis of this correlation. The central topic was to understand whether partial necrosis represented tumor behavior independent of any causes or whether it was caused by etiologic factors. The tumor size and TACE procedure were obviously related to partial necrosis, thanks to a vascular mechanism: the nodules with the highest diameter are more likely to be poorly perfused in some areas, and TACE modifies the vascular supply of the tumor.

We believe our analysis on the biologic mechanisms of partial necrosis to be very indicative. Against our expecta-

TABLE 2. Univariate and multivariate analysis of the variables related to the presence of partial necrosis

Variables		No. patients	No. partial necrosis (percent)	Chi-square
Sex	Male	43	15 (34.9)	P=NS
	Female	11	1 (9.1)	
Age	0–50 years	25	12 (48)	$P<0.05$
	>50 years	29	4 (13.8)	
Preoperative treatment	TACE	19	10 (52.6)	$P<0.05$[a]
	No TACE	35	6 (17.1)	
Etiology of cirrhosis	HCV positive	26	8 (30.8)	P=NS
	HCV negative	28	8 (28.6)	
Waiting time	0–6 months	35	13 (37.1)	P=NS
	>6 months	19	3 (15.8)	
Alfa-feto protein level	0–30 ng/dL	36	9 (25)	P=NS
	>30 ng/dL	18	7 (38.9)	
Tumor diameter	0–2 cm	28	5 (17.9)	$P<0.05$
	>2 cm	26	11 (42.3)	
Peritumoral capsule	Presence	15	6 (40)	P=NS
	Absence	39	10 (25.6)	
Tumor vascular invasion	Presence	34	11 (32.4)	P=NS
	Absence	20	5 (25)	
Satellite nodules	Presence	4	2 (50)	P=NS
	Absence	50	14 (28)	
Edmonson differentiation degree	I°–II°	38	10 (26.3)	P=NS
	III°–IV°	16	6 (37.5)	
E-cadherin expression	Low expression	22	11 (50)	$P<0.05$[a]
	High expression	32	5 (15.6)	
Year of LT	Before 1996	13	4 (30.8)	P=NS
	After 1996	41	12 (29.3)	

[a] Independently related to tumor recurrence on the multivariate analysis (TACE RR=21.9 CI=2.4–204; E-cadherin RR=9.1 CI=1.84).
TACE, transarterial chemoembolization; HCV, hepatitis C virus; LT, liver transplantation; RR, relative rate; CI, confidence interval.

TABLE 3. HCC features after TACE according to the presence of partial necrosis on samples

Variables	TACE with partial necrosis (10 cases)	TACE without partial necrosis (9 cases)	Chi-square or *t* test
Mean waiting time	98±78 days	192±210 days	$P<0.05$
Alfa-feto protein level	32±43 ng/dL	61±147 ng/dL	P=NS
Tumor diameter	2.75±1.4 cm	2.6±0.8 ng/dL	P=NS
Presence of peritumoral capsule	4 (40%)	2 (22.2%)	P=NS
Presence of tumor vascular invasion	3 (30%)	3 (33.3%)	P=NS
Presence of satellite nodules	1 (10%)	0	P=NS
Edmonson differentiation degree III°–IV°	2 (20%)	2 (22.2%)	P=NS
Low E-cadherin expression	5 (50%)	0	$P<0.05$

HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization.

tions, we could not find any relation between partial necrosis and tumor vascular invasion (Table 2). The following succession of events were therefore excluded: partial necrosis, increased proliferation activity of vascular endothelial cells, and higher tumor vascular invasion (*35*).

Conversely, we saw a lower expression of the adhesion molecule E-cadherin in the cases with partial necrosis. A possible explanation is that these tumor cells may be less attached to the surrounding extracellular matrix and could thus be easily dislodged into the bloodstream, favoring recurrence after LT (*17*, *36*, *37*). The statistical relation between the E-cadherin expression and partial necrosis recommends further basic research studies to understand the biologic steps that lead to this correlation. Our final definitive hypothesis was that TACE and low E-

cadherin expression, the only two significant variables related to partial necrosis in the multivariate analysis, were an etiologic factor and a consequence, respectively.

Concerning the percentage of partial necrosis, most of the cases with HCC recurrence presented a rate of partial necrosis equal to or higher than 50% on the samples (Fig. 2). Therefore, unless it is 100%, a high rate of tumor necrosis can not be considered a satisfactory end point, as previously reported in the series of patients not treated by LT (*38*, *39*).

The study design was not designed to assay the efficacy of neoadjuvant therapies for HCC before LT: it was not randomized, the intent-to-treat analysis was not performed, the drop-out rate from the waiting list was not considered, and patients with TACE had longer waiting times. On the other hand, there was an undoubted statistical correlation between partial necrosis, mainly secondary to TACE, as shown by the multivariate analysis, and HCC recurrence after LT. The results consequently emphasize the importance of achieving complete nodule necrosis and selecting procedures and patients. The association between percutaneous therapies and TACE (*40*, *41*), the stratification of patients according to HCC size, and the risk of dropout may be the key topics in achieving this aim, suggesting future studies.

The weak point in our study were those patients treated before LT but without necrosis on the histologic sample. We hypothesize that they had a smaller feeding artery, thus allowing less intra-arterial drug delivery during TACE. They also had a longer waiting time, which probably selected patients with a better tumor prognosis, as demonstrated by the absence of HCC recurrences (Fig. 2).

The efficacy of TACE, which is strongly indicated on the basis of the predominant HCC fed by the hepatic artery (*18*), is strictly related to the type of procedure: subsegmental TACE can obtain a rate of tumor necrosis higher than bilobar TACE (*42–47*). The procedure is also operator dependent, and we do not exclude the possibility that cases without nodule necrosis after TACE were performed with inappropriate techniques.

Unlike other reports, we did not detect any cases with complete necrosis of nodules (*10*, *14*, *15*, *33*), but we were very strict with the preoperative HCC diagnosis, following the Barcelona criteria (*22*). Cases with complete necrosis of nodules were excluded if the nodules did not exceed 2 cm and they had no preoperative biopsy confirming the HCC features. Partial necrosis was also present in more than 15% of cases without TACE (Table 2), suggesting other related mechanisms, such as chronic ischemia. Some HCCs have a faster growth than their vascular support, and they develop partial necrosis, suggesting aggressive tumor behavior, as reported for other types of tumors (*48*, *49*).

In conclusion, our study indicates that partial necrosis is a risk factor for tumor recurrence after LT. Patients and procedures should be selected bearing in mind the side effects of incomplete necrosis of the nodules.

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