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ORIGINAL ARTICLE

Microscopic Portal Vein Invasion in Relation to Tumor Focality and Dimension in Patients with Hepatocellular Carcinoma

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Abstract

Background Microscopic portal vein invasion (microPVI) and tumor multifocality are hepatocellular carcinoma (HCC) prognosis factors. To investigate whether microPVI and multifocality are directly related to each other.

Methods We retrospectively analyzed the relationships between microPVI, multifocality, and maximum tumor diameter (MTD) in prospectively collected transplanted HCC patients.

Results HCCs with 1, 2, or ≥ 3 foci had more microPVI in larger than in smaller HCCs, with microPVI being present in 52.24% of single large foci. Conversely, microPVI patients had similar percentages of single and multifocal lesions. A linear regression model of MTD, showed microPVI best associated with MTD, with 2.49 as coefficient, whereas multifocality had a 0.83 coefficient. A logistic regression model of microPVI showed significant association with tumor multifocality, especially for small HCCs. Trends for microPVI and multifocality in relation to MTD revealed that both increased with MTD but more significantly for microPVI. Survival was similar in patients with small HCCs, with or without microPVI, but was significantly worse in microPVI patients with larger HCCs. No patient survival differences were found in relation to focality.

Conclusions MTD had stronger associations with microPVI than with multifocality. microPVI was associated with worse survival in patients with large HCCs, but survival was not impacted by number of tumor foci. microPVI and multifocality appear weakly related, having different behavior in relation to MTD and survival.

Keywords Hepatocellular carcinoma · Microscopic portal vein invasion · Focality · Survival

Abbreviations

microPVI	Microscopic portal vein invasion
PVT	Portal venous invasion
HCC	Hepatocellular carcinoma
MTD	Maximum tumor diameter
AFP	Alpha-fetoprotein
TMB	Tumor mutation burden
LDLT	Liver donor liver transplant
OR	Odds ratio

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Introduction

Several HCC tumor factors have been shown to influence patient survival, in addition to non-tumor (mainly liver-related) factors. Amongst the best studied tumor factors are maximum tumor diameter (MTD), numbers of tumor foci (focality), portal venous invasion (PVT), degree of tumor differentiation, and serum levels of secreted alpha-fetoprotein (AFP). Several of these parameters were recently combined into a clinically useful HCC aggressiveness index.[1–3]

Molecular markers such as tumor mutation burden (TMB) and microsatellite instability (MSI), amongst others have more recently been used.[4–7] It has previously been shown that macroscopic PVT increases with increase in HCC maximum tumor size (MTD),[8–10] as does microPVI.[8,11,12] Thus, there seems to be a relationship between MTD and either microPVI or macroscopic PVT. Although microPVI is also an important poor prognosis parameter,[13–25] the relationship between microPVI and tumor focality is less well explored. In this study, we examined the relationships between tumor multifocality and microPVI, a presumed precursor to macroscopic PVT, and of both of them in relation to MTD. We also consider the clinical consequences of knowing these relationships.

Methods

Study Population and Parameters

Patients who underwent liver donor liver transplant (LDLT) for HCC at our Liver Transplantation Institute were included in this study. The data were collected prospectively, but analyzed retrospectively (retrospective cohort). Before this study was designed, approval was obtained from the Inonu University Institutional Review Board (Approval no: 2018/1–9). The tumor aggressiveness characteristics of 323 de-identified HCC patients were analyzed, of who 130 had microscopic HCC invasion of the portal vein (microPVI) on post-liver transplant pathological analysis. These characteristics included maximum tumor diameter (MTD), number of tumor foci, presence of microPVI, and serum levels of the HCC biomarker, alpha-fetoprotein (AFP). Patients with radiological pre-transplant evidence of macroscopic portal or hepatic venous invasion were excluded.

Statistical Analysis

All the statistical computations were made using STATA (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC). Patient characteristics were reported as median for continuous variables and as frequencies and percentages (%) for categorical variables. Normal distributions of quantitative variables were tested using the Kolmogorov–Smirnov normality test. For testing the associations between groups, the Chi-square test for categorical variables was used, and the Wilcoxon rank-sum (Mann–Whitney) test was used for continuous variables. The Chi-square method was used to evaluate the trend between categorical levels of MTD for microPVI or multifocality ($n \geq 3$). For categorigal variables, the test for pairwise comparisons of proportions was used the test for pairwise comparisons of proportions to evaluate the statistical differences between the parameters as category for each level. To evaluate the variation in the increase for the percentage of microPVI and multifocality ($n \geq 3$), the equation of the interpolating line for each modification of variation of the increase was used. The logistic regression model of microPVI (positive vs. negative) on tumor foci was used where the lower category (negative) was defined as reference category. Linear regression model of MTD on single variables of microPVI and tumor foci and their combination in HCC patients was designed to examine how the presence of the single factor and/or their combination could be considered as an expression of tumor size. When testing the null hypothesis of no association, the probability level of error at two tails was 0.05.

Results

Tumor Focality and MicroPVI in Relation to MTD

We examined a transplanted HCC cohort ($n = 323$) and the subset of HCC patients with known microPVI ($n = 130$). We initially examined the percent of patients who had unifocality or multifocality and the percent of each of them with microPVI, separately for patients with small or larger MTD (Table 1). We found that for unifocal HCCs, there was a significant increase in the percent of patients with microPVI, when we compared patients with small versus larger MTD HCCs, 15.60% versus 52.24%, $p < 0.001$. This increase in percent microPVI for larger versus small HCCs was also found in patients having 2 or ≥ 3 tumor foci. The 3-cm MTD cutoff was chosen as we had previously shown a change in HCC biology as MTD increases to > 3 cm.[26,27]

For HCC patients with larger MTD, the percent of patients with microPVI increased with increase in focality, from 52.24% for unifocal to 76.60% for patients with ≥ 3 tumor foci, $p = 0.006$ (Table 1, right hand column). A similar increase in percent microPVI with increase in multifocality was also found for smaller MTD patients (Table 1, left hand

Table 1 Comparisons of % microPVI amongst HCC patients with different tumor foci by MTD group

Parameters*	Tumor foci: 1 (unifocality)			
	Maximum tumor diameter (cm)			
	Total	≤3.0	>3.0	p^
MicroPVI (%) positive	30.18	15.69(a)	52.24(d)	<0.001
Parameters*	Tumor foci: 2 (multifocality)			
	Maximum tumor diameter (cm)			
	Total	≤3.0	>3.0	p^
MicroPVI (%) positive	44.07	33.33(b)	51.43(e)	0.17
Parameters*	Tumor foci: ≥3 (multifocality)			
	Maximum tumor diameter (cm)			
	Total	≤3.0	>3.0	p^
MicroPVI (%) positive	55.79	35.42(c)	76.60(f)	<0.001

*All values: frequencies and percentage (%); ^ Chi-square test. Abbreviation: *MTD* maximum tumor diameter, *PVI* microscopic portal vein invasion. Comparisons p: (b) vs. (a) = 0.09, (e) vs. (d) = 0.94, (c) vs. (a) = 0.01, (f) vs. ((d) = 0.006, (c) vs. (b) = 0.86, (f) vs. (e) = 0.02

column), 15.69% versus 35.42%, $p = 0.01$. Thus, increase in microPVI related to both focality and to increase in MTD.

The trends in tumor indices of percent microPVI and percent multifocality in relationship to MTD were then plotted (Fig. 1). The percentage of patients with microPVI or with multifocality ($n \geq 3$) increased with increasing tumor size, although this association was more significant for microPVI ($p < 0.0001$) than for multifocality ($p = 0.02$). This trend was stronger in small tumors. Furthermore, inspection of the slopes of the two interpolating equations shows that microPVI is more associated with MTD, with a slope, y of 11.773, compared to that of the multifocality, which has a slope, y of 5.691 (Fig. 1A). The ratios of percent microPVI to percent multifocality of a range of MTD was then plotted, using data from (Fig. 1A) and shown in (Fig. 1B). The plot clearly shows how the relationship of microPVI to multifocality changes with increase in MTD. Thus, microPVI increases both with increase in MTD and with increase in focality. Despite

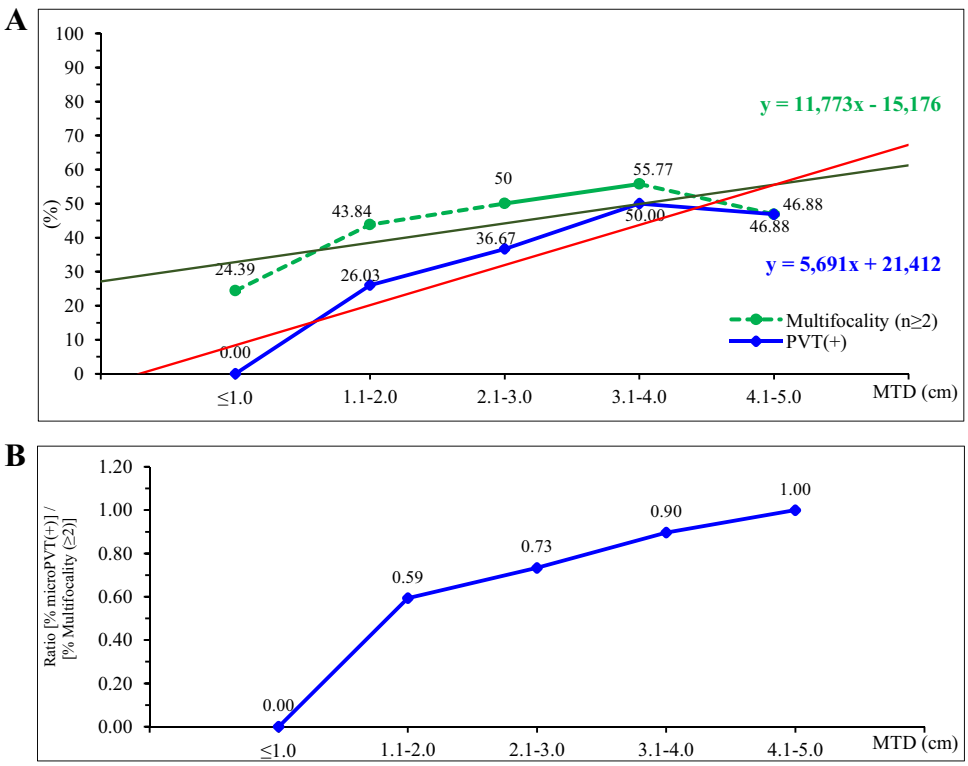

Fig. 1 **A** Trends in HCC patient tumor indices in relationship to MTD. Multifocality ($n \geq 2$) (% of patients) ($p = 0.0200^*$), microPVI (+) (% of patients) ($p < 0.0001^*$). **B** Trend in ratio of percentages between multifocality and microPVI in relationship to MTD in groups

that, even unifocal tumors have an increase in microPVI with increase in MTD/size.

MicroPVI in Relation to Tumor Multifocality

We then examined the relationship directly between percent microPVI and focality, by examining the number of tumor nodules in patients with known microPVI (Table 2). By contrast with Table 1, we found no significant association between focality and MTD ($p = 0.99$), nor in respect of MTD for proportion of patients having unifocal or multifocal HCC (comparison between rows), respectively for the individual MTD groups. Thus, presence of microPVI appears to over-ride other relationships. There is therefore only a weak relationship between percent microPVI and percent focality.

Regression Model of MTD

A linear regression model of MTD was next constructed, on the single variables of microPVI and tumor focality (Table 3). We found that microPVI was best associated with MTD, with 2.49 as coefficient; and tumor multifocality was also associated with MTD but with 0.83 as coefficient (Table 3A). MicroPVI and tumor focality were then considered in combination (Table 3B). Taking microPVI (−) and unifocality (1 tumor focus) as reference, we found that in patients who had microPVI (+) and unifocality, the tumor size (MTD) increased by 2.69 cm; and in patients who had microPVI (+) and multifocality (3 or more tumor foci), MTD increased by 2.70 cm. This formally confirms the stronger association of microPVI with MTD, than of multifocality with MTD.

Logistic Regression Model of MicroPVI (+/−) on Tumor Focality

The association between HCC focality ($n \geq 3$ tumor foci) with microPVI was next considered, separately for HCCs having an MTD ≤ 3.0 cm and MTD > 3.0 cm. We found that there was an association between microPVI and tumor focality (Table 4). The association between these 2 parameters was found to be stronger in small tumors with MTD ≤ 3.0 cm, OR of 2.86, $p = 0.004$, than in larger tumors with MTD > 3.0 cm, OR of 1.76, $p = 0.09$. This shows a different behavior in the 2 MTD groups under consideration. This formally shows an association of microPVI with focality but mainly in smaller MTD HCCs.

Table 2 Comparisons of tumor nodule numbers amongst MTD groups in microPVI (+) HCC patients

Parameters*	Maximum tumor diameter (cm)			$p^{\wedge}$
	Total	≤ 3.0	> 3.0	
Tumor nodules				0.99
1 (unifocal)	39.23	39.02[(a)]	39.33[(c)]	
≥ 3 (multifocal)	40.77	41.46[(b)]	40.45[(d)]	

*All values: frequencies and percentage (%); ^ Chi-square test. Abbreviation: *MTD* maximum tumor diameter, *PVT* microscopic portal vein thrombosis. Comparisons p: (b) vs. (a) = 0.86, (c) vs. (d) = 0.91

Table 3 Linear regression model of MTD on single variables of microPVI and tumor focality (A) and their combination (B) in HCC patients

	β	*se (β)*	*p*	*95% CI*
A)				
MicroPVI				
Negative (Ref)	1			
Positive	2.49	0.32	< 0.001	1.86 to 3.13
Tumor foci				
Unifocality 1 (Ref)	1			
Multifocality ≥ 3	0.83	0.34	0.02	0.15 to 1.50
B)				
Combined				
MicroPVI and tumor foci				
PVI (−) and tumor focus 1 (Ref)	1			
PVI (−) and tumor focus ≥ 3	0.52	0.42	0.22	−0.31 to 1.35
PVI (+) and tumor focus 1	2.69	0.48	< 0.001	1.74 to 3.63
PVI (+) and tumor focus ≥ 3	2.70	0.42	< 0.001	1.88 to 3.52

Abbreviations: *β* coefficient, *se(β)* standard error of β, *MTD* maximum tumor diameter, *PVI* microscopic portal vein invasion. In A, above, age, gender, and liver function tests were also examined, but none were found to be significant

Table 4 Logistic regression model of microPVI (positive vs. negative) on tumor foci (≥ 3 vs. 1). Patients (A) with MTD (≤ 3.0 cm) and (B) with MTD (> 3.0 cm)

	OR (se)	*p*	*95% CI*
A) MTD ≤ 3.0 cm			
Tumor foci			
(= 1) [Ref. cat.]	1	0.004	1.39 to 5.88
(≥ 3)	2.86 (1.05)		
B) MTD > 3.0 cm			
Tumor foci			
(= 1) [Ref. cat.]	1	0.09	0.91 to 3.42
(≥ 3)	1.76 (0.59)		

OR odds ratio, *OR (se)* standard error of OR, *Ref. cat* reference category, *MTD* maximum tumor diameter, *PVI* microscopic portal vein invasion. Age, gender, and liver function tests were also examined, but none were found to be significant

Comparisons Between MicroPVI (−/+) and Focality and Relation Survival

We then focused on tumor focality in the microPVI and non-microPVI patients of the total cohort (Table 5). Patients with small tumors (MTD ≤ 3.0 cm) and larger tumors (MTD > 3.0 cm) were analyzed separately. In the smaller tumor group, 23.56% of patients had microPVI, compared to 59.73% of patients in the larger tumor group ($p < 0.001$). Patients with either small or larger tumors had 41.46% vs. 40.45% multifocality. Thus, there was a change in incidence of microPVI, but not in multifocality, for increase in MTD. However, within both small and larger MTD patient groups, when non-microPVI patients were compared to microPVI patients, the percent in multifocality almost doubled.

Survival was next examined in the various subgroups. We found that in the multifocality tumor groups, 3-year survival was significantly worse for the microPVI group than for the non-microPVI group in patients with larger MTDs. But for the small MTD patients, survival differences were not significant. Interestingly, survival was not significantly different when ≤ 2 foci were compared with ≥ 3 foci, in any focality group or MTD group (see comparisons at the bottom of Table 5; e.g., group a vs. a, group c vs. c, group e vs. e, and group g vs. g). Thus, presence of microPVI had a negative impact in large MTD patients compared with non-microPVI, as expected, but not in small MTD patients. Furthermore, focality seemed to not have a significant impact on survival in either small or large MTD patient groups. We thus found that patients with microPVI had worse survival than patients without microPVI (as expected), but only for patients with larger tumors. MicroPVI had no significant survival effect in patients with small HCCs.

Discussion

Microscopic portal vein invasion (microPVI) and multifocality are poor prognosis factors for HCC patients. We therefore aimed to investigate whether these 2 prognosis parameters were related to each other, in a prospectively collected series of transplanted HCC patients, since microPVI is a pathological diagnosis and we treat HCC patients in a liver transplantation institute. The patients in this study were live donor liver transplantation recipients, with known baseline clinical characteristics and survival. Our initial working hypothesis was that since both microPVI and most tumor multifocality in HCC patients involve tumor invasion, the difference only being in the location-liver parenchyma or portal vein, then these 2 processes should be similar or the same and therefore linked. However, analysis of the data showed that they did not seem to be related parameters.

Table 5 Comparisons between microPVI (−/+) groups in relation to tumor focality and survival

Parameters*	MTD ≤ 3.0 cm			MTD > 3.0 cm		
	microPVI		$p^{\wedge}$	microPVI		$p^{\wedge}$
	Negative	Positive		Negative	Positive	
MTD (median)	1.5	2.4	$0.0002^{\#}$	4.5	5.5	$0.007^{\#}$
% microPVI	–	23.56		–	59.73	$< 0.001^{\Psi}$
# Tumor foci (%)			0.02^{Θ}			0.004^{Θ}
# Foci (≤ 2)	76.69	58.54	0.035^{Ψ}	81.67	59.55	0.003^{Ψ}
Survival time (%)						
3 yr	$45.10^{(a)}$	$66.67^{(c)}$	0.06	$59.18^{(e)}$	$37.74^{(g)}$	0.03
5 yr	$25.49^{(b)}$	$41.67^{(d)}$	0.11	$36.73^{(f)}$	$24.53^{(h)}$	0.18
# Foci (≥ 3)	23.31	41.46	0.035^{Ψ}	18.33	40.45	0.003^{Ψ}
Survival time (%)						
3 yr	$32.26^{(a)}$	$47.06^{(c)}$	0.31	$72.73^{(e)}$	$33.33^{(g)}$	$0.02^{¥}$
5 yr	$32.26^{(b)}$	$41.18^{(d)}$	0.54	$54.55^{(f)}$	$13.89^{(h)}$	$0.01^{¥}$
	Comparisons			Comparisons		
	(a) $p = 0.20^{\Psi}$	(c) $p = 0.21^{\Psi}$		(e) $p = 0.37^{\Psi}$	(g) $p = 0.67^{\Psi}$	
	(b) $p = 0.47^{\Psi}$	(d) $p = 0.97^{\Psi}$		(f) $p = 0.28^{\Psi}$	(h) $p = 0.20^{\Psi}$	
AFP (%)			$0.02^{\neq}$			$0.04^{\neq}$
(≥ 100 IU/mL)	15.27	32.50	0.034^{Ψ}	16.95	32.58	0.026^{Ψ}

*All values: frequencies and percentage (%); $^{\Theta}$ p-value for association between # Tumor Nodules and microPVI; $^{\neq}$ *p*-value for association between AFP and microscopicPVI; $^{\wedge}$ Chi-square test; $^{\#}$ Wilcoxon rank-sum (Mann–Whitney) test; $^{\Psi}$ test for pairwise comparisons of proportions; $^{¥}$ Fisher's exact test. Abbreviations: *PVI* microscopic portal vein invasion, *MTD* maximum tumor diameter, *yr* years, *AFP* alpha-fetoprotein

MicroPVI was found to increase with increase in MTD, whether unifocal or multifocal lesions were compared (Table 1). This is similar to the reported increases in microPVI with increase in MTD[11,12,24] and with macroPVT increase with increase in MTD.[27,28] We found that HCCs with 1, 2, or ≥ 3 foci have an increase in microPVI in larger compared with smaller HCCs, although 52.24% of single large nodules have microPVI. Conversely, in patients having microPVI, a similar percent have single and multifocal lesions. This finding alone shows a dissociation between multifocality and microPVI, since large single tumor foci had such a high percent of microPVI, and when microPVI was present, it was distributed amongst unifocal and multifocal HCCs.

A linear regression model of MTD was created, and microPVI was found to be best associated with MTD, with 2.49 as coefficient, whereas multifocality had a 0.83 coefficient. Similarly, a logistic regression model of microPVI was then created, in which we found an association between microPVI and tumor focality, but the association was stronger in smaller size (≤ 3 cm) HCCs, OR 2.86, than in larger size (> 3 cm) HCCs, OR 1.77 (Tables 2 and 3).

Trends in percent microPVI and percent multifocality in relation to MTD were examined. Both increased with MTD but were more significant for microPVI (Fig. 1A). The difference in the trends became even clearer with the ratios of the 2 parameters plotted against each other (Fig. 1B), when it can be seen that they greatly diverged for smaller MTDs, but approximated each other at an MTD of 3 cm or more. This showed that their biology in relation to MTD differed significantly from each other. Thus, the 2 parameters of microPVI and multifocality may not be directly connected to each other. This would imply the existence of more than a single type of stem cell. Since unifocal larger HCCs had percent of microPVI, it seems unlikely that multifocality can cause microPVI. Since microPVI patients were evenly distributed across unifocal and multifocal tumors (Table 5B), probably microPVI did not cause multifocality. This leaves 2 reasonable hypotheses: A, as MTD increases, a stem cell differentiates into progeny that can cause either microPVI lineage or into a multifocality lineage; or B, that as MTD increases, so do 2 separate stem cell types that produce either microPVI or multifocality. Although the data does not allow a confident choice between hypothesis A and B, the change in the ratio of microPVI to multifocality shown in Fig. 1B makes it more likely that they have unrelated origins and therefore may originate from different progenitors, as postulated in hypothesis B. This choice derives some support from the percent of multifocality shown in Table 5, with a doubling of percent multifocality in presence versus absence of microPVI, in both small (23.31% vs. 41.46%) and larger (18.33 vs. 40.45%) MTD groups.

Survival was similar in patients with small tumors, with or without microPVI, but it was significantly worse in patients with microPVI compared to without it, in larger HCCs regardless of focality (Table 5). Thus, patients with ≤ 2 foci and larger tumors had a 3-year survival of 37.74 months with microPVI vs. 59.18 months without microPVI, $p = 0.03$. Similarly, patients with ≥ 3 foci and larger tumors had a 3-year survival of 33.33 months with microPVI vs. 72.73 months without microPVI, $p = 0.02$. Interestingly, we found no survival differences in patients when unifocal HCC patients were compared with multifocal HCC patients, within microPVI or within non-microPVI groups.

Strengths of this study include the finding of microPVI even in unifocal HCC. Also, although Fig. 1. shows that both microPVI and multifocality increase with respect to MTD, they are not necessarily related to each other. Weaknesses include the retrospective nature and relatively small size of the study. Thus, Table 5 showed a longer survival for small unifocal than multifocal HCC patients, but the difference was not significant.

There are some clinical implications to these findings. Since microPVI cannot be diagnosed with confidence presurgery, yet has a negative prognostic significance, some form of neo-adjuvant HCC therapy pre-transplantation (and probably pre-resection, given the high 5-year recurrence rates post resection) might seem warranted for evaluation. However, the literature does not offer guidance for the time needed. Given the balance between need for transplant of the liver, yet need for therapy of the microPVI, 4–6 months of neo-adjuvant therapy might seem reasonable. Since chemoembolization or even radioembolization have previously been shown to have a positive impact on survival in macroscopic PVT,[29–31] the results might encourage the use of either therapy pre-transplant (or pre-resection) for pre-surgical tumor control. Since even patients within Milan criteria have a 75% reported 5-year survival rate, there exists the opportunity to extend survival of 25% of those patients and possibly even more for extra-Milan criteria patients. Analogous approaches for neo-adjuvant but resectable tumors have been successfully used in tumors of the breast and colon. Ability to diagnose the presence of microPVI would still be helpful, and perhaps advances in "liquid" biopsy might point a way forward for this, as do serum levels of GGT and inflammation markers and the HCC biomarker des-gamma-carboxy prothrombin.[11,32,33] However, even neo-adjuvant HBV therapy may limit the presence of microPVI.[34]

Our conclusions are that there were trends in the parameter relations but mainly for patients with small tumors. We found stronger associations of MTD with microPVI than with multifocality. Although microPVI was associated with worse survival than in patients without microPVI, survival was not impacted by numbers of tumor foci. Thus, though

microPVI and multifocality are poor prognosis factors and both increase with HCC growth, they do not seem to be strongly related to each other.

Clinical conclusions

We showed here that 2 parameters of HCC aggressiveness, namely microPVI and multifocality, are poorly correlated, implying different origins or mechanisms.

Given that both microPVI and multifocality increase with increase in MTD/tumor size, it seems reasonable in light of these findings to consider neo-adjuvant therapy in all patients awaiting liver transplantation, except those with the smallest tumors. This is a major management suggestion. Even for patients having within-Milan criteria, there are currently 25% who do not get to a 5-year survival and who would benefit from this approach.

Serum GGT levels have been shown to be associated with presence of microPVI. If confirmed in future series, this might be a useful clinical biomarker.

Author Contribution BIC—concept, ideas, and writing. VG and RD—biostatistics. VI, SA, VE, SU, IB, and SY—data collection and paper proofing. ES—pathology analysis.

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Declarations

Institutional review board statement This work complies with the guidelines of the World Medical Association, Declaration of Helsinki. This study was reviewed and approved by the Inonu University Institutional Review Board for non-interventional studies (Approval no: 2018/1–9).

Informed Consent Statement Verbal and written consents were obtained from all liver transplant patents before surgery.

Conflict of Interest The authors declare no competing interests.

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