RESEARCH ARTICLE


# Microscopic vascular invasion by hepatocellular carcinoma in liver transplant patients

**Abstract**

**Background:** A characteristic of Hepatocellular Carcinoma (HCC) is to invade the portal venous system in the liver as a means of spread within the liver and systemically. The ensuing Portal Vein Thrombosis (PVT) is a poor prognosis parameter and often diagnosed radiologically pre-treatment. More limited Microvascular Portal Invasion (microPVI) is typically diagnosed on examination of tumors removed after treatment by resection or transplant. The biological characteristics and subsets of PVI are incompletely characterized.

**Aims:** To examine HCC patients with and without microPVI to understand the clinical relationships to other tumor and clinical characteristics and to survival.

**Methods:** A cohort of 270 liver transplant patients with HCC without macroscopic PVT that were available to us was examined. Patients with (165) and without (105) microPVI were compared for survival and clinical features.

**Results:** The mean survival of patients with and without microPVI was significantly different: 86.6 versus 110.5 months, p=0.007. The microPVI+ patients differed from microPVI- patients in having a significantly larger number of tumor nodules, tumor size and higher serum levels of both Alpha-Fetoprotein (AFP) and almost significant for higher Gamma-Glutamyl Transpeptidase (GGT, p=0.053). Survival in microPVI+ patients related significantly to serum GGT (p=0.006) but not to AFP levels. The incidence of microPVI increased with increase in tumor size and survival decreased significantly with increase in tumor size for microPVI patients. Increase in tumor size was also associated with significantly higher serum GGT levels in patients who were microPVI+, but not in those who were microPVI. Furthermore, patients with microPVI who had prolonged survival significantly differed from those with shorter survival in respect only to tumor size and serum GGT levels.

**Conclusion:** These findings draw attention to a group of patients with microPVI who have long survival and to the usefulness of serum GGT levels in their evaluation and prognosis.

**Keywords: HCC, portal vein invasion, survival**

**Abbreviations: HCC: Hepatocellular Carcinoma; MTD: Maximum Tumor Diameter; AFP: Alpha-Fetoprotein; GGT: Gamma Glutamyl Transpeptidase; ALKP: Alkaline Phosphatase; AST: Aspartate Amino Transferase; ROC: Receiver Operating Characteristic Curve; OS: Overall Survival; PVT: Portal Vein Thrombosis (Macroscopic); DCP: Des Gamma Carboxy-Prothrombin; MRI: Magnetic Resonance Imaging; CT: Computerized Axial Tomography**

## Introduction

Microscopic Portal Vein Invasion (MicroPVI) by Hepatocellular Carcinoma (HCC) is currently only diagnosed on tissue histological examination (in contrast to macroscopic portal vein thrombosis, which is diagnosed radiologically). This can be via biopsy, but more typically by examination of HCC tissues after surgical resection or Liver Transplantation (LT). MicroPVI is also considered to be an independent poor prognostic indicator following both hepatic resection for HCC [1-9] and liver transplantation [10]. Several risk factors for microPVI have been reported, and include tumor size and especially serum DCP levels [10-19], as well as AFP levels [14,18]. Several attempts have been made into classifying the degree of microPVI, based on numbers of invaded vessels and numbers of invading cells [20-22]. Altogether, MicroPVI has been considered to be a major mechanism for intra-hepatic spread of HCC [23].

The current work was undertaken to investigate, in a large series of patients after liver transplant for HCC, the factors associated with microPVI in this group of patients and the prognostic factors that might be involved. We found

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a correlation between presence of microPVI and Maximum Tumor Diameter (MTD) and also found a significant association between serum GGT levels and survival in microPVI patients.

## Methods

Patients who underwent LT for HCC at our Liver Transplantation Institute without macroscopic portal vein thrombosis were the subjects of this study. The data were collected prospectively and were analyzed retrospectively. This study has been approved by Inonu University Institutional Review Board (Approval no: 2018/1-9).

The clinical parameters and tumor characteristics of 270 HCC patients who underwent LT were reviewed according to vascular invasion based on explant pathology report. AFP cut off 200 ng/ml and GGT cut off 100 IU/ml. These cutoffs were found by analysis of ROC and were significantly associated with survival post liver transplant [24]. Categorical (qualitative) variables were expressed as count and percentage and were compared using univariate analysis methods (Pearson chi-square test, continuity corrected chi-square test or Fisher's exact test where appropriate). Multivariate binary logistic regression was performed for odds ratio estimations. As recommended by Hosmer and Leme show the variables with p value less than 0.25 in univariate analysis were included in multivariate analysis. Continuous (quantitative) variables were summarized by median, minimum, maximum values and compared using Mann-Whitney U test. Kaplan-Meier survival estimate was used to determine overall survival of the patients. Follow-up period was defined as the interval between LT until the date of last visit to the outpatient department for living patients or until the date of patient death. Statistical tests were considered significant when the corresponding p value was less than 5%. All statistical analyses were performed using IBM SPSS Statistics for Windows version 25.0 (New York, USA).

## Results

The 270 patients who were transplanted for HCC were dichotomized into 165 patients with microscopic vascular invasion (microPVI +ve) and 105 patients without microscopic vascular (microPVI -ve). The survival of the 2 groups was significantly different, 2660 versus 3315 days (86.6 versus 110.5 months), p=0.007 **(TABLE 1 AND FIGURE 1)** accompanying cumulative survival graph). The cumulative proportion of surviving patients was also less for microPVI +ve compared to microPVI -ve patients at 1, 3, 5 and 10 years **(TABLE 2)**.

The associated clinical characteristics of the 2 groups were also compared **(TABLE 3)** The

**TABLE 1. Survival of HCC patients with microscopic vascular invasion (PVI+) versus (PVI), by the Kaplan-Meier method.**

| | Survival time | | Log-Rank |
|---|---|---|---|
| | Mean ± SE | 95% C.I. | p-value |
| MicroPVI (-ve) [n=165] | 3315.43 ± 167.09 | 2987.94-3642.91 | 0.007 |
| MicroPVI (+ve) [n=105] | 2660.36 ± 217.06 | 2234.91-3085.80 | |

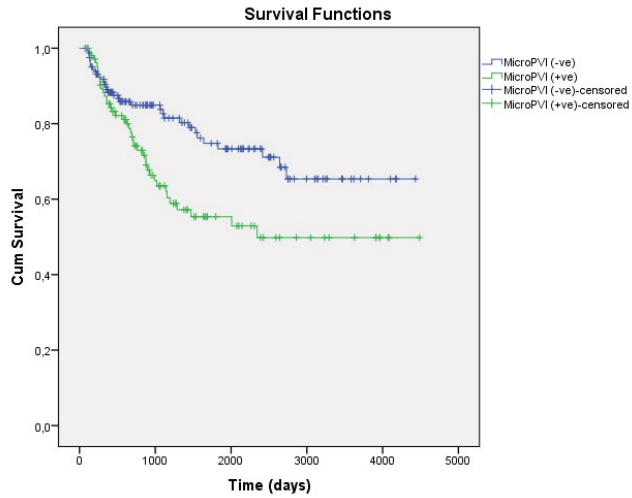


**FIGURE 1. Graphical representation of survival of HCC patients with microscopic vascular invasion (PVI+) versus (PVI), by the Kaplan-Meier method.**

**TABLE 2. Cumulative proportion of surviving patients.**

| Time | microPVI (-ve) | microPVI (+ve) |
|---|---|---|
| 1 year | 89.00% | 85.30% |
| 2 years | 84.90% | 74.10% |
| 3 years | 83.80% | 63.50% |
| 5 years | 74.80% | 55.30% |
| 10 years | 65.40% | 49.80% |

**TABLE 3. Univariate and multivariate analysis of baseline parameter values and tumor characteristics of HCC patients with microscopic vascular invasion (microPVI+) versus without it (microPVI-).**

| | Univariate Analysis | | | Multivariate Analysis | | |
|---|---|---|---|---|---|---|
| | microPVI- | microPVI+ | | | | |
| Parameters | Median (min.-max.) | Median (min.-max.) | p value | OR | 95% C.I. | p value |
| AFP, IU/mL | 11.15 (0.4-6388) | 26.3 (0.2-10800) | <0.001 | 1 | 1.000-1.001 | 0.17 |
| GGT, IU/L | 60 (11-412) | 81 (17-1396) | 0.053 | 0.999 | 0.996-1.002 | 0.678 |
| Platelets, $\times 10^3/\mu L$ | 87 (19-528) | 85 (16-464) | 0.529 | | | |
| Hb, g/dL | 12.9 (6.5-41.3) | 13.1 (6.3-18.7) | 0.307 | | | |
| ALB, g/dL | 2.8 (1.8-4.8) | 3 (1.2-5.2) | 0.616 | | | |
| T. Bili, mg/dL | 1.71 (0.3-28) | 1.84 (0.32-109) | 0.331 | | | |
| AST, IU/L | 55 (9-308) | 63 (22-822) | 0.038 | 0.999 | 0.993-1.006 | 0.845 |
| ALT, IU/L | 39 (5-446) | 47 (10-2088) | 0.014 | 1.006 | 0,998-1,013 | 0.123 |
| ALKP, IU/L | 113 (49-414) | 116 (28-552) | 0.254 | | | |
| MTD (cm) | 2.4 (0.1-10) | 4.1 (1.1-24) | <0.001 | 1.427 | 1.243-1.638 | <0.001 |
| Tumor # | 1 (1-11) | 2 (1-36) | <0.001 | 1.266 | 1.125-1.425 | <0.001 |

GGT: Gamma Glutamyl Transpeptidase (IU/mL); ALB: Albumin (g/dL); AST: Aspartate Aminotransferase (IU/L); ALT: Alanine Aminotransferase (IU/mL); ALKP: Alkaline Phosphatase (IU/mL); T. Bili: Total Bilirubin (mg/dL); Hb: Hemoglobin (g/dL); AFP: Alpha-Fetoprotein (IU/mL); MTD: Maximum Tumor Diameter; microPVI: Microscopic Vascular Invasion

tumor characteristics comprising MTD, tumor number and serum AFP levels were all significantly different between the 2 groups and worse for the microPVI +ve patients: MTD 4.1 *vs.* 2.4 cm, p<0.001; median tumor number was 2 *vs.* 1, p<0.001; serum AFP levels were 26.3 *vs.* 11.15, p<0.001. PVI +ve patients also had significantly higher serum AST, ALT and almost significant GGT levels (81 *vs.* 60, p=0.053) than microPVI -ve patients. Parameters with p value <0.2 were then included in a subsequent multivariate analysis model **(TABLE 3)**. This multivariate analysis showed Odds Ratios (OR) for microPVT of significant parameters: MTD (OR: 1.427, p<0.001) and tumor number (OR: 1.266, p<0.001).

MicroPVI +ve patients were then dichotomized according to high or low serum AFP levels, but no significant survival differences were found **(TABLE 4)**. However, when the microPVI +ve patients were dichotomized according to high or low serum GGT levels, using GGT 100 IU/ml for cutoff as determined by previous ROC curves [24], the 2 microPVI +ve patient groups were found to have significantly different survival (1981.56 *vs.* 2866.59 days, p=0.006) by Kaplan Meier analysis **(TABLE 5)** and associated cumulative survival **(FIGURE 2)**.

The patients were then ordered according to Maximum Tumor Diameter (MTD). **TABLE 6** shows an incremental increase in the percent of patients having microPVI +ve, as MTD increased. Patients with <3 cm MTD tumors had 24% microPVI +ve rate; patients with 3-6 cm MTD had 47.7% microPVI +ve rate; while patients with MTD >6 cm tumors had a 61.5% microPVI +ve rate. There was a significantly different decrease in survival rate as the MTD increased for the microPVI +ve patients, but not for the microPVI -ve patients **(TABLE 7)**. Interestingly, as MTD increased, there was a statistically significant increase in

**TABLE 4. Survival of patients with microscopic portal vein invasion (PVI+ve) by the Kaplan-Meier method, dichotomized by serum AFP levels.**

| | Survival time | | Log-Rank |
|---|---|---|---|
| | Mean ± SE | 95% C.I. | p-value |
| AFP ≤ 200 | 2743.45 ± 258.63 | 2236.54-3250.37 | 0.581 |
| AFP>200 | 2239.55 ± 337.44 | 1578.16-2900.94 | |
| AFP: Alpha-Fetoprotein (IU/mL) | | | |

**TABLE 5. Survival of patients with Microscopic Portal Vein Invasion (PVI +ve) by the Kaplan-Meier method, dichotomized by serum GGT levels.**

| | Survival time | | Log-Rank |
|---|---|---|---|
| | Mean ± SE | 95% C.I. | p-value |
| GGT ≤ 100 | 2866.59 ± 235.98 | 2404.06-3329.11 | 0.006 |
| GGT>100 | 1981.56 ± 331.14 | 1332.52-2630.59 | |
| GGT: Gamma-Glutamyl Transpeptidase (IU/mL) | | | |

**TABLE 6. Percent of patients with microPVI (+) by MTD group.**

| MTD | Micro Vasc. Invasion | |
|---|---|---|
| | # | % |
| <3 cm | 30/124 | 24.2 |
| 3-6 cm | 51/107 | 47.7 |
| >6 cm | 24/39 | 61.5 |
| MTD: Maximum Tumor Diameter; microPVI: Microscopic Portal Vein Invasion | | |

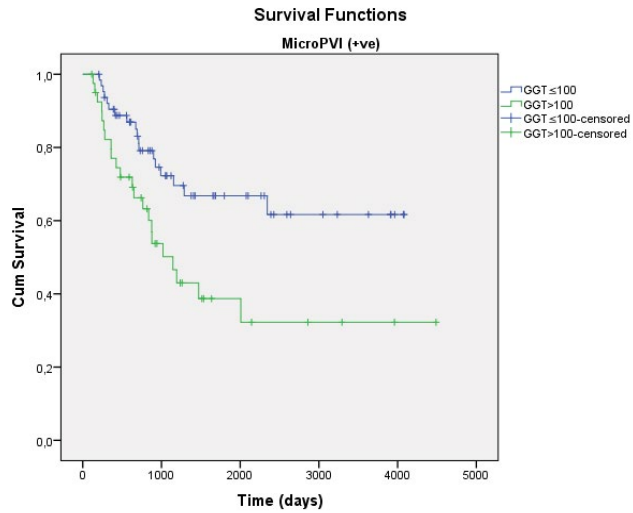


**FIGURE 2. Graphical representation of survival of patients with Microscopic Portal Vein Invasion (PVI +ve) by the Kaplan-Meier method, dichotomized by serum GGT levels.**

**TABLE 7. Survival for different MTD groups, by the Kaplan-Meier method, according to microPVI status.**

| | | Survival time | | Log-Rank |
|---|---|---|---|---|
| | MTD size | Mean ± SE | 95% C.I. | p-value |
| Micro PVI (+ve) | <3 cm (n=30) | 3284.20 ± 288.32[a] | 2719.10-3849.29 | <0.001 |
| n=105 | 3-6 cm (n=51) | 2661.04 ± 288.25[a] | 2096.08-3226.01 | |
| | >6 cm (n=24) | 1237.10 ± 306.36[b] | 636.64-1837.55 | |
| Micro PVI (-ve) | <3 cm (n=94) | 3485.35 ± 217.43 | 3059.19-3911.51 | 0.098 |
| n=165 | 3-6 cm (n=56) | 3192.15 ± 238.35 | 2724.98-3659.33 | |
| | >6 cm (n=15) | 1853.15 ± 401.93 | 1065.37-2640.94 | |
| [a] is significantly different from [b], p<0.05; microPVI: Microscopic Portal Vein Invasion | | | | |

serum GGT levels for the microPVI +ve patients (p<0.001), but not for the microPVI -ve patients with similar MTD (**TABLE 8**).

Inspection of the Kaplan-Meier survival curve associated with (**TABLE 1**), showed that for microPVI +ve patients, there was an inflex-

**Table 8. Comparison of microPVI (+) and microPVI (-) patients in relation to baseline serum GGT levels (IU/ml) in each MTD group.**

| | microPVI (-ve) | microPVI (+ve) |
|---|---|---|
| | GGT median (min.-max.) | GGT median (min.-max.) |
| <3 cm | 58 (11-412) | 54 (18-230) [a] |
| 3-6 cm | 60 (16-301) | 76 (17-330) [a] |
| >6 cm | 83.5 (17-226) | 115.5 (23-1396) [b] |
| | p=0.611 | p=0.001 |

[a] is significantly different from [b], p<0.05; microPVI: Microscopic Portal Vein Invasion; MTD: Maximum Tumor Diameter; GGT: Gamma Glutamyl Transpeptidase

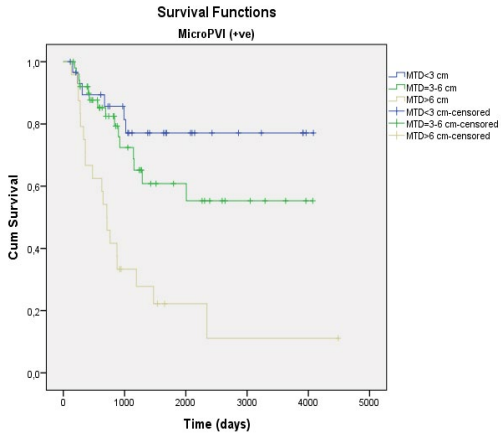


**FIGURE 3. Graphical representation of comparison of microPVI (+) patients in relation to baseline serum GGT levels (IU/ml) in each MTD group.**

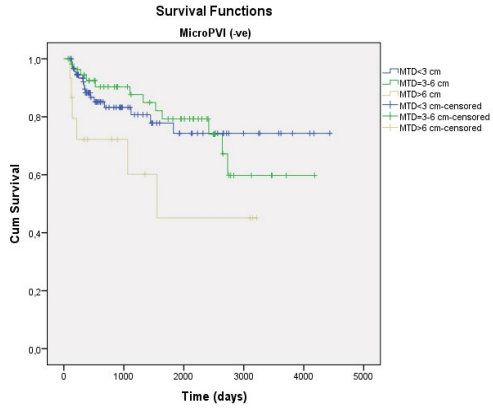


**FIGURE 4. Graphical representation of comparison of microPVI (-) patients in relation to baseline serum GGT levels (IU/ml) in each MTD group.**

**TABLE 9. Comparison of clinical characteristics between long (≥ 2000 days) vs. short survivors (<2000 days) post liver transplantation, all with microscopic portal vein invasion (microPVI+).**

| | ≥ 2000 days | <2000 days | |
|---|---|---|---|
| | Median (min.-max.) | Median (min.-max.) | p-value |
| AFP, IU/mL | 85.35 (1.9-6461) | 23.9 (0.2-10800) | 0.272 |
| GGT, IU/L | 43 (18-242) | 88 (17-1396) | 0.012 |
| Platelets, × $10^3$/µL | 73 (39-295) | 93.5 (16-464) | 0.215 |
| Hb, g/dL | 12.8 (8.4-16.9) | 13.3 (6.3-18.7) | 0.219 |
| Albumin, g/dL | 2.95 (1.8-4) | 3 (1.2-5.2) | 0.858 |
| Total Bili, mg/dL | 1.9 (0.37-39.64) | 1.835 (0.32-109) | 0.628 |
| AST, IU/L | 68.5 (22-537) | 63 (23-822) | 0.726 |
| ALT, IU/L | 38 (14-196) | 51(10-2088) | 0.059 |
| ALKP, IU/L | 115 (43-268) | 122 (28-552) | 0.424 |
| MTD (cm) | 4 (1.5-9.5) | 5 (1.1-24) | 0.042 |
| Tumor # | 2 (1-10) | 2 (1-36) | 0.521 |

GGT: Gamma Glutamyl Transpeptidase (IU/mL); ALB: Albumin (g/dL); AST: Aspartate Aminotransferase (IU/L); ALT: Alanine Aminotransferase (IU/mL); ALKP: Alkaline Phosphatase (IU/mL); T. Bili: Total Bilirubin (mg/dL); Hb: Hemoglobin (g/dL); AFP: Alpha-Fetoprotein (IU/mL); MTD: Maximum Tumor Diameter; microPVI: Microscopic Portal Vein Invasion

ion point, suggesting the presence of shorter and longer survivors. The clinical characteristics of these 2 microPVI +ve groups of patients were compared **(TABLE 9)** and longer survivors were found to have significantly lower serum GGT levels and also significantly smaller tumors.

## Discussion

Currently, microPVI+ is diagnosed after biopsy, most frequently, on pathological examination of a specimen obtained post resection or transplantation. That is the reason for focusing on these transplanted patients, since the diagnosis is usually made unambiguously, although only after treatment. Several questions therefore arise, including the prognostic significance, the possibility of non-surgical diagnosis, and the reasons for its association with larger MTD and higher levels of serum Des Gamma Carboxy Prothrombin (DCP) and the possibility of its prevention.

Most reports of the presence of microPVI +ve patients indicate a worse prognosis than those patients who do not have microPVI or macroPVT [1-15]. We found similarly a worse prognosis in patients with microPVI than in patients without it **(TABLE 1)**. However, the worse prognosis also may depend in part on the degree of microPVI+ [8,25] and has been reported to be present in between 15 and 57% of HCC specimens [25].

Several reports are available on pre-operative and non-surgical, radiological diagnosis of microPVI+, including incomplete HCC capsule and typical dynamic HCC pattern on contrast-enhanced MRI scan [26-30]. Furthermore, molecular markers for microPVI+ have also been recently reported [23,31].

Many reports showed a significant association between increase in MTD and microPVI+ [11-14,32]. We also found a significant relation on multivariate analysis, as well as an increased percent of patients with microPVI+ with increase in MTD **(TABLE 6)**. There has been little published investigation that we are aware of, as to the reasons for increase in incidence of microPVI with increasing MTD. Perhaps it is due to presence of constant microPVI+ per unit of tumor mass, so that as MTD increases so does the probability of diagnosing microPVI+. Possibly, as the tumor enlarges, there is a change in biology (as happens with tumor angiogenesis), so that increase in tumor size and microPVI+ are both inextricably involved in the mechanisms of increasing tumor aggressiveness, and thus of decrease in survival. The clinical pathology results of **TABLE 1** give some support to this idea, as the patients with microPVI+ compared to microPVI- have significantly higher levels of serum AFP, as well as liver inflammation and damage parameters AST, ALT and almost, GGT.

Perhaps the most useful serum marker for microPVI+ and PVT is an increase in levels of the HCC marker des-gamma carboxy prothrombin or DCP [12,13,15,18,19,32]. Unfortunately, access to the DCP assay was not available to us. The reasons for the strong association of microPVI and PVT with high DCP levels in HCC patients have not been thoroughly investigated. However, vitamin K, which corrects the biochemical defect that causes DCP production by HCCs, also inhibits HCC migration [33-35].

The main novel findings in this work are the relationships of GGT in microPVI+ patients. Although it was not statistically significant in multivariate analysis, possibly due to small patient numbers, serum GGT was significantly associated with microPVI+ as compared with microPVI- patients **(TABLE 1)** and it was a significant factor for survival in microPVI+ patients **(TABLE 5)**. Serum GGT levels were also associated with survival in microPVI+ patients, but not in microPVI- patients **(TABLE 8, FIGURES 3 AND 4)**, was the only significant poor prognosis factor, together with MTD, when long versus short survival patients with microPVI+ were compared **(TABLE 9)**. The role of GGT in HCC survival has been shown previously and is associated with parameters of HCC aggressiveness [36,37] and its HCC expression may also offer a growth advantage [38] and it has previously been shown to be significantly associated with presence of PVT [39]. Thus, GGT levels might be usefully investigated in future analyses for both their association and their prognostic significance in patients with both microPVI+ and with PVT.

## Conclusion

These findings draw attention to a group of patients with microPVI who have long survival and to the usefulness of serum GGT levels in their evaluation and prognosis.

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## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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