Microvascular Invasion in Hepatocellular Carcinoma and Liver Transplant

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Abstract

Objectives: Curative therapy for hepatocellular carcinoma is liver transplant. To date, the Milan Criteria remain the best pretransplant clinical surrogate for tumor behavior and overall prognosis. Microvascular invasion portends a poor prognosis; however, it is often undetectable before transplant. Furthermore, its pretransplant indicators are not well established. In this study, we investigated the presurgical and pathologic predictors of microvascular invasion in patients with hepatocellular carcinoma.

Materials and Methods: Between August 2000 and August 2013, 156 liver transplants were performed for hepatocellular carcinoma at the Johns Hopkins Medical Center. Information on clinical characteristics and pathology data, including microvascular invasion, were available for 107 patients on liver explants. Logistic regression was used to assess the effects of Milan Criteria, alpha-fetoprotein, tumor differentiation, and multilobar involvement on the presence of microvascular invasion on explant pathology.

Results: In 107 patients, 24 (22%) had microvascular invasion on pathology. In patients with microvascular invasion, 41% were outside of Milan Criteria versus 19.3% of patients within but without microvascular invasion. In patients with microvascular invasion, the rate of poor differentiation and alpha-fetoprotein level > 1000 ng/mL were more common than in patients without microvascular invasion; however, on univariate and multivariable analyses, Milan Criteria, alpha-fetoprotein level, multilobar involvement, and differentiation did not reach statistical significance in predicting microvascular invasion on pathology.

Conclusions: In this study, potential predictors of microvascular invasion, including Milan Criteria, alpha-fetoprotein level, tumor differentiation, and multilobar involvement, were not predictive. Preoperative prediction of microvascular invasion remains a challenge, suggesting the need for future studies.

Key words: Alpha-fetoprotein, Hepatitis C, Liver cancer, Milan Criteria

Introduction

Hepatocellular carcinoma (HCC) is the 6th most common cancer worldwide and the 3rd most common cause of cancer-related mortality. In the United States, the incidence of HCC has nearly doubled over the past 2 decades. Liver transplant (LT) remains the most effective treatment for early-stage HCC. Nearly one-quarter of all adult LT recipients were transplanted for HCC in the United States. Liver transplant is only indicated in HCC patients at early stages, but unfortunately some patients are diagnosed with locally advanced or metastatic stage. Patients who are transplanted for HCC are at risk for recurrence after transplant. Although the liver containing HCC is replaced with a healthy one, most patients will continue to carry on the existing predisposing factors. In addition, post-LT immunosuppressive medications increase the risk of HCC recurrence, as well as tumor progression. Post-LT HCC recurrence is an important topic, and various risk factors have been described in the literature that can predict cancer recurrence (summarized in Table 1).

To improve allograft and patient recurrence-free survival, various criteria have been developed. The most commonly used criteria is Milan Criteria (MC), which limits LT to patients with 1 nodule ≤ 5 cm or 3
or fewer tumors with the largest not exceeding 3 cm and no evidence of macrovascular invasion or extrahepatic metastasis. After MC was put into use in 1996, excellent post-LT recurrence-free survival was achieved. Subsequently, many other criteria have been developed such as the University of California, San Francisco criteria to extend the life-saving benefits of LT beyond MC (Table 2).

With these criteria, many patients have undergone successful LT. However, HCC recurrence after transplant is still a significant cause of allograft loss and recipient mortality, in approximately 8% to 18% of patients. Milan and other similar criteria consider radiographic tumor size, which does not give enough information about pathologic characteristics and tumor biology, such as vascular invasion and differentiation/grade. Therefore, these criteria do not adequately predict post-LT HCC recurrence. As mentioned above, many other factors are implicated in HCC recurrence following LT (Table 1). Microvascular invasion (MVI) of tumor in native liver can predict tumor recurrence, and it portends a poor prognosis. However, pre-LT detection of MVI is difficult and almost impossible without pathologic examination. Furthermore, the pre-LT predictors of MVI in this context are not well established. To date, MC and pre-LT alpha-fetoprotein (AFP) levels have been used as clinical surrogates for tumor behavior and prognosis; however, their accuracy in predicting MVI remains unclear. Hence, there is a need to identify its presurgical predictors. In this study, we aimed to explore the pre-LT predictors of MVI of HCC, such as demographic characteristics and AFP levels. In addition, we investigated pathologic characteristics that might predict MVI.

**Materials and Methods**

We performed a retrospective review of our transplant database for patients who underwent LT for HCC from August 2000 to August 2013. During this period, 156 LTs were performed for HCC at the Johns Hopkins University Comprehensive Liver Transplant Center. The information on clinical characteristics and pathology data, including MVI, were available for 107 (67%) of these patients on liver explants. In several patients, MVI could not be evaluated on pathology, as a result of prior locoregional therapies by interventional radiology. Logistic regression analysis was used to assess the effect of MC, AFP levels, histopathologic differentiation, and multilobar involvement in univariate analysis. In 2 separate multivariable analyses, we assessed the effects of differentiation and/or multilobar involvement on the presence of MVI.

**Results**

Characteristics of patients are summarized in Table 3. The mean age of patients in the MVI-negative group was 56 years versus 53 years in MVI-positive group. No statistically significant differences were shown between MVI-positive and MVI-negative groups in terms of sex, race, and cause of underlying liver disease. Overall, 74.7% of patients had hepatitis C with or without other concomitant causes of liver disease. On multivariable analysis, AFP, MC, histopathologic differentiation, and multilobar involvement did not reach statistical significance in predicting MVI on pathology.

In our study group of 107 patients, 24 (22%) had MVI on explant pathology, with 14 of these 24 patients (58.3%) within the MC. Of 81 patients in total within MC, 14 patients (17.2%) were positive for MVI. In general, in our study group of 107 patients, 81 (75.7%) were within MC and 26 (24.3%) were outside of MC. Of the 26 patients outside of MC, 10 patients (38.5%) had evidence of MVI on pathology and 16 patients (61.5%) did not have MVI (Table 3).

Pretransplant AFP level was measured in all of the 107 patients. Overall, 90% had AFP levels ≤ 400 ng/mL. Seventy-seven of the 83 patients (93%) in the MVI-negative group had AFP ≤ 400 ng/mL.

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**Table 1. Risk Factors of Hepatocellular Carcinoma Recurrence After Liver Transplant**

<table>
<thead>
<tr>
<th>No. of Lesions</th>
<th>Milan Criteria</th>
<th>UCSF-SC</th>
<th>UCSF-EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≤ 5 cm</td>
<td>≤ 6.5 cm</td>
<td>≤ 8 cm</td>
</tr>
<tr>
<td>2 or 3</td>
<td>≤ 3 cm</td>
<td>≤ 4.5 cm*</td>
<td>≤ 5 cm*</td>
</tr>
<tr>
<td>4 or 5</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>≤ 3 cm*</td>
</tr>
</tbody>
</table>

*Maximum total tumor diameter ≤ 8 cm.

**Table 2. Selection Criteria of Liver Transplant Candidates for Hepatocellular Carcinoma**

<table>
<thead>
<tr>
<th>No. of Lesions</th>
<th>Milan Criteria</th>
<th>UCSF-SC</th>
<th>UCSF-EC</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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</tr>
<tr>
<td>4 or 5</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>≤ 3 cm*</td>
</tr>
</tbody>
</table>

*Maximum total tumor diameter ≤ 8 cm.

**Abbreviations:** EC, expanded criteria; SC, standard criteria; UCSF, University of California-San Francisco

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**Table 3.**

Assignments are based on HCC recurrence after LT.

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**Table 4.**

Assignments are based on HCC recurrence after LT.

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**Table 5.**

Assignments are based on HCC recurrence after LT.
Nineteen of the 24 patients (79%) in the MVI-positive group had AFP ≤ 400 ng/mL. Eleven patients (10%) had AFP > 1000 ng/mL, with 5 patients in the MVI-positive group and 6 in the MVI-negative group (Table 3).

Differentiation was evaluated on histology by the pathologist and categorized into well (18.7%), moderate (74.8%), and poorly differentiated tumors (6.5%). Nineteen patients (22.9%) in the MVI-negative group had well-differentiated tumors versus 1 patient (4.2%) in the MVI-positive group. Of 7 patients with poorly differentiated tumors, 5 patients (71.4%) had positive MVI on pathology (Table 3).

**Discussion**

Liver transplant is the mainstay of treatment for patients with HCC confined to the liver and within MC. Tumor recurrence remains a major challenge after LT, with many previously described factors in the literature (Table 1).

The presence of MVI has been reported to be a key risk factor in predicting tumor recurrence and prognosis after liver resection or LT. Sumie and associates reported 110 patients who underwent curative liver resection for HCC without macrovascular invasion, with 45% of patients having evidence of MVI. On multivariable analysis, cirrhosis and MVI were independent risk factors for recurrence-free survival. The 5-year recurrence-free survival rates were 20.8% and 52.6% for patients with and without MVI, and the 5-year disease-specific survival rates for patients with and without MVI were 59.3% and 92.0%. In a study by Jonas and associates that reported on 120 patients transplanted for HCC in the context of cirrhosis, overall 1-, 3-, and 5-year survival rates were 90%, 71%, and 60%. In multivariable analysis, histologic grade and MVI were determined as prognostic factors. In the same study, histologic grade and maximal diameter showed significant correlation with vascular invasion.

Despite the significance of MVI in predicting tumor recurrence, it is difficult to determine the presence of MVI preoperatively as it is a pathologic diagnosis. Recognition of preoperative predictors of MVI might help to identify patients who are appropriate for LT with lower risk of tumor recurrence. Several groups have evaluated the preoperative predictors of MVI on pathology in patients who undergo liver resection as treatment for HCC. Zhao and associates studied 265 patients who underwent resection of multinodular HCC and found 24% with MVI on pathology. Patients with MVI had significantly lower overall and recurrence-free survival rates than those without MVI. On multivariable analysis, serum AFP level > 400 μg/L, serum gamma-glutamyltransferase level > 130 U/L, total tumor diameter > 8 cm, and tumor number > 3 were independent predictors of MVI.

In this study, we investigated the clinical and pathologic predictors of MVI in patients who underwent LT for HCC. Age, sex, race, and cause of underlying liver disease could not predict the presence of MVI. On multivariate analysis, none of the variables reached statistical significance in predicting MVI on pathology, including histopathologic differentiation, multilobar involvement, AFP levels, and MC. Our findings were not consistent with the observations of others in identifying AFP, tumor differentiation, and multifocality as predictors of MVI on histology.
In a study that evaluated 120 patients who underwent LT for HCC, histologic grading and maximal diameter of tumor showed a significant correlation with MVI. The rates of MVI were lower in patients with well-differentiated tumors (25%) versus patients with moderately to poorly differentiated tumors (100%) \( (P < .01) \). In our study, overall, 75% of patients had moderately differentiated tumors. The rate of MVI was more common in poorly differentiated tumors, at 71%, versus 5% in well-differentiated tumors; however, in regression analysis, histologic differentiation did not reach statistical significance in predicting MVI.

Alpha-fetoprotein has been evaluated in predicting MVI on histology with conflicting results in transplant settings. In a study of 101 patients with HCC, AFP > 100 ng/mL was associated with MVI (odds ratio of 5, 95% confidence interval 1.4-18.1; \( P = .006 \)). However, in a study by Chou and associates, AFP levels were not associated with MVI. In our study, MVI was more common in patients with AFP > 1000 ng/mL versus patients with AFP ≤ 400 ng/mL; however, it did not reach statistical difference in predicting MVI on pathology. We also investigated multilobar involvement as a potential predictor of MVI, but it did not reach statistical significance. In a study of 60 patients with HCC who underwent LT, tumor multifocality, on both magnetic resonance imaging and pathologic examination, was the only variable that predicted MVI (odds ratio of 2.43 and \( P = .013 \) for magnetic resonance imaging; odds ratio of 1.94 and \( P = .013 \) for pathologic examination). All other tumor characteristics with magnetic resonance imaging failed to predict MVI.

The main limitation of our study is its retrospective nature. Data on some of the variables were not available, leading to the exclusion of part of the original cohort. Our study also did not include radiologic findings on the patients. These observations should be further investigated in larger prospective studies evaluating the clinical, radiologic, and pathologic data.

**Conclusions**

We retrospectively evaluated liver explants to potentially identify the pretransplant and pathologic predictors of MVI among patients who underwent LT for HCC. In this study, MC, AFP, multilobar tumor involvement, and histologic differentiation were not predictive of MVI on explant histology. These variables should be further studied in larger groups of patients prospectively, as preoperative prediction of MVI remains a challenge in the LT setting and may provide significant insight in those patients most likely to benefit from LT.

**References**