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Predictive Value of Alpha-Fetoprotein Change Rates for Hepatocellular Carcinoma Recurrence After Liver Transplant

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ABSTRACT

Background: Liver transplantation offers a conclusive solution for individuals diagnosed with Hepatocellular carcinoma (HCC). Alterations in alpha-fetoprotein (AFP) levels prior to transplantation could serve as an indicator for the potential recurrence of HCC.

Objective: This study aimed to evaluate the capacity of variations in the pre-transplant AFP levels as a prognostic indicator for tumor recurrence after transplant.

Patients and methods: 144 HCC patients had liver transplant over a 20-year period at our institute. Their mean age at time of transplant was 54.8 years, and 124 patients were males. 71 patients (49.3%) received interventions for HCC (group 1), while 73 patients (50.7%) had no HCC treatment (group 2).

Results: The recurrence-free survival rate was 86.8%. HCC recurrence was reported in 19 patients (13.2%), including 17 males and 2 females. Among these 19 patients, 11 were in group 1 (15.5%), and 8 were in group 2 (11%).
The predictors of recurrence were a tumor volume greater than 115 cc ($P = 0.010$), and AFP > 400 ng/ml ($P = 0.009$). While AFP gradient (AFP-G) did not exhibit a significant correlation with tumor recurrence in the entire cohort, it demonstrated a notable correlation with recurrence in untreated patients. A specific AFP-G threshold of 60 ng/ml/month was chosen. An AFP-G of 60 ng/ml/month displayed a sensitivity of 83.3% and a specificity of 94.7% for predicting HCC recurrence.

Conclusion: It was noted that using an AFP-G exceeding 60 ng/ml/month should be regarded as a prognostic tool rather than a strict selection criterion for transplant candidates.

Keywords: Gradient, Dynamic, Alfa-fetoprotein, Hepatocellular carcinoma, Liver transplant

INTRODUCTION

Liver transplant has emerged as a definitive treatment for Hepatocellular carcinoma (HCC). Over the years, numerous studies have sought to optimize candidate selection and enhance the outcome of transplant for HCC patients [(1)].

The criteria used for selecting transplant candidates have evolved, with the Milan Criteria serving as the gold standard adopted by many centers [(2, 3)]. Despite, their widespread use, Milan criteria are often limited in predicting patient outcomes accurately. Some patients with tumors beyond Milan criteria may still have a good prognosis after transplantation, while others within Milan criteria may experience HCC recurrence and poor outcomes [(4)]. This led to the development of various selection criteria, primarily varying in the permissible tumor morphology, particularly concerning tumor number and maximum diameter. Factors such as tumor progression and pre-transplant HCC management strategies were found to influence post-transplant recurrence rates, irrespective of whether patients met the Milan criteria.

This prompted the incorporation of additional parameters into selecting the patients, including Alpha-fetoprotein (AFP) levels and tumor grade [(3)]. This is consistent with observations that histological features, such as tumor differentiation, vascular invasion, and satellite nodules, are more robust predictors of recurrence risk [(5)]. Unfortunately, these histological features can only be determined by examining explanted livers, rendering them unsuitable as pre-transplant selection tools [(6)]. High AFP levels have been associated with poor histological features and HCC recurrence after transplant [(7)].

However, there is no agreement on an AFP cutoff value for selecting transplant candidates. Recent research has suggested that the rate of AFP increase before liver transplant might be a better predictor of tumor recurrence than static AFP values [(8)]. While, pre-transplant therapeutic interventions can reduce AFP levels, they may not be necessarily associated with a lower recurrence rate [(9)]. This study aimed to study the pre-transplant AFP dynamics on HCC recurrence after transplant considering patients with and without therapeutic interventions for HCC.

PATIENTS AND METHODS

Between 1998 and 2019, patients who had HCC and underwent liver transplant at the University of Alberta were enrolled in this study. At the start of the program, selection criteria of HCC patients included those with a single lesion not larger 7.5 cm, or multiple lesions not

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exceeding 5 cm in diameter, regardless of the number of lesions [10]. As poorly differentiated HCC has been considered a contraindication for liver transplant, tumors > 5 cm underwent biopsy. After January 2007, selection criteria evolved to include composite criteria, combining a tumor volume of < 115 cc, and AFP < 400 ng/ml[3].

Presence of macrovascular invasion and/or metastases disqualified HCC patient from undergoing liver transplant during both time periods. Patients with HCC received locoregional therapies while on the liver transplant waitlist, assessed case by case by a multidisciplinary team.

The immunosuppression protocol initially relied on induction with steroids, which was later replaced with an induction therapy using anti-CD25 monoclonal antibody, along with the use of calcineurin inhibitors for maintenance, transitioning to Sirolimus-based regimens. Screening for HCC recurrence after transplant was routinely conducted for all HCC patients using AFP and imaging studies every 3-6 months.

The collected data included demographic information (gender and age at transplant), diagnosis for transplant, Child-Pugh score, MELD score, HCC data (number, size, and location of lesions), locoregional HCC treatment data, AFP data (including maximum reading, timing, and values of the last two AFP readings before liver transplant), date of diagnosis of tumor recurrence, graft and patient survivals. We divided the patients into 2 groups: group 1 had interventions while waiting for transplant, while group 2 did not.

To calculate the AFP-gradient (AFP-G), the difference between the last two values of the pre-transplant AFP was divided by the time duration (expressed as ng/ml/month).

Ethical approval: The study received approval from The Research Committee at the University of Alberta Hospital in Canada, and the whole research process adheres to the Helsinki Declaration.

Statistical Analysis

Data are summarized as frequency (%) or mean ± SD. Predictors of HCC recurrence were analyzed using Cox's proportional hazard regression model. ROC analysis was used to assess AFP-G as a prognostic marker, both in all patients and within the intervention and no-intervention groups separately. The cutoff value for AFP-G was determined using the AFP-G with the highest maximum likelihood ratio. A P-value of ≤ 0.05 is considered significant.

RESULTS

This study reviewed 144 patients who had HCC and received liver transplant at University of Alberta over a 20-year period. The mean age was 54.8 ± 8.9 years, and 124 (86.1%) were males.

Table (1) presents the patients' characteristics and describes the different pre-transplant interventions for HCC while waiting for liver transplant. The mean follow-up was 4.99 ± 4.23 years. The intervention group (group 1) comprised 71 (49.3%) patients, while the no-intervention group (group 2) consisted of 73 (50.7%) patients.

Table 1: Characteristics of patients

Variable	summary
Age at time of transplant (years)	54.8 ± 8.9
Male Gender (%)	124 (86.1)
Underlying liver disease	
HCV	76
HBV	27
HCV, HBV	7
Alcoholic liver cirrhosis	17
Primary sclerosing cholangitis	5
Hemochromatosis	7
Cryptogenic	5
Meld score	19.5 ± 9.3
Child Pugh score	7.9 ± 2.4
Waiting time on the transplant list (days)	198.3 ± 281.5
Number of tumors	2.6 ± 3.1
Maximal tumor diameter (cm)	3.5 ± 1.9
Total tumor volume pretransplant (cm^3)	51.3 ± 68.6
Low AFP	96.5 ± 376.8
High AFP	345.3 ± 1028.5
Rate of change	45.9 ± 291.6
Pre-transplant Intervention for HCC	71 (49.3)
Percutaneous Ethanol Injection	26
Trans-arterial chemoembolization	30
Radiofrequency Ablation	10
Liver Resection	5
Follow-up (years)	4.99 ± 4.23

Recurrence-free survival after transplant was 86.8% (figure 1), 19 patients (13.2%) had HCC recurrence; 17 males and 2 females. Out of these 19 patients, 11 were in group 1 (15.5%), and 8 were in group 2 (11%). Comparing the main characteristics between both groups showed no difference regarding the pre-transplant AFP values, AFP-G, and the TTV. Nevertheless, group (1) exhibited notably larger tumors when comparing the maximum tumor diameter to that of group (2) (2.86 ± 1.6 cm) (*P*=0.041).

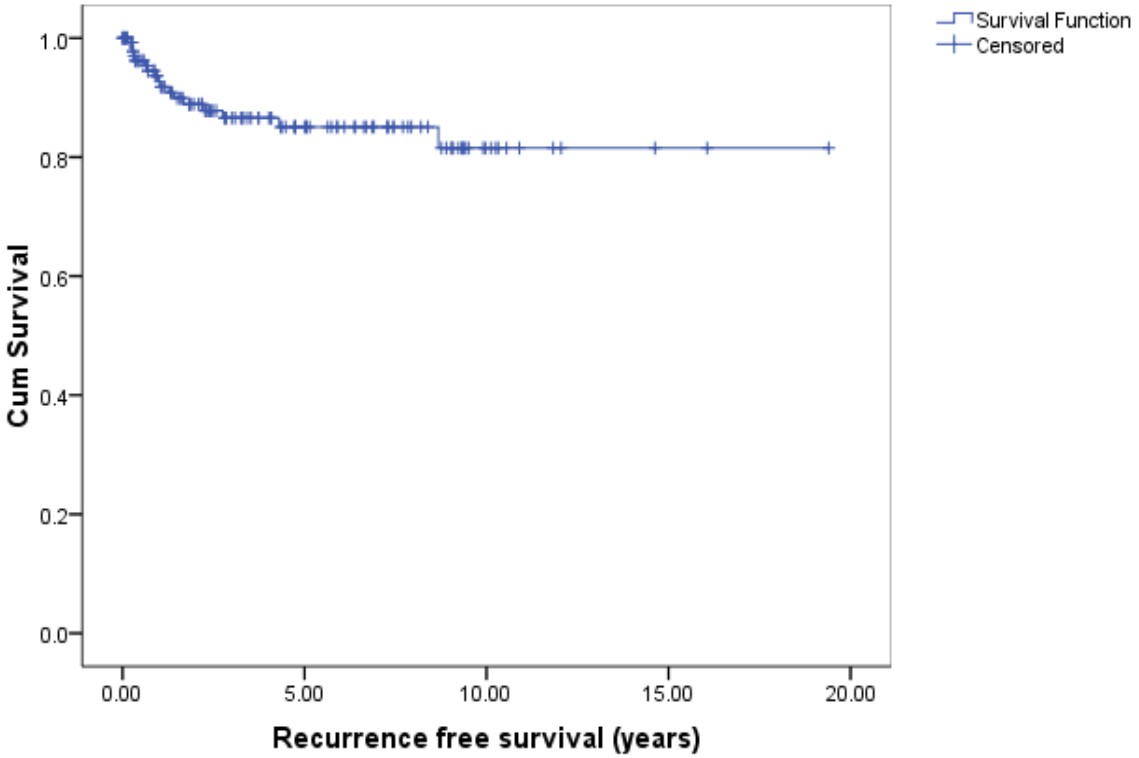

Figure 1: Recurrence-free survival of patients after liver transplant.

Predictors of HCC recurrence post-transplant: We conducted an analysis to investigate potential risk factors for tumor recurrence following transplant. The Milan criteria, pre-transplant AFP levels exceeding 400 ng/ml, total tumor volume (TTV) exceeding 115 cc, AFP-G, and vascular invasion emerged as potential indicators for HCC recurrence. Conversely, factors such as age, gender, MELD score, the etiology of liver cirrhosis, Child score, tumor number or location, and pre-transplant interventional treatments for HCC showed no significant correlation with disease recurrence.
Subsequently, a multivariate analysis was done to further assess the predictors of recurrence. This analysis revealed only two independent predictors of HCC recurrence after transplant: TTV exceeding 115 cc and AFP levels exceeding 400 ng/ml, with respective *P*-values of 0.010 and 0.009 (Table 2).

Table 2: Predictors of HCC recurrence after liver transplant

Variable	HR	95% CI	*P*-value
AFP >400	6.329	1.581 – 25.337	0.009
Beyond Milan criteria	0.676	0.181 – 2.524	0.560
AFP-G	0.721	0.218 – 1.537	0.458
Vascular invasion	2.315	0.963 – 7.648	0.056
Total tumor volume >115	10.731	1.769 – 25.130	0.010

Relation between AFP-G and recurrence of HCC after liver transplant: To assess the relationship between pre-transplant AFP-G levels and HCC recurrence post-transplant, we employed ROC analysis. The results indicated no correlation between pre-transplant AFP-G and disease recurrence in the overall patient population (Area under the curve (AUC) = 0.600, $P = 0.228$), as well as in group 1 patients (AUC = 0.384, $P = 0.290$) (Figures 2 and 3).

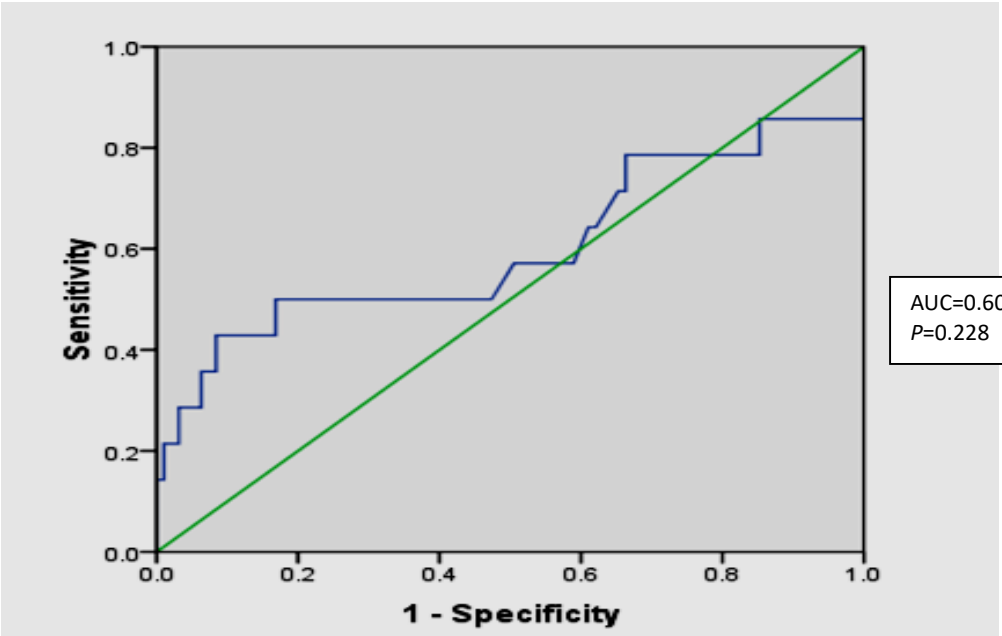

Figure 2: ROC Analysis of AFP gradient in all patients.

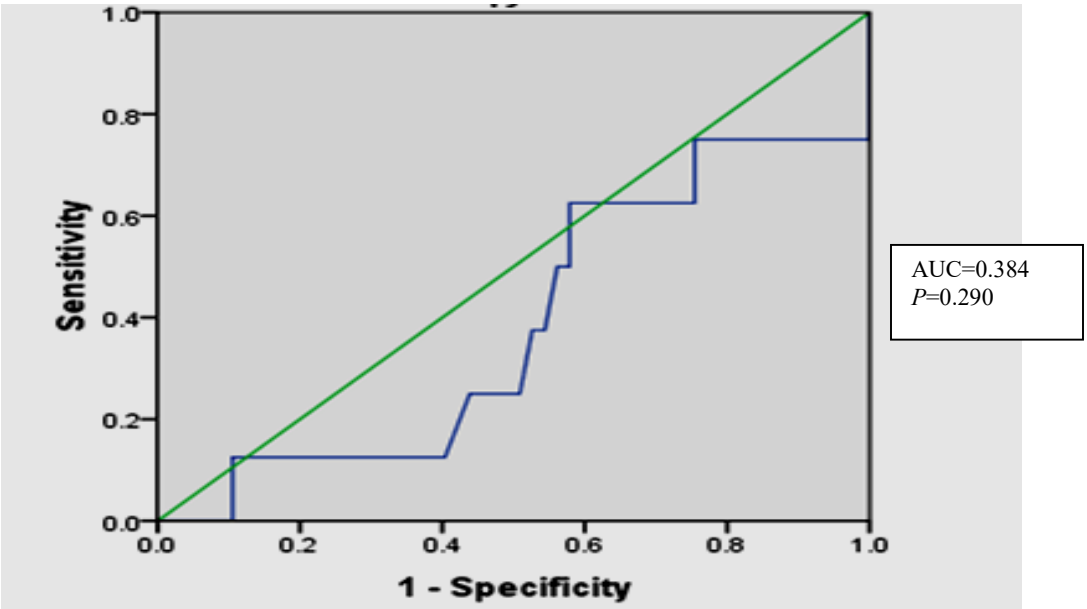

Figure 3: ROC analysis of AFP gradient in the intervention group

However, in the case of group (2) patients, AFP-G demonstrated the ability to predict recurrence with a high degree of accuracy (AUC = 0.965, $P < 0.001$) (Figure 4). Consequently, we were unable to establish a conclusive link between AFP-G and HCC recurrence in all patients or in those who underwent pre-transplant therapeutic interventions for HCC. Nevertheless, it is worth noting that AFP-G emerged as a highly predictor of recurrence in group (2) patients.

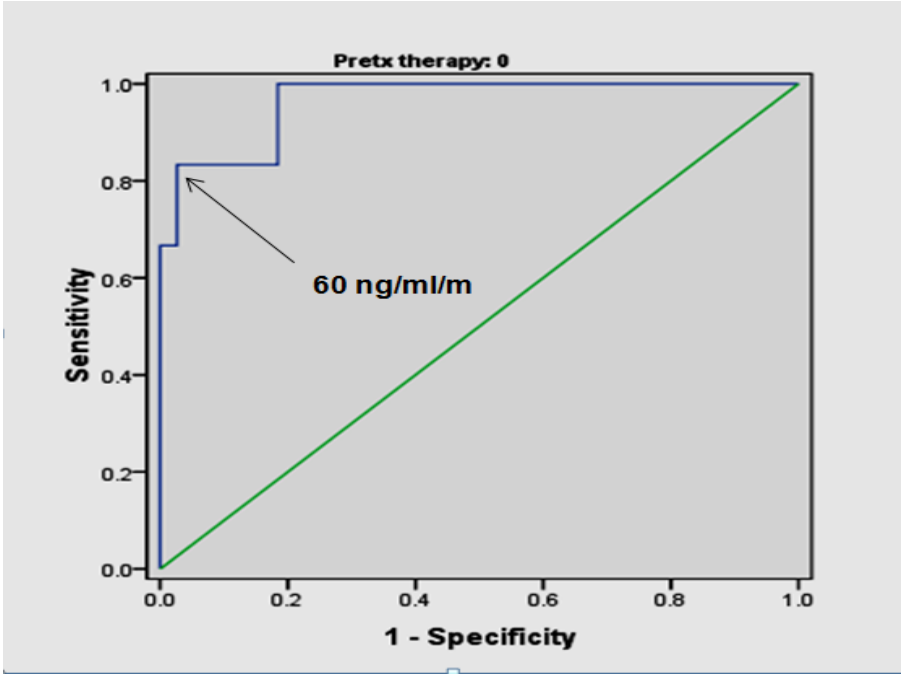

Figure 4: ROC Analysis of AFP gradient in the no-intervention group

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Determination of AFP-G cutoff Value:

Using the ROC curve, an AFP-G cutoff value of 60 ng/ml/month was selected. An AFP-G of 60 ng/ml/m could predict HCC recurrence. The sensitivity was 83.3%, while specificity was 94.7%. The positive and negative predictive values were 71.4%, and 97.3%, respectively.

- **Sponsoring financially:** Nil
- **Competing interest:** Nil

DISCUSSION

Our study highlighted a clear correlation between increasing AFP values and tumor recurrence, which was statistically significant when no pre-transplant HCC treatment was given. However, this relationship was not as consistent in patients who received treatment. Several factors may account for this variation, including the different treatment modalities, the number of treatment sessions, and the interval between treatment and transplant. Based on our data, an AFP-G of > 60 ng/ml/m was associated with HCC recurrence after transplant in untreated patients, ultimately leading to significantly reduced survival.

To optimize the usage of the limited donor pool, various studies have proposed different criteria for selection of HCC patients for liver transplant, with the aim of reducing tumor recurrence and improving survival rates [4]. An increasing serum AFP by > 50 µg/L/month [5] or > 15 µg/L/month [11] were identified as a valuable preoperative predictor of recurrence and the most significant predictor of mortality after transplant.

Given the accelerated growth and vascular invasion of tumors, AFP levels are not expected to increase linearly. A patient who presents early with a stable or slowly increasing AFP may not maintain that profile until the time of transplant. In our series, patients who exhibited an accelerated rate of AFP secretion just before transplant had a less favorable prognosis in terms of recurrence-free survival. It remains uncertain whether these patients would fare better if they received treatment before transplant. While, some reports indicate no survival benefits of pre-transplant HCC treatments, others suggest the opposite[9].

Our study also revealed that pre-transplant interventional treatment for HCC on the waiting list altered AFP levels and the rate of change but did not conclusively improve post-transplant recurrence-free survival. The correlation between increasing AFP values and tumor recurrence was clear in untreated patients but was less apparent in patients who had interventions, likely due to the impact of the treatment. It is important to consider that the AFP-G value was based on the latest AFP values before transplant, rather than the values at the time of listing. Additionally, we did not have definitive data on how these patients would have fared if they had received treatment before transplant. Therefore, it may not be appropriate to use AFP-G > 60 ng/ml/m as an absolute criterion for excluding the transplant candidates. It may be more appropriate as a prognostic tool rather than a selection tool. This discovery holds clinical significance, enabling the consideration of a broader range of

candidates while only slightly elevating the risk of recurrence.

Han *et al.* [5] found that the preoperative AFP slope alone can predict HCC recurrence, whether patients received preoperative therapy or not. The study centered on analyzing the AFP slope before transplant in a group of 48 patients who underwent liver transplants for HCC. The results disclosed that, while the absolute AFP value failed to predict HCC recurrence, the AFP slope emerged as the exclusive preoperative predictor of HCC recurrence. Furthermore, this AFP slope displayed a significant correlation with the presence of large tumors surpassing 7 cm in diameter and the occurrence of vascular invasion. However, the reported median waiting time in their study was relatively short (30 days), which may not allow for a sufficient decrease in AFP levels in treated patients to result in a negative or stable AFP slope. Moreover, it was not reported how many patients who received pre-transplant therapy developed HCC recurrence, and pre-transplant therapy was not statistically associated with HCC recurrence.

We did not exclude patients with incidental HCC from our study to explore the significance of AFP gradient in this specific patient group as a diagnostic tool. The study should further investigate how many patients exceeding Milan criteria with a low AFP-G did well and how many within the Milan criteria with a high AFP-G developed recurrence.

Our data revealed that eight out of the eleven patients who developed HCC recurrence in group (1) had a negative or zero pre-transplant AFP-G, indicating that treatment may temporarily alter AFP progression but may not necessarily prevent post-transplant recurrence. This explains our inability to establish a clear relationship between pre-transplant AFP-G and HCC recurrence in the interventional group.

CONCLUSION

This study underscored the significance of dynamic AFP levels as a predictive factor for HCC recurrence following transplant. Although a pre-transplant AFP greater than 60 ng/ml/m was strongly linked to recurrence in untreated patients, additional research is warranted to elucidate the specific influence of pre-transplant treatments on AFP levels and post-transplant outcomes. It is advisable to regard AFP levels exceeding 60 ng/ml/m as a prognostic tool rather than a rigid selection criterion for transplant candidates.

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