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Differences in Post-Transplant Hepatocellular Carcinoma Recurrence by Etiology of Liver Disease

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Abstract

Background and Aims: The five-year incidence of post-transplant hepatocellular carcinoma (HCC) recurrence is 8-20%. Several studies have evaluated pre-transplant risk factors for HCC recurrence, but nearly all data have treated HCC as a homogeneous condition across all etiologies of liver disease despite differences in tumor biology and baseline incidence of HCC. We sought to evaluate the impact of etiology of liver disease, maximum pre-transplant alpha fetoprotein (AFP), and the interaction of the two factors on the risk of HCC recurrence.

Methods: We performed a retrospective cohort study of HCC transplant recipients using United Network for Organ Sharing (UNOS) data from 2002-2016. Competing risks regression was performed to identify variables associated with HCC recurrence, and an interaction term between etiology and maximum AFP category.

Results: Among 18,406 recipients, 1,484 patients experienced HCC recurrence over 3.1 years of median follow-up time. There was a significant interaction between AFP category and etiology of liver disease ($p < 0.001$). Among patients with a maximum AFP <100ng/mL, those with alcoholic liver disease had the lowest risk of recurrence; by contrast, in patients with a maximum AFP of 100-499, 500-1,000, or >1,000ng/mL, those with alcoholic liver disease had the highest risk of HCC recurrence among all etiologies.

Conclusion: Risk of HCC recurrence differs by etiology of liver disease, and the significance of elevated pre-transplant AFP varies by etiology. Patients with alcoholic liver disease and elevated maximum AFP are at uniquely high risk of HCC recurrence. These findings have potential UNOS policy implications, as the transplant selection process may ultimately benefit from etiology-specific criteria.

Keywords

alpha fetoprotein (AFP); interaction; risk factors; United Network for Organ Sharing (UNOS); competing risks regression

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Conflicts of Interest

The authors of this manuscript have no conflicts of interest to disclose as described by *Liver Transplantation*.

Introduction

Hepatocellular carcinoma (HCC) is a primary liver tumor that occurs in the setting of cirrhosis in 80-90% of cases in the United States.[1,2] Among possible treatments, liver transplantation (LT) offers the highest recurrence-free survival rates.[3,4] However despite strict selection criteria, the five-year post-LT recurrence risk is 8-20%,[5,6,7]with less than one year median survival once diagnosed.[8,9,10]HCC recurrence is thought to occur because of circulating tumor cells not eliminated through transplant.[11,12] Numerous studies have aimed to identify predictors of HCC recurrence to improve LT patient selection and minimize adverse outcomes. Risk prediction has focused on pre-transplant laboratory criteria (e.g., alpha fetoprotein [AFP]), imaging criteria, and explant pathology.[13,14,15] These findings have LT selection implications as they inform Organ Procurement and Transplantation Network (OPTN) HCC exception policies, such as those for patients with high AFP levels (>1,000ng/mL).[16] Importantly, the literature on predicting HCC recurrence has treated HCC as a homogeneous condition across all liver disease etiologies, despite data suggesting otherwise.

The diagnosis and biology of HCC vary based on underlying chronic liver disease (CLD). HCC incidence differs between viral and non-viral etiologies of CLD.[17,18] Furthermore, AFP serves as a marker of HCC as well as hepatic regeneration.[19] Not only is AFP known to be elevated in viral CLD, even in the absence of HCC,[20] but the sensitivity and specificity of AFP in diagnosing HCC varies significantly among viral and non-viral etiologies of CLD. [21,22,23]These disparities speak to potentially different immunological pathways leading to HCC. Indeed, recent work suggests that viral CLD results in a necroinflammatory process whereas non-viral CLD causes cell death leading to a deregulated liver immune network.[24] These mechanistic differences underscore the need to evaluate risk factors for HCC recurrence through a different lens— based on etiology of liver disease.

We sought to explore whether etiology of liver disease reflects differences in HCC tumor biology and behavior, in particular its propensity for recurrence. Because the LT model entails the removal of the entire liver and that recurrence must be due to pre-transplant metastasis, we elected to operationalize our research question using national registry data. We aimed to determine: 1) whether the risk of HCC recurrence differs by etiology of liver disease, and 2) whether pre-transplant AFP predicts HCC recurrence differently based on etiology of liver disease.

Methods

Design, Patient Selection, and Variable Collection

We conducted a retrospective cohort study using United Network for Organ Sharing (UNOS) data between 2/2002 and 9/2016. Although prioritization for patients with HCC has changed over time, this can be accounted for in models and would not be expected to confound associations between etiology of liver disease, AFP, and HCC recurrence. We included patients aged ≥18 who underwent LT with standardized T2 Model for End-Stage

Liver Disease (MELD) exceptions. We excluded patients without recorded AFP values and those with non-standardized HCC exceptions.

Demographic variables (age, sex, race) and body mass index (BMI) were collected, as were pre-LT MELD score, tumor characteristics including number of tumors, largest tumor diameter, adherence to the Milan criteria immediately prior to transplant, locoregional therapy prior to transplant (including embolization, ablation, and radiation-based approaches), downstaging prior to transplant, and prior surgical resection. Etiologies of liver disease were classified as hepatitis C (HCV), hepatitis B (HBV), alcoholic (EtOH), non-alcoholic fatty liver (NAFLD), autoimmune (including autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis), and other (comprised of numerous less common etiologies including hemochromatosis, sarcoidosis, alpha-1 antitrypsin, and rare inborn metabolic liver diseases). Maximum pre-transplant AFP (max AFP) was classified as a four-level categorical variable (<100ng/mL, 101-499ng/mL, 500-1,000ng/mL, and >1,000ng/mL), adapted from numerous prior studies.[5,13,15,16] The max AFP variable performs similarly to AFP immediately prior to transplant (pre-transplant AFP) in HCC recurrence models,[25] and reflects current OPTN policy which provisionally disallows standard exception points for patients with max AFP >1,000ng/mL.[16] However, as a sensitivity analysis, pre-transplant AFP was also analyzed in models (detailed below), classified as a four-level categorical variable with the same thresholds as max AFP. Waiting time and cold ischemia time were evaluated in both continuous and categorical formats, based on prior literature.[26,27]

Outcome Definition

HCC recurrence was determined based on the designation of (1) post-transplant death from HCC or metastatic malignancy, or (2) post-transplant recurrence of pre-transplant malignancy. These fields are derived from LT recipient follow-up data that must be submitted annually by transplantation centers. Importantly, this HCC recurrence outcome ascertainment algorithm has been previously validated[28] and utilized in numerous studies, including the recent validation of the RETREAT score.[29] While UNOS does not specify imaging requirements for post-transplant HCC recurrence surveillance, it is standard to perform computed tomography (CT) or magnetic resonance imaging (MRI) on an annual or biennial basis. Furthermore, although tissue diagnosis of HCC recurrence is not available in the UNOS dataset, a recurrence diagnosis is based on imaging criteria in nearly all cases.

Patient Characteristics

Patients stratified by HCC recurrence were compared across a range of the aforementioned variables. Kaplan-Meier survival estimates were generated for 1, 3, and 5-year recurrence by max AFP category and etiology of liver disease. Descriptive statistics were computed as medians and interquartile ranges (IQR). Wilcoxon rank-sum and chi-squared tests were used to compare continuous and categorical data, respectively. For these and subsequent tests, a two-tailed alpha level = 0.05 was used as the threshold for statistical significance unless otherwise stated. All data management and computations were performed using STATA/IC version 14.2.

Cox Regression Analysis

Variables potentially associated with HCC recurrence were first analyzed with univariate Cox proportional hazards regression. Explant pathology characteristics were not included in any models, as these are not available for risk stratification in the pre-transplant setting. An alpha = 0.10 was maintained for potential inclusion in multivariable modeling. After identification of candidate variables, multivariable Cox regression was performed. Multiple selection methods were used, including researcher-driven, forward selection, and reverse selection, with a threshold alpha = 0.05 used for variable retention. As per the second study objective, an *a priori* interaction term between max AFP category and etiology of liver disease was included in the model. The Cox proportional hazards assumption was evaluated using log-log survival plots and plotting of Schoenfeld residuals over time. No serious violations were observed.

Competing Risks Regression Analysis

The final multivariable Cox regression model was subsequently modeled in a competing risks framework, identifying death as a competing event. The pre-specified interaction term was included, and linear combinations were used to derive subhazard ratio (SHR) estimates for each AFP-etiology level. Statistical comparisons between AFP levels, among different etiologies of liver disease, were Bonferroni-adjusted for multiple comparisons. Cumulative incidence functions were plotted for each liver disease etiology, stratified by AFP category. Of note, because the data missingness was less than 5% in the final regression models, imputation methods were not used.

Sensitivity Analyses

To determine the impact of using max AFP as opposed to pre-transplant AFP in the primary analysis, Cox regression and competing risks regression analyses were also performed using pre-transplant AFP as defined previously. Additionally, to determine if HCC recurrence dynamics have changed as a result of directly administrated antiretroviral therapy (DAART), models were produced for the pre- and post-DAART era, using January 1st, 2014 as the transition point (based on the sofosbuvir Food and Drug Administration [FDA] approval date). In both cases, tables were produced for the interaction term between AFP and etiology of liver disease, with Bonferroni-adjusted alpha thresholds used for statistical comparisons to low-AFP reference groups.

Exploratory Analysis

Explant data from the UNOS registry, catalogued since 4/2012, was utilized to perform an exploratory analysis on HCC patients with AFP ≥100ng/mL. This threshold was chosen given the smaller sample size constraints imposed by the explant dataset. Several variables were compared among etiologies based on prior literature identifying explant predictors of HCC recurrence, including number of tumors (solitary versus multiple),[30,31] macrovascular invasion,[32] poor tumor differentiation,[33] adherence to Milan criteria on pathology, and maximum tumor size.[34,35] An aggregate binary variable (termed *poor prognosis*) indicating the presence of multiple tumors, macrovascular invasion, or poor tumor differentiation was also included for analysis, again because of the smaller sample size. Chi-squared and

Kruskal Wallis tests were performed for categorical and continuous data analysis, respectively, with a Dunn's test used for post-hoc pairwise comparisons.

Results

Patient Characteristics

After applying selection criteria, a total of 18,406 patients were included in the analytic cohort (Supplemental Figure 1). Over median 3.1 years of follow-up, 1,484 patients were diagnosed with HCC recurrence, yielding a recurrence rate of 19.5 cases per 1,000 person-years. The characteristics of patients with HCC recurrence differed from those without recurrence across a range of demographic, laboratory, and clinical factors (Table 1). Kaplan-Meier survival estimates for HCC recurrence at 1, 3, and 5 years post-LT, stratified by max AFP category and etiology of liver disease, are presented in Supplemental Tables 1a and 1b.

Cox Regression and Competing Risks Analyses

By all selection methods, the final multivariable Cox regression and competing risks regression models included etiology of liver disease, max AFP range, the etiology-AFP interaction term, and were adjusted for the following covariates: age, sex, race, waiting time, largest tumor size at transplant, adherence to Milan criteria, locoregional therapy prior to transplant, and downstaging prior to transplant (Table 2; univariate analysis available in Supplemental Table 2). The recurrence risk was higher in males, patients with increased max AFP, and patients who received locoregional therapy or who were downstaged prior to transplant; recurrence risks were lower in blacks, Hispanics, patients with alcoholic liver disease, and those within Milan criteria.

Interaction between Etiology of Liver Disease and AFP

The etiology-AFP interaction term was significant in both Cox and competing risks regression models ($p < 0.01$ and $p = 0.02$, respectively). When max AFP category was stratified by liver disease, HCC patients with EtOH and NAFLD were less likely to produce AFP at the highest levels (Figure 1; $p < 0.001$). The HCC recurrence risk with increased max AFP differed by etiology of liver disease (Table 3). For example, among HCV patients, the recurrence risk was more than 3.5 times higher for those with AFP >1,000ng/mL versus AFP <100ng/mL (SHR: 3.73, 95% CI: 2.92 – 4.77, $p < 0.001$). However, for EtOH patients, those with AFP >1,000ng/mL had a more than seven times increased risk of recurrence versus those with AFP <100ng/mL (SHR: 7.20, 95% CI: 3.75 – 13.81, $p < 0.001$). Estimates for recurrence risk with high AFP in HBV, NAFLD, or autoimmune disease were generally lower than those for HCV or EtOH, and often there were no significant differences in risk compared to low AFP values. When stratified by max AFP category, EtOH patients had the lowest HCC recurrence risk for AFP levels <100ng/mL, but the highest risk among etiologies for elevated AFP levels (Figures 2a, b, c, d). In both sensitivity analyses, the interaction term between etiology of liver disease and max AFP was statistically significant in multivariable Cox and competing risks regression models (all $p < 0.05$). Similar trends to those noted above were observed using pre-transplant AFP (Supplemental Table 3) as well as using max AFP in the pre- and post-DAART eras (Supplemental Table 4).

Exploratory Analysis

In patients with AFP ≥100ng/mL with explant data, maximum tumor size was significantly different among all etiologies (Table 4; p = 0.04). EtOH patients had the smallest maximum tumor size (median: 2.2cm, IQR: 1.7 – 3.4cm), although pairwise testing did not meet statistical significance owing to multiple comparisons (data not shown). There were significant differences in the number of tumors on explant (p = 0.04) as well as poor prognosis characteristics (p = 0.02), where EtOH patients had the highest proportions among all etiologies for both variables (64.1% multiple tumors and 76.9% poor prognosis). Again, owing to multiple comparisons, the pairwise analyses were not statistically significant (data not shown). There were no significant differences in macrovascular invasion, poorly differentiated tumors, or explant adherence to Milan criteria. Note that explant predictors based on tumor size were not included in the aggregate variable given the differences found in maximum tumor size.

Discussion

In this analysis of 15 years of HCC transplant data, we found that etiology of liver disease was significantly associated with HCC recurrence, with EtOH patients having the lowest recurrence risk overall. This finding is in opposition to other published literature. In the RETREAT study,[15] etiology was not statistically significant on univariate analysis. There are several possible explanations for this difference, including the much greater power in the present study (nearly 20-fold larger sample size), as well as the relative enrichment of EtOH in our dataset. In the US Multicenter HCC Transplant Consortium, etiology was not a significant predictor of recurrence,[36] but the multivariable models adjusted for explant pathology factors (where we found relevant differences in our exploratory analysis); this would be expected to abolish the association. Moreover, our finding that EtOH patients have a lower risk of HCC recurrence is not altogether unexpected given known differences in baseline risk of HCC by CLD. Indeed, the literature suggests that viral CLD confers a 20 – 25 relative risk of HCC, in contrast to 1.5 – 3 for EtOH patients.[37]

The key novel finding of this study was a significant interaction between AFP and etiology of liver disease. Although the risk of HCC recurrence generally increased with rising AFP for each etiology, the degree of increased risk was dramatically higher for EtOH patients. There is biological plausibility for these findings. First, we demonstrated that AFP production differs by etiology; fewer HCC patients with EtOH or NAFLD produced high levels of AFP, a finding consistent with prior literature.[23] Second, our exploratory analysis revealed objective differences in explant pathology by etiology among patients with elevated AFP. While pairwise analyses were not statistically significant, the data suggest that alcoholic HCC patients with high AFP may have smaller maximum tumor sizes, poorer pathology characteristics, and more often have multiple tumors on explant. This finding implies that there may be features of the HCC tumor burden in high-AFP alcoholic patients that are unlike counterpart tumors in other liver diseases. Here it is intriguing to recall that the Milan criteria were derived from an almost exclusively viral CLD cohort which may not adequately serve HCC patients with non-viral CLD.[3] Finally, immunological research supports the possibilities of different pathways to HCC, and articulates how this might occur

on the basis of underlying mechanisms of disease.[38] HCV and HBV cause chronic non-cytopathic damage and a necroinflammatory response, while EtOH and NAFLD cause primary hepatocyte death with production of disease-associated molecules and a deregulated innate immune system.[24] Although the common result is chronic inflammation and HCC risk, the signaling and cytokine pathways that activate the inflammasome are context-specific.[39] It is therefore plausible that the likelihood of pathway-specific AFP elevation and its interpretation could be different as well.

Our findings also have potential policy implications. In particular, they are of import to the current OPTN/UNOS HCC exception criteria, where AFP >1,000ng/mL *provisionally* disqualifies a patient for standardized HCC exception points.[40] This policy is applied uniformly across all etiologies of liver disease, however our data suggest that etiology-specific AFP thresholds may be more appropriate. For example, because of the uniquely high HCC recurrence risks associated with elevated AFP in EtOH patients, an AFP ≥100ng/mL may be more reasonable as a provisional exclusion threshold in these patients, as the degree of increased risk relative to AFP <100ng/mL at this level was higher than any other etiology for any degree of AFP elevation. Likewise, the AFP thresholds should potentially be *liberalized* in patients with HBV, NAFLD, and autoimmune liver disease, because in many cases an elevated AFP did not correspond to an increased risk of HCC recurrence.

There are several limitations to our study. First, the retrospective design precludes causal inferences regarding exposure and outcome. Second, there is potential for exposure misclassification, in particular etiology of CLD. For example, some patients classified with HCV may also have alcoholic liver disease, or that EtOH patients may also have NAFLD. However, this misclassification is likely to be non-differential (i.e. not dependent on the HCC recurrence outcome). Furthermore, it is unlikely that patients labeled as having alcoholic liver disease are misclassified, and if some patients with concomitant, unlabeled alcoholic liver disease are classified under other etiologies, this would be expected to bias estimates towards the null. Third, some degree of outcome misclassification is likely. Although we employed a validated algorithm for ascertainment, there are certainly undiagnosed cases of recurrent HCC that are therefore unreported, or cases that are diagnosed but reported in a fashion not captured by our algorithm. However, because transplant centers apply uniform surveillance protocols dictated by UNOS policies, this misclassification would be non-differential and expected to underestimate HCC recurrence risks overall. Finally, our exclusion criteria may produce some selection bias. Patients without AFP values recorded in the UNOS dataset were excluded, as were those without standard HCC exception data. However, the majority of the patients without AFP data were listed prior to 5/2003, when AFP was not a required element of the HCC exception submission.[41] Missing data in this case result from structural changes in submission requirements, and should be randomly distributed amongst patients with differing etiologies of liver disease. Regarding the patients excluded for non-standard HCC exceptions, this step would likely identify patients with HCC cases referred to regional review boards. These cases would be more likely to contain disease outside of the Milan criteria, and presumably a higher risk of HCC recurrence. Excluding these patients would have the effect of biasing

overall regression results towards the null, making the estimates in this study more conservative than would otherwise be expected.

In conclusion, our data suggest that we should view HCC as a condition with a multitude of biological phenotypes requiring a highly tailored approach. With respect to post-LT recurrence, the significance of a high AFP differs by etiology of liver disease, and LT selection criteria may need to address these differences. Further research is undoubtedly required to validate these findings in prospective cohorts, establish acceptable AFP thresholds for etiology-specific risks, and to further elucidate the biological/immunological underpinnings of HCC that arises in the context of different chronic liver diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

HCC	hepatocellular carcinoma
AFP	alpha fetoprotein
UNOS	United Network for Organ Sharing
LT	liver transplantation
OPTN	Organ Procurement and Transplantation Network
CLD	chronic liver disease
MELD	model for end-stage liver disease
BMI	body mass index
HCV	hepatitis C
HBV	hepatitis B
EtOH	alcoholic liver disease
NAFLD	non-alcoholic fatty liver disease
CT	computed tomography
MRI	magnetic resonance imaging
IQR	interquartile range

SHR	subhazard ratio
DAART	directly administrated antiretroviral therapy
FDA	Food and Drug Administration

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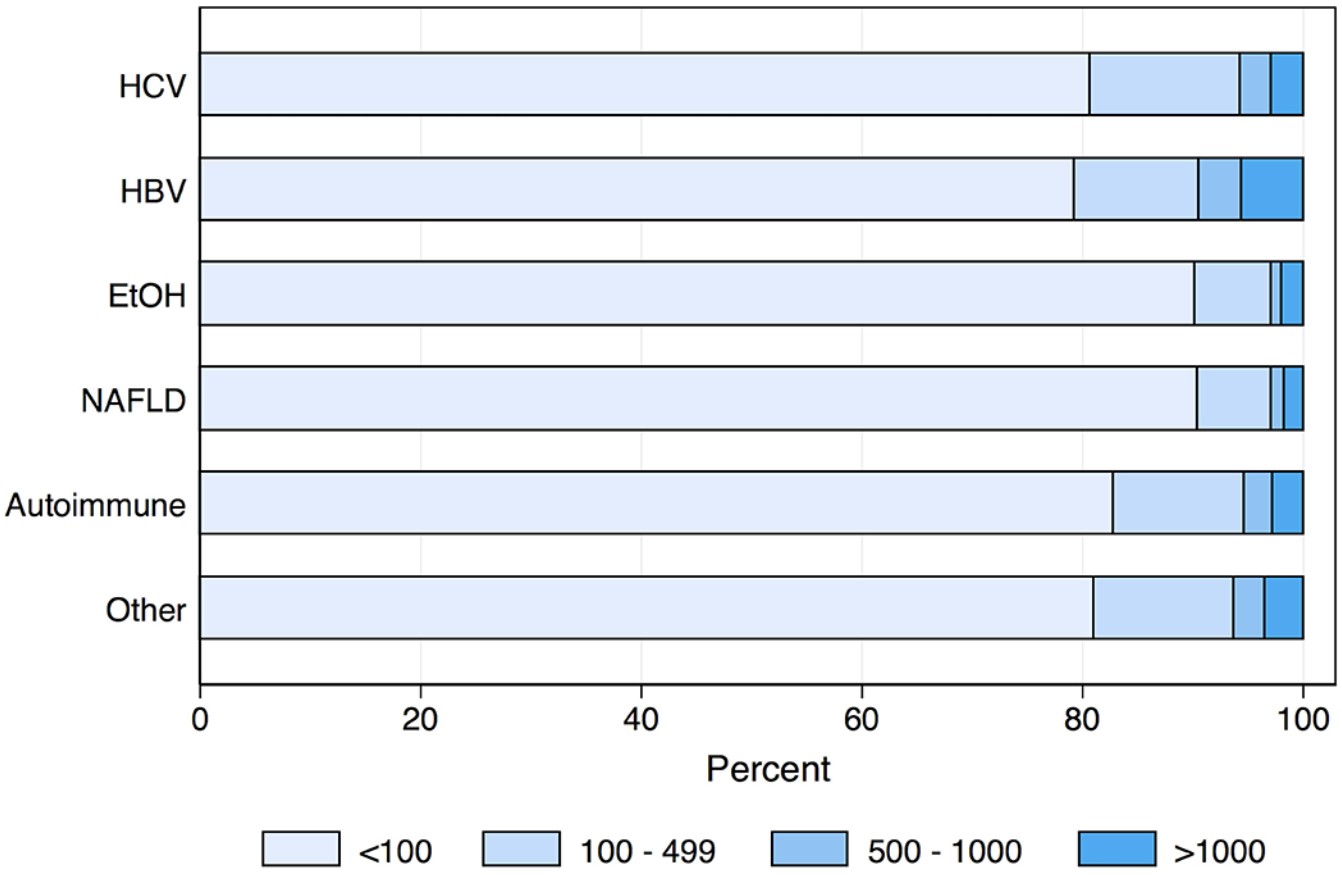

Figure 1 –.
Proportional Categorizations of Pre-transplant AFP (ng/mL), Stratified by Etiology Liver Disease

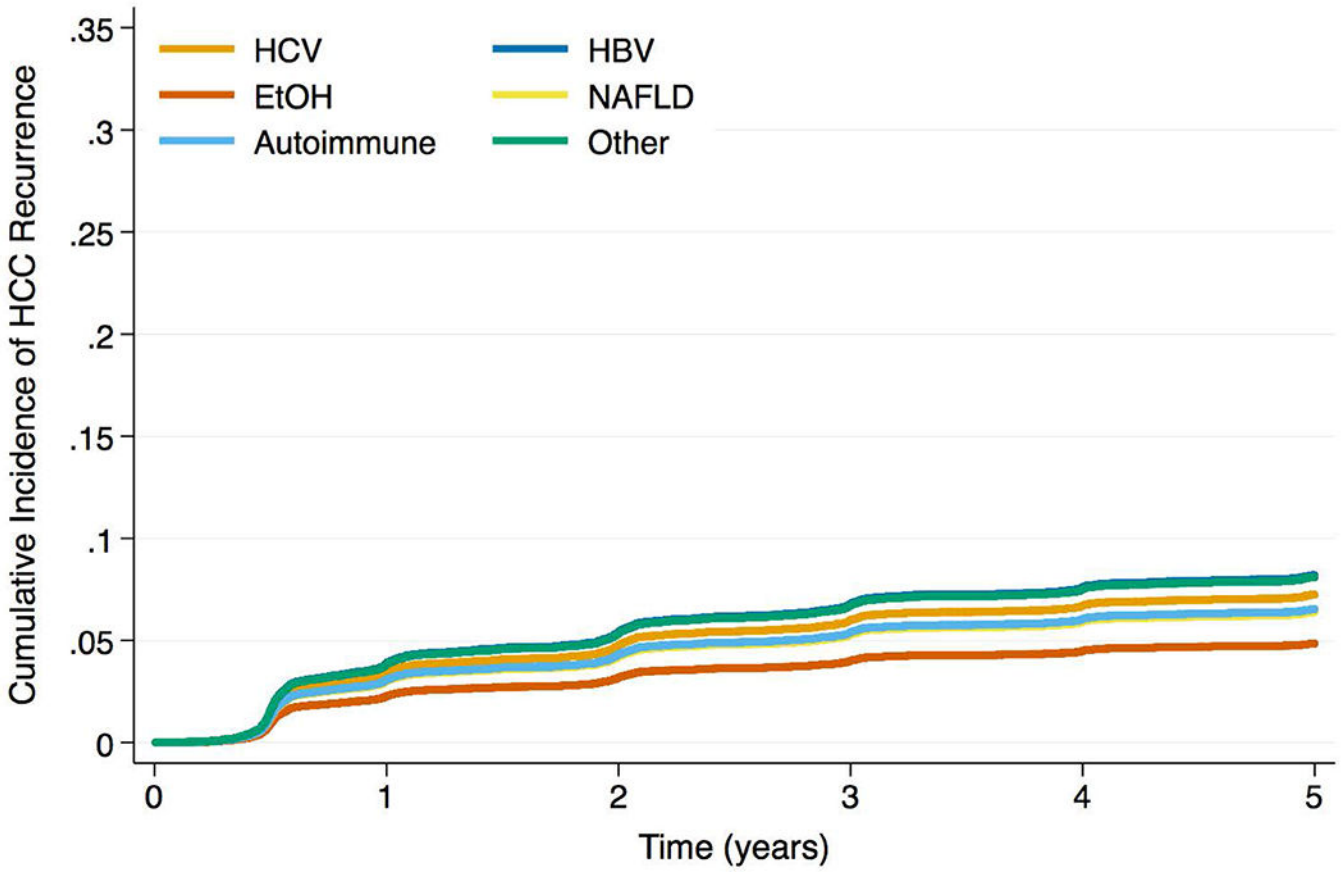

Figure 2a -
Cumulative Incidence Functions for HCC Recurrence by Max AFP Category: AFP <100ng/mL

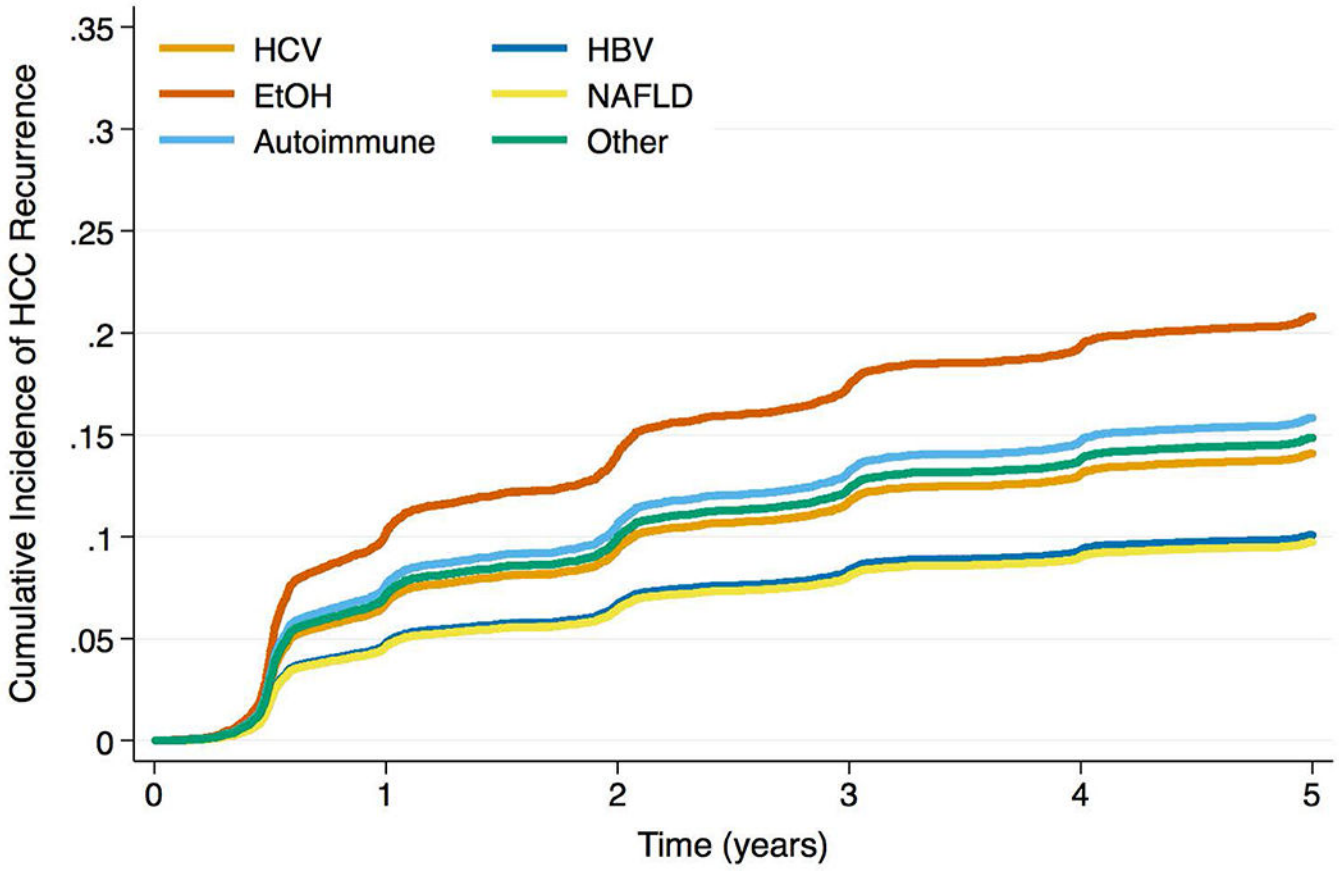

Figure 2b -
Cumulative Incidence Functions for HCC Recurrence by Max AFP Category: AFP 100 – 499ng/mL

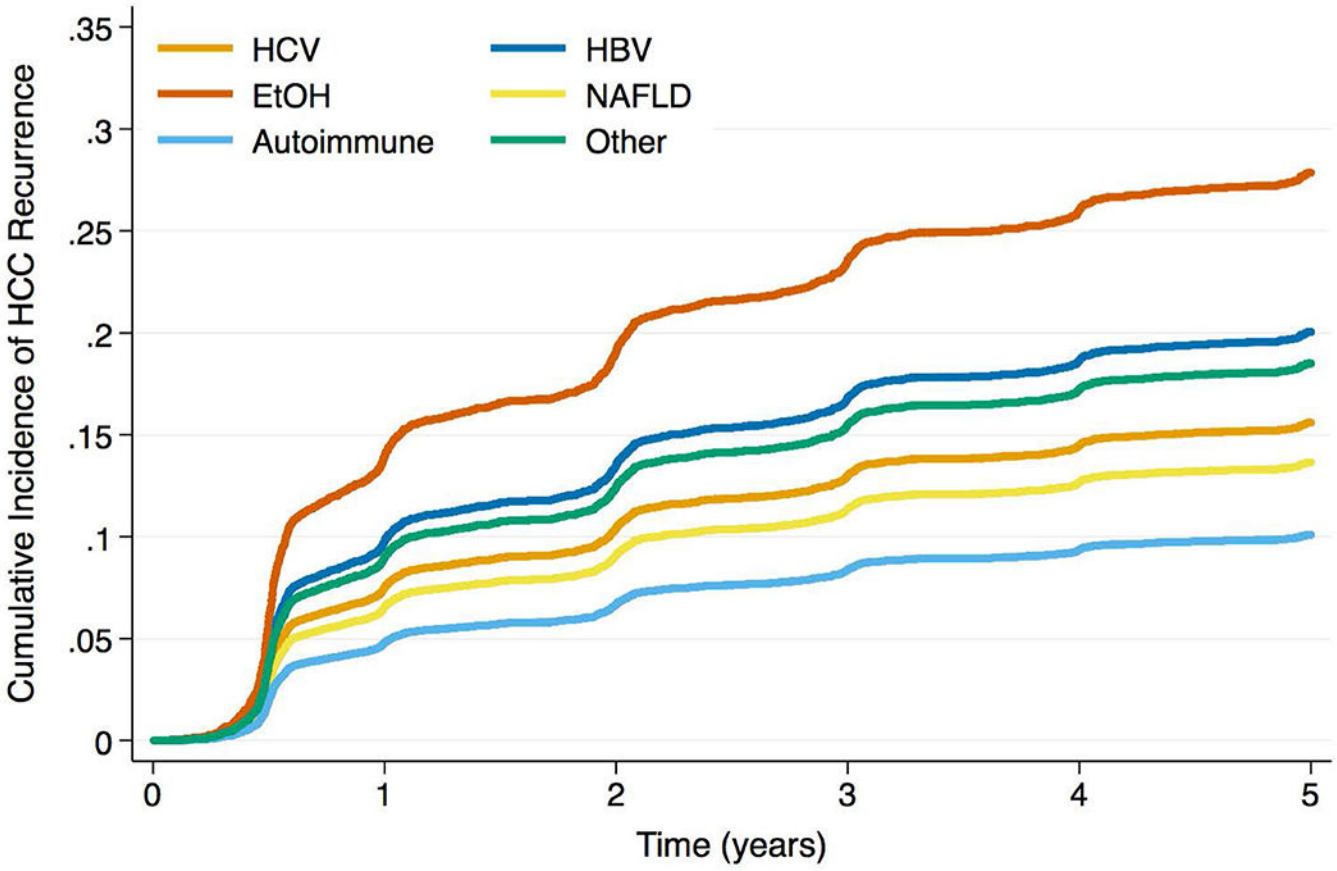

Figure 2c -
Cumulative Incidence Functions for HCC Recurrence by Max AFP Category: AFP 500 – 1000ng/mL

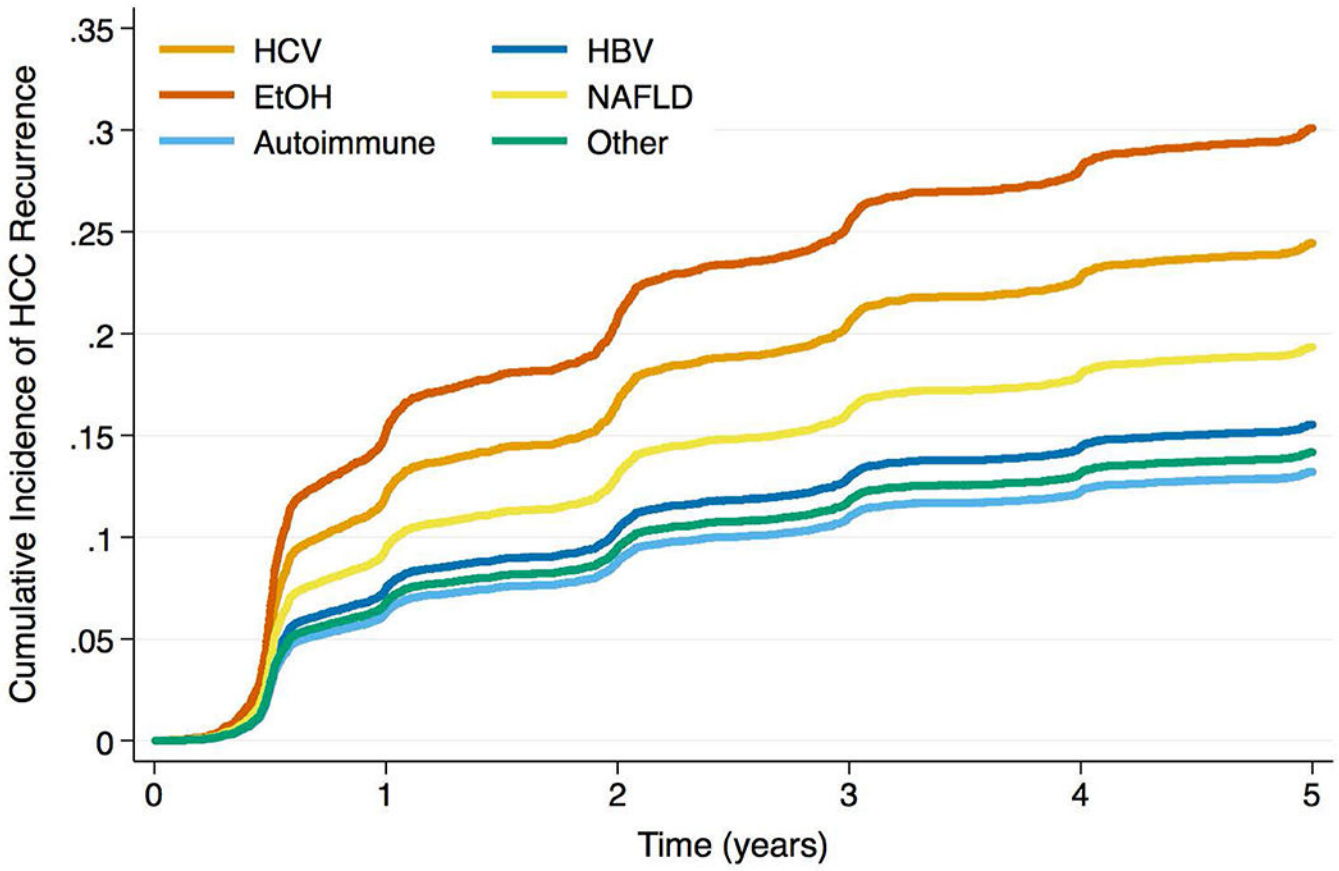

Figure 2d -
Cumulative Incidence Functions for HCC Recurrence by Max AFP Category: AFP >1000ng/mL

Table 1 –

Patient Characteristics by Hepatocellular Carcinoma Recurrence Status

Variable	No Recurrence (N = 16922)	HCC Recurrence (N = 1484)	p-value
Age at Listing, median (IQR)	58.0 (53.0, 62.0)	57.0 (53.0, 62.0)	0.640
Sex			<0.001 *
Female	3920 (23.2%)	271 (18.3%)	
Male	13002 (76.8%)	1213 (81.7%)	
Race			0.015 *
White	11202 (66.2%)	1036 (69.8%)	
Black	1609 (9.5%)	121 (8.2%)	
Hispanic	2480 (14.7%)	180 (12.1%)	
Asian	1409 (8.3%)	131 (8.8%)	
Other	222 (1.3%)	16 (1.1%)	
Etiology of Chronic Liver Disease			0.002 *
HCV	10224 (60.4%)	921 (62.1%)	
HBV	1023 (6.0%)	96 (6.5%)	
EtOH	1556 (9.2%)	111 (7.5%)	
NAFLD	1564 (9.2%)	106 (7.1%)	
Autoimmune	504 (3.0%)	36 (2.4%)	
Other	2051 (12.1%)	214 (14.4%)	
MELD at Listing, median (IQR)	12.0 (9.0, 16.0)	11.0 (8.0, 15.0)	0.002 *
BMI at Listing, median (IQR)	28.1 (25.0, 31.6)	27.8 (25.0, 31.6)	0.400
Months on Waiting List, median (IQR)	5.5 (2.0, 12.4)	4.2 (1.5, 9.5)	<0.001 *
Waiting Time <6 Months	8894 (52.6%)	905 (61.0%)	<0.001 *
Waiting Time >18 Months	2531 (15.0%)	172 (11.6%)	<0.001 *
Pre-transplant AFP, median (IQR)	9.0 (5.0, 31.0)	25.0 (7.0, 136.0)	<0.001 *
Pre-transplant AFP Range (ng/mL)			<0.001 *
<100	14101 (87.5%)	1018 (71.3%)	
100 – 499	1441 (8.9%)	243 (17.0%)	
500 – 1000	287 (1.8%)	64 (4.5%)	
>1000	284 (1.8%)	102 (7.1%)	
Max AFP, median (IQR)	12.0 (5.0, 47.0)	31.0 (9.0, 166.5)	<0.001 *
Max AFP Range (ng/mL)			<0.001 *
<100	14166 (83.7%)	1001 (67.5%)	

Variable	No Recurrence (N = 16922)	HCC Recurrence (N = 1484)	p-value
100 – 499	1935 (11.4%)	293 (19.7%)	
500 – 1000	399 (2.4%)	74 (5.0%)	
>1000	422 (2.5%)	116 (7.8%)	
Number of Viable Tumors at Transplant			<0.001 *
1	12775 (75.5%)	1064 (71.7%)	
2	2998 (17.7%)	282 (19.0%)	
3	1104 (6.5%)	130 (8.8%)	
4 or more	45 (0.3%)	8 (0.5%)	
Largest Tumor at Transplant (cm), median (IQR)	2.1 (1.1, 2.8)	2.5 (1.7, 3.3)	<0.001 *
Within Milan Criteria at Transplant	16397 (96.9%)	1393 (93.9%)	<0.001 *
Locoregional Therapy Prior to Transplant	11973 (70.8%)	1071 (72.2%)	0.250
Downstaged Prior to Transplant	264 (1.6%)	35 (2.4%)	0.020 *
Prior Surgical Resection	200 (1.2%)	21 (1.4%)	0.430
Cold Ischemia Time (Hours), median (IQR)	6.2 (4.9, 8.0)	6.5 (5.0, 8.1)	0.004 *
Cold Ischemia Time >10 Hours	2213 (13.1%)	233 (15.7%)	0.004 *

* Statistically significant at the alpha = 0.05 level

Table 2 –

Multivariable Cox Regression and Competing Risks Regression Analyses: Variables Associated with Post-transplant HCC Recurrence

	Multivariable Cox Regression		Competing Risks Regression	
	Hazard Ratio (95% CI)	**p-value**	**Subhazard Ratio (95% CI)**	**p-value**
Variables of Interest				
Etiology (ref = HCV)				
HBV	1.10 (0.83 – 1.45)	0.514	1.14 (0.86 – 1.51)	0.359
EtOH	0.65 (0.51 – 0.83)	0.001*	0.66 (0.52 – 0.85)	0.001*
NAFLD	0.87 (0.69 – 1.09)	0.223	0.88 (0.70 – 1.10)	0.267
Autoimmune	0.87 (0.58 – 1.31)	0.510	0.90 (0.60 – 1.36)	0.612
Other	1.10 (0.92 – 1.32)	0.294	1.13 (0.94 – 1.35)	0.202
Max AFP Range (ng/mL; ref <100)				
100 – 499	2.08 (1.77 – 2.45)	<0.001*	2.02 (1.72 – 2.38)	<0.001*
500 – 1000	2.34 (1.73 – 3.17)	<0.001*	2.26 (1.65 – 3.08)	<0.001*
>1000	4.06 (3.20 – 5.16)	<0.001*	3.73 (2.92 – 4.77)	<0.001*
Etiology-AFP Range Interaction		0.006*		0.015*
Covariates				
Age (per 5 years)	1.05 (1.01 – 1.09)	0.008*	1.04 (1.00 – 1.08)	0.041*
Male Sex	1.36 (1.18 – 1.55)	<0.001*	1.36 (1.19 – 1.55)	<0.001*
Race (ref = white)				
Black	0.77 (0.64 – 0.93)	0.007*	0.73 (0.60 – 0.88)	0.001*
Hispanic	0.82 (0.70 – 0.96)	0.015*	0.82 (0.70 – 0.97)	0.017*
Asian	0.84 (0.68 – 1.04)	0.107	0.87 (0.70 – 1.08)	0.211
Other	0.69 (0.42 – 1.13)	0.136	0.69 (0.41 – 1.15)	0.152
Waiting Time (per 3 months)	0.99 (0.98 – 1.00)	0.013*	0.99 (0.98 – 1.00)	0.022*
Largest Tumor at Transplant (cm)	1.11 (1.08 – 1.13)	<0.001*	1.11 (1.07 – 1.15)	<0.001*
Within Milan Criteria at Transplant	0.76 (0.60 – 0.96)	0.020*	0.77 (0.61 – 0.98)	0.031*
Locoregional Therapy Prior to Transplant	1.27 (1.13 – 1.43)	<0.001*	1.29 (1.14 – 1.44)	<0.001*
Downstaged Prior to Transplant	1.53 (1.09 – 2.14)	0.014*	1.53 (1.09 – 2.16)	0.014*

*Statistically significant at the alpha = 0.05 level

**MELD pre-transplant and number of viable tumors at transplant were not statistically significant at the alpha = 0.05 level and were removed from these models

Table 3 –

Multivariable Competing Risks Regression Results: Subhazard Ratios Derived from Interaction between Etiology of Liver Disease and Max AFP range (ng/mL)

Variable	Subhazard Ratio (95% CI)	p-value
HCV		
AFP <100	1 (reference)	
AFP 100 – 499	2.02 (1.72 – 2.38)	<0.001*
AFP 500 – 1000	2.26 (1.65 – 3.08)	<0.001*
AFP >1000	3.73 (2.92 – 4.77)	<0.001*
HBV		
AFP <100	1 (reference)	
AFP 100 – 499	1.24 (0.67 – 2.29)	0.489
AFP 500 – 1000	2.62 (1.27 – 5.40)	0.009*
AFP >1000	1.97 (0.97 – 4.03)	0.062
EtOH		
AFP <100	1 (reference)	
AFP 100 – 499	4.69 (2.92 – 7.52)	<0.001*
AFP 500 – 1000	6.57 (2.75 – 15.68)	<0.001*
AFP >1000	7.20 (3.75 – 13.81)	<0.001*
NAFLD		
AFP <100	1 (reference)	
AFP 100 – 499	1.55 (0.83 – 2.89)	0.168
AFP 500 – 1000	2.22 (0.76 – 6.52)	0.145
AFP >1000	3.26 (1.39 – 7.65)	0.007*
Autoimmune		
AFP <100	1 (reference)	
AFP 100 – 499	2.55 (1.18 – 5.50)	0.017*
AFP 500 – 1000	1.58 (0.23 – 10.65)	0.640
AFP >1000	2.10 (0.52 – 8.52)	0.300
Other		
AFP <100	1 (reference)	
AFP 100 – 499	1.90 (1.35 – 2.69)	<0.001*
AFP 500 – 1000	2.42 (1.33 – 4.40)	0.004*
AFP >1000	1.81 (1.01 – 3.23)	0.045

* Statistically significant at the alpha = 0.017 level (Bonferroni corrected)

Table 4 –

Explant Characteristics for Patients AFP ≥100ng/mL

Variable	HCV N = 650	HBV N = 49	EtOH N = 39	NAFLD N = 44	Autoimmune N = 18	Other N = 90	p-value
Number of Tumors							0.039 *
Solitary	286 (44.0%)	33 (67.3%)	14 (35.9%)	19 (43.2%)	9 (50.0%)	41 (45.6%)	
Multiple	364 (56.0%)	16 (32.7%)	25 (64.1%)	25 (56.8%)	9 (50.0%)	49 (54.4%)	
Macrovascular Invasion							0.240
No	630 (96.9%)	47 (95.9%)	37 (94.9%)	41 (93.2%)	18 (100.0%)	83 (92.2%)	
Yes	20 (3.1%)	2 (4.1%)	2 (5.1%)	3 (6.8%)	0 (0.0%)	7 (7.8%)	
Poorly Differentiated							0.150
No	570 (87.7%)	45 (91.8%)	29 (74.4%)	37 (84.1%)	16 (88.9%)	75 (83.3%)	
Yes	80 (12.3%)	4 (8.2%)	10 (25.6%)	7 (15.9%)	2 (11.1%)	15 (16.7%)	
Poor Prognosis‡							0.019 *
No	252 (38.8%)	29 (59.2%)	9 (23.1%)	18 (40.9%)	9 (50.0%)	34 (37.8%)	
Yes	398 (61.2%)	20 (40.8%)	30 (76.9%)	26 (59.1%)	9 (50.0%)	56 (62.2%)	
Within Milan Criteria							0.620
No	213 (32.8%)	12 (24.5%)	12 (30.8%)	17 (38.6%)	7 (38.9%)	34 (37.8%)	
Yes	437 (67.2%)	37 (75.5%)	27 (69.2%)	27 (61.4%)	11 (61.1%)	56 (62.2%)	
Max Tumor Size (cm), median (IQR)	2.6 (2, 3.7)	2.5 (1.3, 3.5)	2.2 (1.7, 3.4)	3.2 (2.1, 4.3)	3.1 (2.5, 3.5)	3 (2, 4.2)	0.040 *

* Statistically significant at the alpha = 0.05 level

‡ Aggregate variable including the presence of: multiple tumors, macrovascular invasion, or poor tumor differentiation