Association of Tumor Grade With Long-Term Survival in Patients With Hepatocellular Carcinoma After Liver Transplantation

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ABSTRACT

Background. Association of tumor grade and the prognosis of hepatocellular carcinoma (HCC) patients after liver transplantation (LT) had not been clearly illustrated. The objective of the current study was to investigate the impact of tumor grade on the long-term survival of patients with HCC receiving LT.

Methods. Data from Surveillance, Epidemiology, and End Results Program (SEER) 18 registry for 2004–2015 was extracted for the present study. Propensity score matching was performed to eliminate possible bias. In addition, multivariable analysis was utilized to adjust for confounding factors. The primary endpoints were overall survival (OS) and disease-specific survival (DSS).

Results. A total of 802 patients diagnosed with HCC receiving LT between 2004–2015 included in the SEER registry were analyzed in the study. In the multivariable adjusted cohort (OS: n = 802; DSS: n = 640), a worse prognosis was observed in OS (HR, 1.57; 95% CI, 1.13–2.19; *P* = .008) and DSS (HR, 2.62; 95% CI, 1.44–4.77; *P* = .002) for patients with moderate/poor-differentiated tumors compared to patients with well-differentiated tumors. In stratified analyses, the salutary effects of well-differentiation on OS and DSS were consistent across all examined subgroups. In the propensity-matched cohort, the univariable analysis showed that patients with moderate/poor-differentiated tumors still had worse OS (HR 1.60, 95% CI 1.12–2.28, *P* = .010) and DSS (HR 2.54, 95% CI 1.36–4.74, *P* = .003) compared to patients with well-differentiated tumors.

Conclusion. Tumor grade of differentiation had a statistically significant effect on the long-term prognosis of HCC after LT.

HEPATOCELLULAR carcinoma (HCC) is one of the most common malignancies worldwide [1]. Potentially curative therapy for HCC includes liver resection, liver transplantation (LT), and local ablation [2]. Since LT may potentially cure both the tumor and the underlying liver disease, it is recommended as the first-line treatment for HCC meeting the Milan criteria but unsuitable for resection [3,4]. Tumor grade of differentiation is an indicator of how closely the tumor cells resemble the morphologic and functional characteristics of parent tissues from organ of origin [5]. Well-differentiated tumor cells are closely similar to the tissues from the organ of origin. Poor-differentiated and undifferentiated tumor cells are disorganized and they have little (poorly differentiated) or no (undifferentiated) resemblance to the tissues from the organ of origin.

Several studies have demonstrated an association of tumor grade and patient survival after LT for HCC [6–15]. However, most publications lack sufficient power for statistical analyses and the results were inconclusive. No previous studies have explored the independent role of tumor grade in the long-term prognosis of patients with HCC after LT in a population with large sample size. In the present study, we aimed to investigate the impact of tumor grade on the long-term survival of patients with HCC (receive LT) diagnosis in the US general population using the most

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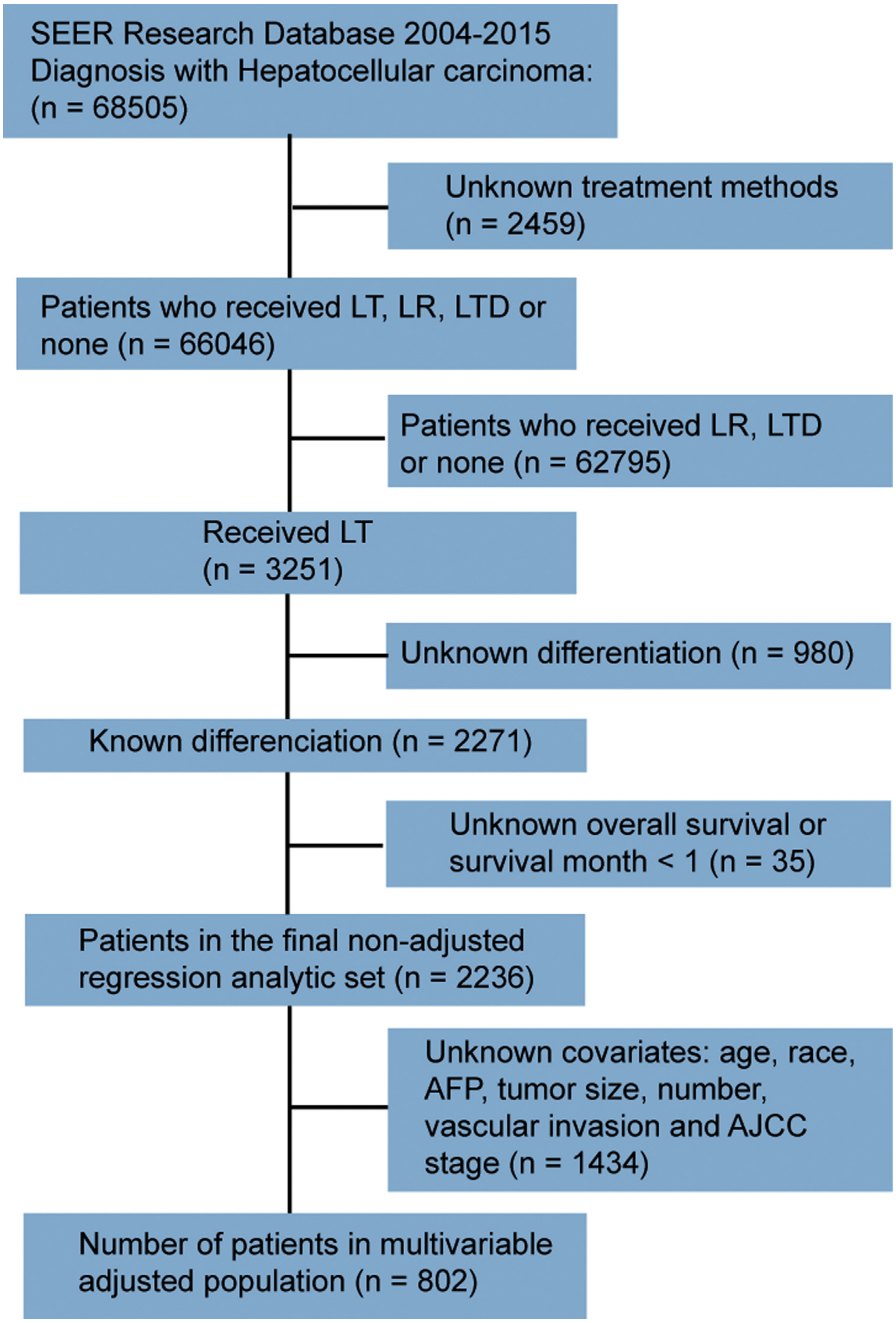

Fig 1. Flowchart representing selection process of patients included in this study.

current Surveillance, Epidemiology, and End Results Program (SEER) 18 registry.

PATIENTS AND METHODS

Patient Selection

Data from SEER 18 registry database for 2004–2015 was extracted for the present study. The SEER program collects data regarding cancer incidence, patient demographics, tumor parameters, patient treatment, and survival from 18 population-based cancer registries covering approximately 28% of the US population (seer.cancer.gov/about/overview.html). We first identified 68,505 patients whose pathological diagnosis as HCC (International Classification of Diseases for Oncology, 3rd Edition [ICD-O-3], site code C22.0 and histologic type ICD-O-3 codes 8170–8175) between 2004–2015 from the SEER database. In the SEER database, tumor grade is coded as grade 1 (well differentiated), grade 2 (moderately differentiated), grade 3 (poorly differentiated), or grade 4 (undifferentiated). American Joint Commission on Cancer (AJCC) tumor node metastasis (TNM) stage is based on the 6th edition. The detailed inclusion and exclusion criteria was shown in Fig 1. A total of 802 patients with HCC undergoing LT matching the specified eligibility criteria were included in the present study. The SEER code for LT were 61.

STATISTICAL ANALYSIS

The primary endpoint was overall survival (OS), defined as the interval from the date of diagnosis to the date of death of any cause. Disease-specific survival (DSS), defined as time until death attributed to HCC, was evaluated as a secondary outcome. Continuous variables were presented as mean ± SD and tested by *t* test or Kruskal-Wallis H test. Categorical variables were expressed as number (%) and tested by χ^2 test or Fisher's exact test.

The survival curves were determined by the Kaplan-Meier method and compared by the log-rank test. Multivariable Cox proportional hazards regression models were employed to calculate hazard ratios (HR) and 95% confidence intervals (CI) for comparisons between groups. The interaction test was performed to detect the influence of each stratified factor on the relationship between surgical methods and patient survival. *P* value for interaction less than .05 means interaction exists between that factor and the relationship (all potential confounding factors were adjusted during interaction tests).

Propensity score matching (PSM) was performed with the following variables: age, race, sex, year of diagnosis, alpha-fetoprotein level, fibrosis score (Ishak), tumor size, tumor number, vascular invasion and AJCC-TNM stage. Patients were matched with the closest estimated propensity score within .02 of the standard deviation of the logit-transformed propensity score. After PSM selection, a new cohort including 2 comparable groups were obtained and compared by univariable Cox regression.

P value less than .05 was deemed statistically significant. All statistical analyses were performed by R (http://www.R-project.org) and EmpowerStats software (www.empowerstats.com, X&Y solutions, Inc. Boston, Mass, United States).

RESULTS

Demographics

A total of 802 patients diagnosed with HCC receiving LT between 2004–2015 included in the SEER registry were analyzed in the study. Demographic features of patients were summarized in Table 1. Patients were divided into well-differentiated (grade 1) group, moderate-differentiated

Table 1. Clinical Features of the Included Patients With Hepatocellular Carcinoma

	Well-differentiated (n = 264)	Moderate-differentiated (n = 446)	Poor-differentiated (n = 92)	*P* Value
Sex (female/male)	63/201	90/356	22/70	.452
Age (years)	56.2 ± 10.2	57.6 ± 7.3	56.7 ± 8.1	.088
Marriage status (married/divorced or separated/single)	181/33/43	296/70/64	62/17/11	.553
Insurance (yes/no)	201/2	360/0	64/0	.181
Race (white/black/other/unknown)	217/18/28/1	349/43/54/0	57/6/28/1	<.001
Year of diagnosis (2004–2009/2010–2015)	126/138	180/266	49/43	.029
AFP (ng/mL negative/positive)	122/142	170/276	24/68	.002
Tumor size (cm)	2.6 ± 1.5	2.7 ± 1.4	3.0 ± 1.5	.027
<3	170 (64.4%)	286 (64.1%)	52 (56.5%)	.531
≥3, <5	77 (29.2%)	127 (28.5%)	29 (31.5%)	
≥5, <7	12 (4.5%)	28 (6.3%)	9 (9.8%)	
≥7	5 (1.9%)	5 (1.1%)	2 (2.2%)	
Tumor number (single/multiple)	144/120	213/233	46/46	.217
One lesion in one lobe (yes/no)	131/133	179/267	31/61	.009
Vascular invasion (no/yes)	197/67	304/142	54/38	.013
FS (0–4/5–6)	35/229	41/405	14/78	.110
AJCC-TNM stage (I/II/III/IV)	140/114/10/0	194/228/21/3	36/50/5/1	.129

Data are shown as mean ± SD or n (%).
AJCC-TNM stage is based on the 6th edition.
Abbreviations: AFP, alpha-fetoprotein; AJCC-TNM, American Joint Commission on Cancer tumor node metastasis; FS, fibrosis score.

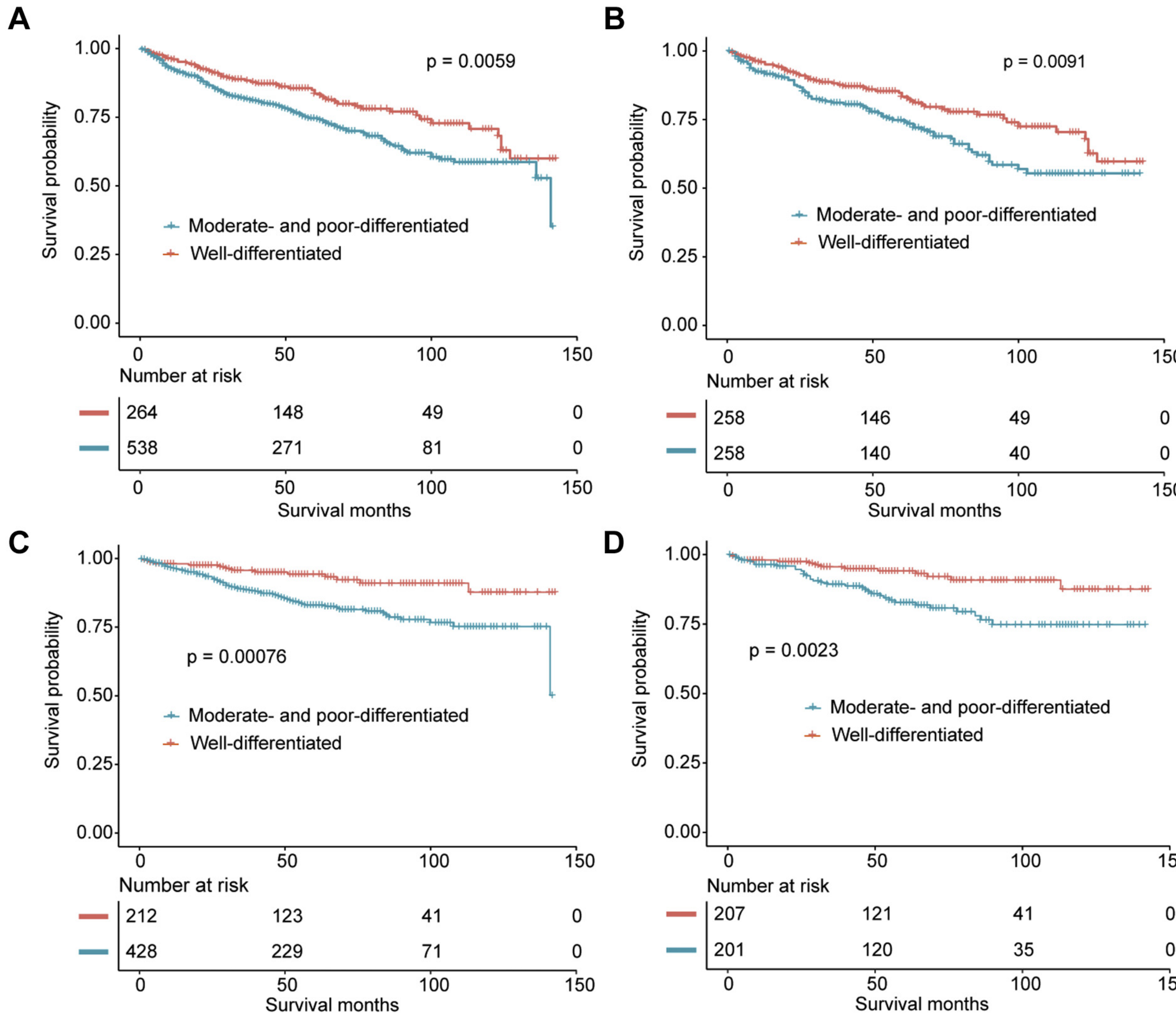

Fig 2. (**A**) Overall survival analysis for patients with well-differentiated and moderate/poor-differentiated tumors in non-adjusted population. (**B**) Overall survival analysis for patients with well-differentiated and moderate/poor-differentiated tumors in propensity score matched population. (**C**) Disease-specific survival analysis for patients with well-differentiated and moderate/poor-differentiated tumors in non-adjusted population. (**D**) Disease-specific survival analysis for patients with well-differentiated and moderate/poor-differentiated tumors in propensity score matched population.

Table 2. Association of Tumor Differentiation With Patient Survival

	OS			DSS		
	Number	HR (95% CI)*	*P* Value	Number	HR (95% CI)*	*P* Value
Non-adjusted	2236	1.33 (1.12, 1.57)	.001	1780	2.02 (1.51, 2.69)	<.001
Multivariable adjusted model†	802	1.57 (1.13, 2.19)	.008	640	2.62 (1.44, 4.77)	.002
Matched on propensity score‡	516	1.60 (1.12, 2.28)	.010	408	2.54 (1.36, 4.74)	.003
Regression adjusted with propensity score						
Propensity score, continuous	802	1.49 (1.08, 2.06)	.016	640	2.30 (1.28, 4.11)	.005
Propensity score, quintile	802	1.52 (1.10, 2.11)	.012	640	2.28 (1.26, 4.10)	.006

Data are shown as HR (95% CI) *P* value.

Abbreviations: AFP, alpha-fetoprotein; AJCC-TNM, American Joint Commission on Cancer tumor node metastasis; DSS, disease-specific survival; FS, fibrosis score; OS, overall survival; PSM, propensity score matching.

*Moderate- and poor-differentiated versus well-differentiated (HR: 1).

†Adjusted model was adjusted for age, race, sex, year of diagnosis, AFP level, FS, vascular invasion, tumor size, tumor number, and AJCC-TNM stage.

‡PSM model was based on the following variables: age, race, sex, year of diagnosis, AFP level, FS, vascular invasion, tumor size, tumor number, and AJCC-TNM stage.

(grade 2) group and poor-differentiated group (grade 3 and 4). There were differences in race, year of diagnosis, alpha-fetoprotein level, tumor size, tumor distribution, and vascular invasion among 3 groups, whereas age, sex, tumor number, fibrosis score, and AJCC-TNM stage showed no significant differences among three groups.

Multivariable Cox Regression

A total of 802 patients with known prognostic data were included in survival analyses. In the total cohort, the mean OS times for patients in the well-differentiated group and moderate/poor-differentiated group were 114.5 months and 102.8 months, respectively. The mean DSS times for patients in the well-differentiated group and moderate/poor-differentiated group were 132.3 and 118.6 months, respectively. Patients with well-differentiated tumors had longer OS ($P < .001$) and DSS ($P < .001$) compared to patients with moderate/poor-differentiated tumors (Fig 2A and 2C).

As shown in Table 2, in the multivariable adjusted cohort (OS: n = 802; DSS: n = 640), after adjusting potential confounders, a worse prognosis was observed in OS (HR, 1.57; 95% CI 1.13–2.19; $P = .008$) and DSS (HR, 2.62; 95% CI 1.44–4.77; $P = .002$) for patients with moderate/poor-differentiated tumors compared to patients with well-differentiated tumors.

STRATIFIED ANALYSES

According to multivariable Cox analyses, Figs 3 (OS) and 4 (DSS) showed the associations of tumor grade and long-term survival stratified by clinicopathologic variables. In stratified analyses, the salutary effects of well-differentiation on OS and DSS were consistent across all examined subgroups (all the interaction P values $> .05$).

Outcomes in Propensity Score Matched Population

As shown in Supplementary Table 1, in the matched cohort, potential prognostic variables became well-balanced for most baseline characteristics. In the PSM cohort, patients with moderate/poor-differentiated tumors showed worse OS and DSS (both P values $< .001$) compared to patients with

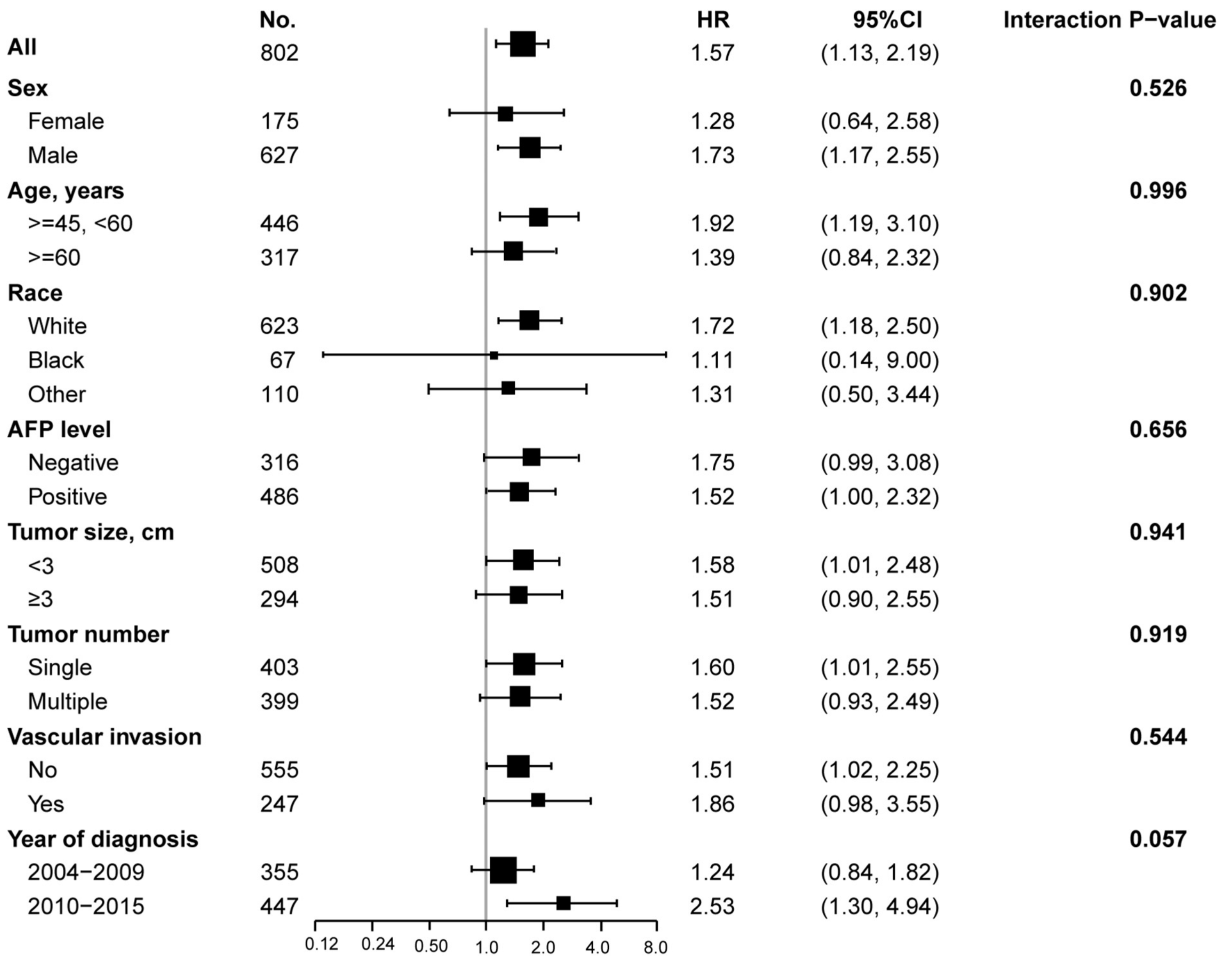

Fig 3. Stratified analysis based on clinicopathologic features (overall survival). Abbreviation: AFP, alpha-fetoprotein. In subgroup analyses, all identified confounding factors were adjusted except for the factor that the subgroup was based on.

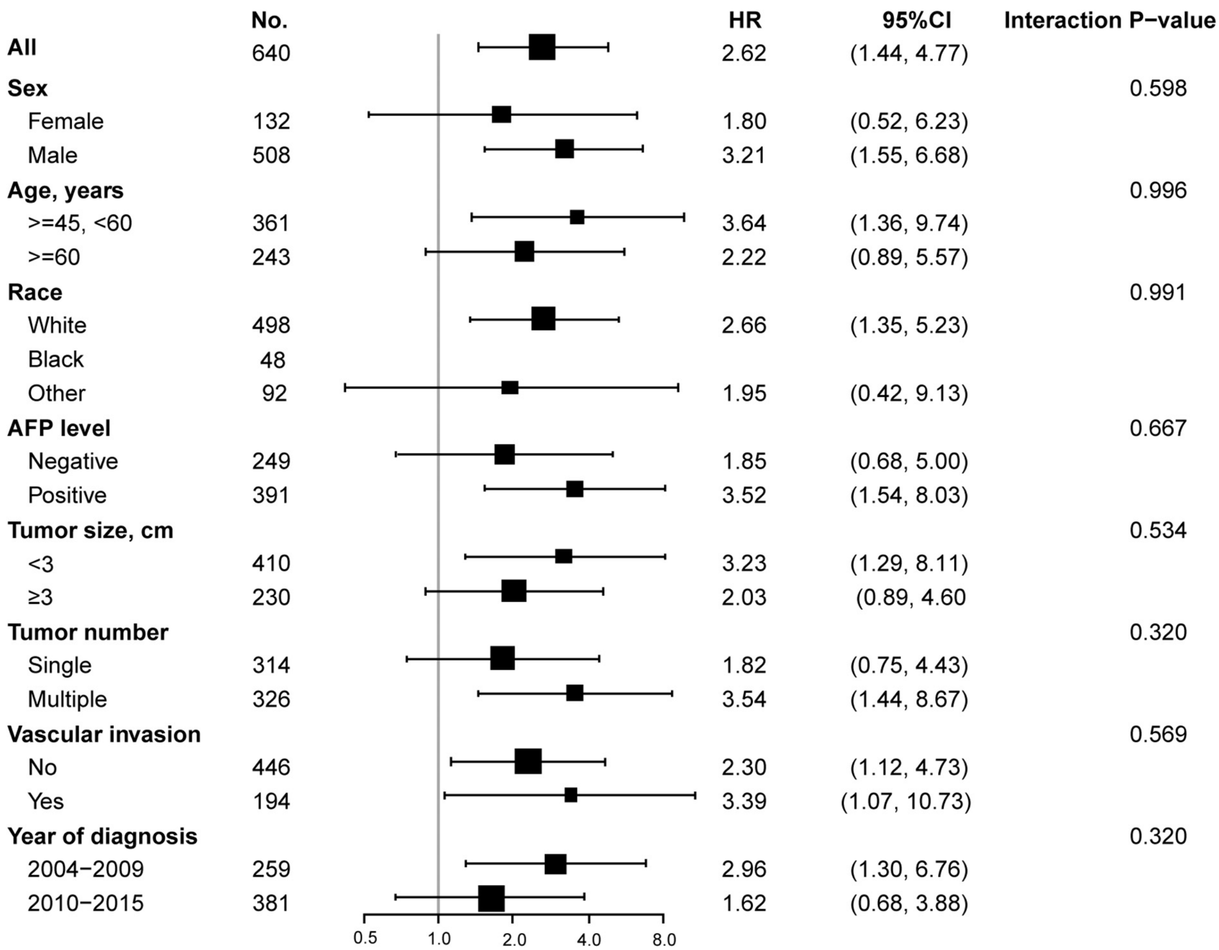

Fig 4. Stratified analysis based on clinicopathologic features (disease-specific survival). Abbreviation: AFP, alpha-fetoprotein. In subgroup analyses, all identified confounding factors were adjusted except for the factor that the subgroup was based on.

well-differentiated tumors (Fig 2B and 2D). In the propensity-matched cohort, the univariable analysis showed that patients with moderate/poor-differentiated tumors still had worse OS (HR 1.60, 95% CI 1.12–2.28, P = .010) and DSS (HR 2.54, 95% CI 1.36–4.74, P = .003) compared to patients with well-differentiated tumors (Table 2).

The HRs (moderate- and poor-differentiated versus well-differentiated) adjusted by propensity score demonstrated both shorter OS (continuous: HR 1.60, 95% CI 1.49–2.06, P = .016; quintile: HR 1.52, 95% CI 1.10–2.11, P = .012) and DSS (continuous: HR 2.30, 95% CI 1.28–4.11, P = .005; quintile: HR 2.28, 95% CI 1.26–4.10, P = .006) associated with moderate- and poor-differentiation (Table 2).

DISCUSSION

In the present study, we aimed to explore the independent role of tumor grade in long-term prognosis for patients with HCC undergoing LT. Both multivariable regression analysis and the propensity score methods showed that patients with well-differentiated tumors had better OS and DSS compared to patients with moderate/poor-differentiated tumors. Additionally, the stratified analyses showed that the conclusion was consistent across all subgroups.

In several published studies, tumor grade was a significant risk factor for long-term survival (both disease-free survival and overall survival) in patients with HCC undergoing LT [10–12,14]. However, up to date, the published data are insufficient to illustrate the prognostic difference of patients with different tumor grade. In previous studies, multivariable analyses were usually utilized to adjust confounding factors. However, there was inherent defect of regression adjustment because application of regression analysis was considerably limited by sample size. In this study, to clarify the oncological benefit of well tumor differentiation for HCC after LT, tumor-specific survival was utilized as a predominant outcome. Besides, PSM analysis allowed balancing of a large number of covariates (eg, tumor size and vascular invasion) without the common statistical concerns for regression analysis. Additionally, we conducted stratified analysis and interaction test to get rid of the factors that affected the relationship between tumor grade and long-term prognosis.

Several publications have demonstrated that mitotic index and histologic grade were significant predictors of microvascular invasion (MVI) [5,16], while MVI was a well-recognized risk factor for patient survival [17–19]. However, neither MVI nor tumor grade had been included in the main criteria for LT including the Milan criteria and University of California San Francisco criteria [2,6,20]. These criteria only recommended excluding patients with major vascular invasion for LT, while they did not consider sufficiently about the biological behavior of tumors. Based on our retrospective experience, tumor grade of differentiation had a statistically significant effect on the prognosis of HCC after LT. However, before altering the current LT selection criteria for patients with HCC, prospective studies are still needed to confirm the impact of tumor grade on clinical outcomes.

Admittedly, this study has several limitations inherent to its design. First, the SEER database does not provide data of liver function, comorbidities, performance status for HCC patients, thus we cannot evaluate the influence of these factors in multivariable analyses. However, the SEER database did provide Ishak score in our analysis, which is an efficient parameters indicating liver cirrhosis. Second, we cannot acquire the data related to pre- or post-operative treatment (such as transcatheter arterial chemoembolization and chemotherapy), thus the influence of adjuvant treatment was not adjusted in the multivariable models. Third, the findings of the current study should be interpreted with caution because a number of patients were excluded from our main analyses due to having an unknown covariates in the SEER database. Despite these limitations of the SEER database, it includes nearly 30% of the population from various geographic regions in the US and is therefore a good tool to evaluate the research questions in this study. By combining multivariate COX regression and propensity score modeling, our outcomes indicated that patients with well differentiated tumors had better OS and DSS compared to those with moderate/poor-differentiated tumors.

SUPPLEMENTAL DATA

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.transproceed.2018.12.033

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