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Original Article

Histology of Hepatocellular Carcinoma: Association with Clinical Features, Radiological Findings, and Locoregional Therapy Outcomes

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ABSTRACT

Objective: The objective of the study was to investigate whether hepatocellular carcinoma (HCC) histology is associated with clinical and computed tomographic/magnetic resonance imaging features and locoregional therapy (LRT) outcomes.

Subjects and Methods: This single-center retrospective study included 124 consecutive patients (92 men, median age 59 years) with 132 HCC diagnosed by biopsy between 2008 and 2017 before LRT. Patients underwent chemoembolization (n = 51, 41%), ablation (n = 41, 33%), yttrium-90 radioembolization (n = 17, 13%), and chemoembolization/ablation (n = 15, 12%). Barcelona clinic liver cancer (BCLC) stage was 0/A (n = 48, 38%), B (n = 33, 26%), C (n = 27, 22%), and D (n = 16, 13%). Edmondson-Steiner (ES) grade and cytology were correlated with baseline features and radiologic response using logistic regression. Time to progression (TTP) and transplant-free survival (TFS) were analyzed using Cox proportional hazard models.

Results: High ES grade was associated with α-fetoprotein (AFP) >50 ng/ml (odds ratio [OR] 4.6, 95% confidence interval [CI]: 1.5–13.9; $P < 0.01$), tumor diameter >5 cm (OR 3.1, 95% CI: 1.1–9.0; $P < 0.05$), infiltrative appearance (OR 5.0, 95% CI: 1.5–16.2; $P < 0.01$), and BCLC Stage C (OR 4.5, 95% CI: 1.3–16.4; $P = 0.02$). Clear-cell subtype was associated with non-viral cirrhosis (OR 5.3, 95% CI: 1.6–17.2; $P < 0.01$) and atypical enhancement (OR 3.1, 95% CI: 1.0–9.3; $P < 0.05$). AFP, BCLC Stage B, and diameter were associated with reduced TTP and TFS ($P < 0.05$). Neither ES grade nor clear-cell subtype was associated with objective response (OR 2.3, 95% CI: 0.7–7.4; $P = 0.15$ and OR 1.1, 95% CI: 0.4–3.4; $P = 0.87$, respectively), TTP ($P > 0.20$), or TFS ($P > 0.90$) on univariate or stratified analysis.

Conclusion: Histologic grade is associated with aggressive tumor features, while clear-cell HCC is associated with non-viral cirrhosis and atypical enhancement. Unlike AFP, BCLC stage, and tumor size, histologic features were not associated with LRT outcomes, supporting biopsy deferral for imaging diagnosed HCC.

Keywords: Hepatocellular carcinoma, Locoregional therapy, Edmondson-Steiner grade

INTRODUCTION

Histological grades and cytological subtypes of hepatocellular carcinoma (HCC) have been established.[1] Previous studies suggest that high tumor grade is associated with tumor recurrence and decreased survival after orthotopic liver transplantation (OLT) among patients within Milan criteria and higher local tumor progression rates after radiofrequency ablation (RFA) among

Barcelona clinic liver cancer (BCLC) Stage A patients.[2-4] High tumor grade on explant is also associated with lack of odds ratio (OR) to transcatheter arterial chemoembolization (TACE).[5] In regard to cytological subtype, the clear-cell variant has been associated with improved survival after surgical resection[6-8] though data conflicts.[9,10] However, aforementioned studies predominantly include patients with early-stage disease and are not fully reflective of HCC populations treated with locoregional therapy (LRT). Further, tumor grade on explant after LRT may differ from before therapy. There is also a paucity of literature on the prognostic value of cytological subtype in patients treated with LRT. This study was undertaken to assess the relationship between baseline histological features of HCC and clinical stage, computed tomographic (CT) and magnetic resonance imaging (MRI) features, and outcomes in patients treated with LRT.

SUBJECTS AND METHODS

Study cohort

This single-center retrospective cohort study was approved by our Institutional Review Board and in compliance with the Health Insurance Portability and Accountability Act, with a waiver of informed consent for retrospective review of medical records. Consecutive patients (n = 124, mean age 61 years; age range 30–85 years) at the University of Illinois Hospital with 132 percutaneous biopsy-proven HCC who subsequently underwent LRT were identified through retrospective chart review from 2008 to 2017. The cohort included 92 men with mean age of 59 years (range 30–85 years) and 32 women with mean age of 65 years (range 47–83 years). LRT modalities were transarterial chemoembolization (TACE, n = 51, 41%), percutaneous thermal ablation (n = 41, 33%), yttrium-90 (Y90) radioembolization (n = 17, 13%), and combination TACE/ablation (n = 15, 12%). Baseline BCLC stage was 0/A (n = 48, 38%), B (n = 33, 26%), C (n = 27, 22%), and D (n = 16, 13%). Mean tumor diameter was 4.8 cm ± 4.1 cm. The most common etiology of HCC was hepatitis C virus (38.7%). Ethnicity, gender, age, baseline laboratory values, Child-Pugh (CP) class, cirrhosis etiology, and type of LRT were tabulated [Table 1]. The mean time from biopsy to LRT was 34 days (standard deviation, 60 days).

Table 1: Baseline demographic characteristics.

	n **(%)**
Mean age (range)	
60 (30–85)	
Gender	
Male	92 (74.2)
Female	32 (25.8)
Ethnicity	
African-American	34 (27.4)
Caucasian	54 (43.6)
Hispanic	9 (7.3)
Asian	2 (1.6)
Other	25 (20.2)
Diabetes mellitus	
Yes	51 (41.1)
No	73 (58.9)
Cirrhosis etiology	
HCV	48 (38.7)
HBV	6 (4.8)
ASH	12 (9.7)
NASH	12 (9.7)
Mixed (viral, ASH, and NASH)	38 (30.7)
Other	8 (6.5)
Mean tumor diameter (cm)	
4.9±4.1	
Tumor diameter (cm)	
≤5	87 (71.9)
>5	34 (28.1)
Total number of tumors	
1	59 (47.6)
2	27 (21.8)
3	10 (8.1)
4	5 (4.0)
5	1 (0.8)
6	3 (2.4)
>6	19 (15.3)
Within Milan criteria	
Yes	65 (52.4)
No	59 (47.6)
AFP, median (IQR) (ng/ml)	
13.1 (6.0–44.3)	
AFP >50 ng/ml	
No	91 (76.5)
Yes	28 (23.5)
Child-Pugh class	
A	53 (42.7)
B	56 (45.2)
C	15 (12.1)
BCLC stage	
Stages 0, A	48 (38.7)
Stage B	33 (26.6)
Stage C	27 (21.8)
Stage D	16 (12.9)
Locoregional therapy	
Y90	17 (13.7)

(*Contd...*)

Locoregional therapies

LRT was performed by board-certified interventional radiologists with 2–20 years attending experience. Treatment modality was determined based on the BCLC staging algorithm and discussion in multidisciplinary tumor board. Y90 radioembolization was reserved for patients with multifocal and/or infiltrative disease, macrovascular invasion, and total bilirubin of <2.0 mg/dL. RFA under

Table 1: (*Continued*)

	n (%)
Ablation	41 (33.1)
RFA	38 (30.6)
MWA	3 (2.4)
TACE	51 (41.1)
TACE/ablation	15 (12.1)
TACE/RFA	14 (11.3)
TACE/PEI	1 (0.8)

HBV: Hepatitis B virus, HCV: Hepatitis C virus, ASH: Alcoholic steatohepatitis, NASH: Non-alcoholic steatohepatitis, AFP: Alpha-fetoprotein, IQR: Interquartile range, MWA: Microwave ablation, RFA: Radiofrequency ablation, PEI: Percutaneous ethanol injection, TACE: Transarterial chemoembolization, Y90: Yttrium-90, BCLC: Barcelona clinic of liver cancer, ethnicity other: Alaska Native, Native Hawaiian, unavailable, cirrhosis etiology other: Primary (*n*=1), cryptogenic (*n*=5), sarcoidosis (*n*=1), hemochromatosis (*n*=1)

ultrasound (US) and CT guidance was performed using 14-gauge multi-tined electrodes (Starburst®, Angiodynamics, Inc. Latham, NY) per manufacturer protocol. Conventional TACE was performed as previously described.[11] Three thermal ablations were performed with microwave antennas (PR 15, NeuWave Medical, Madison, WI) per manufacturer protocol. For Y90 radioembolization, all patients underwent planning mesenteric angiography with Tc-99 macroaggregated albumin administration and predetermined dose of Y90 was administered at lobar (n = 16) or segmental level (n = 1) using SIR-Spheres® (n = 13) (SIRTex, Sydney, NSW) or Theraspheres® (BTG, London, England) (n = 4). For patients treated with TACE/ablation, ablation was performed within 24 h of TACE.

Radiological assessment

Baseline contrast-enhanced multiphase CT (n = 74) or MR (n = 38) obtained within 1 month before LRT was assessed for arterial-phase hyperenhancement, venous or delayed phase washout, delayed capsular enhancement, macrovascular invasion, and tumor diameter as per Liver Imaging Reporting and Data System definitions.[12] Follow-up CT or MR images were prescribed 1 month after LRT and every 3 months thereafter to assess for residual tumor or recurrence. Tumor response and progression were determined according to modified Response Evaluation Criteria in Solid Tumors (mRECIST) by board-certified radiologists with 4–9 years attending experience who were blinded to histological data.[13] Complete response (CR) and partial response (PR) were considered an objective response. For patients with more than one CT or MRI before LRT (n = 50), tumor volume doubling time was determined based on the Schwartz equation $([T\times\log_2]/3 \times [\log(M_1)-\log(M_0)]$; where, T is the interval in days between two MR or CT studies, M0 is the 1st time maximum diameter, M1 is the 2nd time maximum diameter).[14] Median value of tumor volume doubling time was utilized to dichotomize the variable.[15]

Histological analysis

Percutaneous biopsy specimens were obtained under US guidance with an 18-gauge core needle (BioPince™, Argon Medical Devices, Frisco, TX) and immediately fixed in formalin. Histological analysis was performed by a board-certified pathologist (G.G). Eight patients (6%) had two biopsied HCC; one tumor was randomly selected per patient for inclusion in statistical analysis to maintain the independence of analyzed observations. Histological grade was classified by the Edmondson-Steiner (ES) system:[1] Grade 1 (well differentiated), Grade 2 (moderately differentiated), Grade 3 (poorly differentiated), and Grade 4 (pleomorphism). ES grade of 3 or 4 was defined as high grade. Cytology was classified as usual, clear, sclerosing, sarcomatoid, pleomorphic, fibrolamellar, steatohepatitis, or inflammatory type; specimens containing two or more subtypes were classified as mixed type.[1] Cytology was further categorized into three groups: 100% clear cell, focal clear cell (combination of clear cell and other variants), and absence of clear-cell components.[8] Architectural subtypes were classified as trabecular, pseudoglandular, and solid and specimens containing two or more subtypes were classified as mixed.[1]

Clinical outcomes

Primary outcome measures were time to progression (TTP) after LRT and transplant-free survival (TFS). TTP was defined as the time from LRT to the detection of progression on imaging by mRECIST. TFS was defined as time from LRT to death or last clinical encounter; patients were censored at time of OLT.

Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS) (2013, version 9.4; SAS Institute, Cary, NC). The association between two measurements was assessed on univariate analysis and by binary or multinomial logistic regressions when one of the measures was dichotomized or separated into >2 categories, respectively. To examine the impact of measurements on TTP and TFS, both univariate and stratified Cox proportional hazard models were fitted accordingly and Kaplan–Meier curves were created. Two-sided $P < 0.05$ was considered statistically significant. A two-sided $P = 0.05–0.10$ was considered a statistical trend.

RESULTS

Baseline biopsy and imaging characteristics

Tumors were ES Grade 1 (n = 13, 10.6%), 2 (n = 94, 76.4%), or 3 (n = 16, 13%) [Table 2]. Twenty-four specimens

(20.9%) were comprised entirely of clear cells, 23 (20%) with focal clear cell, and 68 (59.1%) contained no clear cells. Architectural subtypes were trabecular (n = 44, 37.6%), pseudoglandular (n = 31, 26.5%), and solid or mixed solid pattern (n = 20, 17.1%). Baseline CT and MR imaging features are summarized in Table 2. Median value of tumor volume doubling time was 155 days (interquartile range [IQR] 100–221 days).

Baseline features associated with high ES grade and clear-cell cytology by univariate analysis

High ES grade was associated with baseline α-fetoprotein (AFP) level >50 ng/ml (OR 4.6, 95% confidence interval [CI] 1.5–13.9; P = 0.007), BCLC Stage C (OR 4.5, 95% CI 1.3–16.4; P = 0.02), tumor diameter >5 cm (OR 3.1, 95% CI 1.1–9.0; P = 0.035), and infiltrative appearance (OR 5.0, 95% CI 1.5–16.2; P = 0.0007) [Table 3]. There was a trend toward shorter tumor volume doubling time (<155 days) with high ES grade (P = 0.09). About 100% clear-cell cytological variant was associated with non-viral cirrhosis (OR 5.3, 95% CI 1.6–17.2; P = 0.005) and both 100% clear cell and focal clear cell were associated solid architectural subtype (OR 6.3, 95% CI 1.8–21.9; P = 0.004 and OR 5.5, 95% CI 1.5–19.7; P = 0.009, respectively). Atypical contrast enhancement (lacking arterial-phase hyperenhancement or washout) was associated with 100% clear cell (OR 3.1, 95% CI 1.0–9.3; P = 0.046) but not with focal clear cell (OR 1.6, 95% CI 0.5–5.0; P = 0.44) in comparison to complete absence of clear cells. A trend was observed of clear-cell HCC being associated with lack of arterial-phase hyperenhancement (OR 3.2, 95% CI 0.9–11.1; P = 0.07) and comorbid diabetes mellitus (OR 2.6, 95% CI 1.0–6.7, P = 0.05). Clear-cell HCC was not associated with high tumor volume doubling time (OR 0.5, 95% CI 0.1–2.5, P = 0.39).

Treatment response

LRT achieved an objective response in 80 (75.4%) of the 106 tumors, in which follow-up imaging was available. High ES grade was not associated with objective response after LRT (OR 2.5, 95% CI 0.7–8.4; P = 0.15) by univariate analysis. The presence of 100% clear cell (OR 1.1, 95% CI 0.36–3.36; P = 0.87) or focal clear cell (OR 1.4, 95% CI 0.5–4.0; P = 0.57) on biopsy was not associated with objective response to LRT.

TTP and TFS by univariate and stratified analysis

Median TTP was 178 days (IQR 81–311 days), with overall disease progression rate of 59.8% with median follow-up of 183 days (range: 12–1799 days).

On univariate analysis [Table 4], tumor burden exceeding Milan criteria (hazard ratio [HR] 2.6, 95% CI 1.6–4.4; P < 0.001), baseline serum AFP level >50 ng/ml (HR 2.5, 95% CI 1.4–4.4; P = 0.002), BCLC Stage B (HR 3.0, 95% CI 1.6–5.7; P < 0.001), infiltrative appearance (HR 2.4, 95% CI 1.2–4.8; P = 0.01), and macrovascular invasion (HR 2.9, 95% CI 1.4–6.2; P = 0.006) were associated with disease progression. A trend was observed of a higher hazard of progression in tumors with microvascular invasion (HR 2.5, 95% CI 0.9–6.9; P = 0.08). High ES grade (HR 1.5, 95% CI 0.7–3.1; P = 0.33) [Figure 1a]

Table 2: Baseline biopsy and imaging characteristics.

	n **(%)**
ES grade	
1	13 (10.6)
2	94 (76.4)
3	16 (13.0)
4	0 (0)
Cytologic variant	
Usual	64 (55.7)
Clear	24 (20.9)
Sclerosing	1 (0.9)
Inflammatory	1 (0.9)
Fibrolamellar	0 (0)
Pleomorphic	0 (0)
Steatohepatitis	0 (0)
Sarcomatoid	0 (0)
Mixed	25 (21.7)
Architecture subtypes	
Trabecular	44 (37.6)
Pseudoglandular	31 (26.5)
Mixed trabecular	22 (18.8)
Solid and mixed solid	20 (17.1)
Clear-cell amount	
100%	24 (20.9)
Focal	23 (20.0)
None	68 (59.1)
Microvascular invasion	
Yes	5 (9.1)
No	50 (90.9)
Arterial phase hyperenhancement	
Yes	81 (82.6)
No	17 (17.4)
Infiltrative appearance	
Yes	17 (14.2)
No	103 (85.8)
Washout	
Yes	95 (85.6)
No	16 (14.4)
Delayed capsular enhancement	
Yes	44 (39.6)
No	67 (60.4)
Macrovascular invasion	
Yes	13 (10.7)
No	109 (89.3)

Cytology mixed=Combination of any variant type (usual, clear, sclerosing, etc.) Clear cell 100%=Only clear cell, clear cell focal=Combination of clear cell+other cytological subtype, none=Absence of clear cell. ES: Edmondson-Steiner

Table 3: Histological associations with baseline characteristics by univariate analysis.

	ES grade high versus low		100% clear cell versus none		Focal clear cell versus none	
	OR	**Significance**	**OR**	**Significance**	**OR**	**Significance**
Age	1.0	*P*=0.25	1.0	*P*=0.80	1.03	*P*=0.27
Gender						
Male	1.0		1.0		1.0	
Female	0.4	*P*=0.26	1.1	*P*=0.79	1.2	*P*=0.71
Ethnicity						
American	1.0		1.0		1.0	
Caucasian	1.5	*P*=0.59	1.2	*P*=0.69	1.6	*P*=0.44
Other	1.9	*P*=0.37	0.8	*P*=0.78	1.6	*P*=0.46
DM						
No	1.0		1.0		1.0	
Yes	1.5	*P*=0.41	2.6	*P*=0.05	1.4	*P*=0.48
Cirrhosis etiology						
HCV	1.0		1.0		1.0	
HBV	1.5	*P*=0.70	0.0	*P*=1.0	0.6	*P*=0.59
ASH	1.3	*P*=0.74	5.0	*P*=0.05	0.9	*P*=0.92
NASH	0.8	*P*=0.81	7.5	*P*<0.05	0.6	*P*=0.59
Mixed	0.7	*P*=0.61	3.0	*P*=0.10	0.8	*P*=0.73
Other	1.1	*P*=0.92	11.3	*P*<0.05	2.7	*P*=0.34
Viral cirrhosis						
Yes	1.0		1.0		1.0	
No	0.8	*P*=0.59	5.3	*P*<0.01	1.0	*P*=0.95
AFP >50 ng/ml						
No	1.0		1.0		1.0	
Yes	4.6	*P*<0.01	0.5	*P*=0.30	1.5	*P*=0.49
Child-Pugh class						
A	1.0		1.0		1.0	
B	0.4	*P*=0.09	0.7	*P*=0.43	0.4	*P*=0.09
C	0.1	*P*=0.16	0.8	*P*=0.71	0.2	*P*=0.14
BCLC stage						
0, A	1.0		1.0		1.0	
B	1.5	*P*=0.57	1.5	*P*=0.52	1.1	*P*=0.83
C	4.5	*P*<0.02	1.4	*P*=0.63	0.3	*P*=0.15
D	0.3	*P*=0.44	1.0	*P*=0.98	0.2	*P*=0.18
Tumor diameter						
≤5 cm	1.0		1.0		1.0	
>5 cm	3.1	*P*<0.05	1.1	*P*=0.85	1.5	*P*=0.49
Within Milan criteria						
Yes	1.0		1.0		1.0	
No	2.0	*P*=0.21	0.8	*P*=0.56	0.7	*P*=0.44
Arterial-phase hyperenhancement						
Yes	1.0		1.0		1.0	
No	0.7	*P*=0.69	3.2	*P*=0.07	1.8	*P*=0.38
Washout						
No	1.0		1.0		1.0	
Yes	1.7	*P*=0.56	0.8	*P*=0.75	1.1	*P*=0.86
Delayed capsular enhancement						
No	1.0		1.0			
Yes	2.2	*P*=0.17	1.0	*P*=0.95	1.8	*P*=0.24
Enhancement pattern						
Typical	1.0		1.0		1.0	
Atypical	0.4	*P*=0.33	3.1	*P*<0.05	1.6	*P=0.44*

(*Contd...*)

Table 3: (*Continued*)

	ES grade high versus low		100% clear cell versus none		Focal clear cell versus none	
	OR	Significance	OR	Significance	OR	Significance
Macrovascular invasion						
No	1.0		1.0		1.0	
Yes	2.4	*P*=0.22	0.7	*P*=0.63	0.7	*P*=0.66
Infiltrative appearance						
No	1.0		1.0		1.0	
Yes	5.0	*P*<0.01	1.2	*P*=0.84	1.2	*P*=0.79
TVDT >155 days						
No	1.0		1.0		1.0	
Yes	0.2	*P*=0.09	0.5	*P*=0.39	2.0	*P*=0.35

Age: Age at LRT, DM: Diabetes mellitus, ethnicity other: Hispanic, Asian, Alaska Native, Native Hawaiian, unavailable, HBV: Hepatitis B virus, HCV: Hepatitis C virus, ASH: Alcoholic steatohepatitis, NASH: Non-alcoholic steatohepatitis, BCLC: Barcelona clinic of liver cancer, Typical enhancement pattern: Presence of arterial-phase hyperenhancement and washout, TVDT: Tumor volume doubling time, LRT: Locoregional therapy, ES: Edmondson-Steiner, OR: Objective response

and 100% clear-cell variant (HR 1.4, 95% CI 0.8–2.6; $P = 0.28$) [Figure 2a] were not associated with disease progression by univariate analysis. High ES grade or 100% clear cell also were not associated with progression when stratified by LRT modality or BCLC stage [Supplemental Table 1].

Median TFS was 329 days (IQR 184–660 days). At the conclusion of the study period, 58 (47%) were alive, 40 (32%) were deceased, and 26 (21%) had undergone orthotopic liver transplant.

Decreased TFS was observed with baseline tumor diameter >5 cm (HR 2.0, 95% CI 1.0–3.8; $P = 0.05$), tumor burden exceeding Milan criteria (HR 3.1, 95% CI 1.6–6.0; $P = 0.001$), CP Class C (HR 2.9, 95% CI 1.1–7.6; $P = 0.03$), BCLC Stage B (HR 3.9, 95% CI 1.7–9.0; $P = 0.002$), Stage C (HR 2.9, 95% CI 1.2–6.9; $P = 0.02$), Stage D (HR 5.2, 95% CI 1.8–15.0; $P = 0.002$), and macrovascular invasion (HR 3.0, 95% CI 1.2–7.9; $P = 0.02$) [Table 4]. In addition, stable or progressive disease after LRT was associated with lower TFS (HR 2.4, 95% CI 1.2–4.9; $P = 0.02$).

High ES grade (HR 1.0, 95% CI 0.3–2.8, $P = 0.96$) and 100% clear-cell variant (HR 1.02, 95% CI 0.5–2.2, $P = 0.95$) were not associated with TFS on univariate analysis [Figures 1b and 2b]. High ES grade and 100% clear cell also were not associated with survival stratified by LRT modality or BCLC stage [Supplemental Table 1]. When stratified by BCLC stage, there was a trend toward longer TFS with 100% clear cell in comparison to no clear cell in BCLC Stage B patients (HR 3.2, 95% CI 0.9–11.5; $P = 0.08$).

DISCUSSION

The baseline histological features of HCC underlying clinical presentation, radiologic features, and clinical outcomes following LRT are not fully understood. Prior studies that have demonstrated an association between histological subtype of HCC on recurrence and survival after resection, RFA, and transplant predominantly represent early-stage disease and histological assessment on surgical resection specimens or explant.[2-5] In this study, histological information was assessed on percutaneous biopsy before LRT, and most patients (61%) were BCLC Stage B or greater. High tumor grade was significantly associated with aggressive tumor features such as large tumor diameter, elevated baseline serum AFP, and infiltrative appearance. In addition, the clear-cell variant was significantly associated with non-viral etiologies of cirrhosis and atypical contrast enhancement patterns on CT or MR images. While these findings suggest that tumor grade and cytological subtype in part underlie differences in baseline clinical and imaging features, such conventional histological information on percutaneous biopsy did not impart additional prognostic information from tumor stage and high-risk imaging features.[16]

High ES has been associated with lack of objective response after drug-eluting embolic TACE in a retrospective cohort study of 93 patients who subsequently underwent OLT; however, the majority of patients were early stage, and histological differentiation was determined after TACE on explant.[5] In this study, no association was observed between high ES grade on percutaneous biopsy before LRT and objective response (OR 2.3, 95% CI 0.7–7.4; $P = 0.15$), suggesting that the predictive value of ES grade on post-therapy explant cannot be extrapolated to percutaneous biopsy specimens before LRT or patient populations with intermediate- or advanced-stage disease.

Poor histologic differentiation was associated with tumor diameter >5 cm, infiltrative appearance, and elevated serum AFP, supportive of prior studies. AFP is considered to be secreted by dedifferentiated HCC,[17] and infiltrative appearance on imaging is known to be associated with higher AFP levels

Table 4: Time to progression and transplant-free survival by Univariate analysis.

	TTP		TFS	
	HR	**Significance**	**HR**	**Significance**
Age	0.97	*P*<0.05	1.0	*P*=0.79
Gender				
Male	1.0		1.0	
Female	0.7	*P*=0.18	1.0	*P*=0.93
Ethnicity				
African-American	1.0		1.0	
Caucasian	1.2	*P*=0.64	1.5	*P*=0.31
Other	0.7	*P*=0.35	0.9	*P*=0.78
DM				
No	1.0		1.0	
Yes	0.7	*P*=0.19	0.9	*P*=0.78
Cirrhosis etiology				
HCV	1.0			
HBV	1.5	*P*=0.45	0.7	*P*=0.68
ASH	0.8	*P*=0.52	0.5	*P*=0.33
NASH	0.4	*P*=0.11	0.6	*P*=0.42
Mixed	1.0	*P*=0.92	1.00	*P*=0.99
Other	1.6	*P*=0.36	0.7	*P*=0.59
Tumor diameter (cm)				
≤5	1.0			
>5	1.7	*P*=0.06	2.0	*P*<0.05
Within Milan criteria				
Yes	1.0		1.0	
No	2.6	*P*<0.001	3.1	*P*<0.001
AFP >50				
No	1.0		1.0	
Yes	2.5	*P*<0.005	1.4	*P*=0.36
Child-Pugh class				
A	1.0		1.0	
B	1.5	*P*=0.14	1.3	*P*=0.41
C	2.1	*P*=0.13	2.9	*P*<0.05
BCLC stage				
0/A	1.0		1.0	
B	3.0	*P*<0.001	3.9	*P*<0.005
C	1.6	*P*=0.15	2.9	*P*<0.05
D	2.5	*P*=0.06	5.2	*P*<0.005
ES grade				
Low	1.0		1.0	
High	1.5	*P*=0.33	1.0	*P*=0.96
Architecture subtypes				
Trabecular	1.0		1.0	
Mixed trabecular	1.3	*P*=0.49	0.7	*P*=0.50
Pseudoglandular	1.0	*P*=0.89	1.3	*P*=0.55
Solid and mixed solid	1.2	*P*=0.64	1.5	*P*=0.37
Clear-cell amount				
None	1.0		1.0	
Focal	1.0	*P*=0.91	1.0	*P*=0.97
100%	1.4	*P*=0.28	1.0	*P*=0.95
Microvascular invasion				
No	1.0		1.0	
Yes	2.5	*P*=0.08	1.6	*P*=0.47
Arterial phase hyperenhancement				
Yes	1.0		1.0	
No	0.7	*P*=0.19	1.1	*P*=0.75
Infiltrative HCC				
No	1.0		1.0	
Yes	2.4	*P*<0.05	1.7	*P*=0.30
Venous phase washout				
No	1.0		1.0	
Yes	1.6	*P*=0.18	1.5	*P*=0.41
Macrovascular invasion				
No	1.0		1.0	
Yes	2.9	*P*<0.01	3.0	*P*<0.05
Delayed capsular enhancement				
No	1.0		1.0	
Yes	1.1	*P*=0.81	0.7	*P*=0.37
OR after the first LRT				
Yes	1.0		1.0	
No	2.3	*P*<0.005	2.1	*P*<0.05
OR after all LRTs				
Yes	1.0			
No	2.7	*P*<0.001	2.4	*P*<0.05
Progression within 100 days				
No	-		1.0	
Yes	-	-	5.9	*P*<0.001

TTP: Time to progression, TFS: Transplant-free survival, HBV: Hepatitis B virus, HCV: Hepatitis C virus, ASH: Alcoholic steatohepatitis, NASH: Non-alcoholic steatohepatitis, ethnicity other: Alaska native, Native Hawaiian, unavailable, cirrhosis etiology other: Primary, cryptogenic, sarcoidosis, DM: Diabetes mellitus, BCLC: Barcelona clinic liver cancer, ES grade: Edmondson-Steiner grade, LRT: Locoregional therapy, OR: Objective response

and worse survival after intra-arterial therapy.[18] While tumor size, infiltrative appearance, and elevated AFP were associated with reduced TTP and TFS in this cohort, no such association was observed with histological features on univariate or stratified analysis. The predictive value of histologic differentiation on prognosis was limited by the fact that 70% of tumors with elevated AFP (>50 ng/ml), 75% of infiltrative HCC tumors, and 76% of tumors >5 cm were histologically low grade on percutaneous biopsy in this study. This is consistent with a retrospective study of 63 patients showing that tumor grade and microvascular invasion did not predict clinical response or overall survival after transarterial embolization or TACE in 50 patients with prior percutaneous core biopsy.[19] While poor tumor differentiation has been associated with local tumor progression in a study of 95 BCLC Stage 0/A patients treated with RFA, these findings are confounded by high-grade tumors being larger than low-grade tumors in the cohort and more frequent use of internally cooled electrodes with a higher-powered generator in the low-grade group.[4]

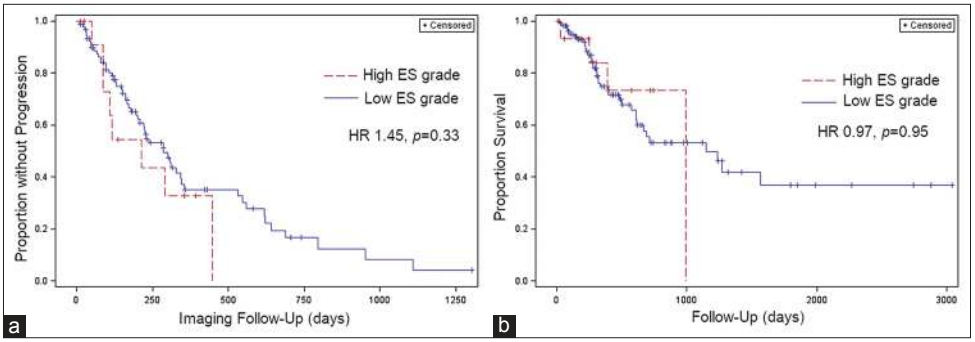

Figure 1: (a) Time to progression by Edmondson-Steiner (ES) grade after locoregional therapy. Kaplan–Meier curves of time to progression in patients with hepatocellular carcinoma of high ES grade (n = 13, median 118 days) or low ES grade (n = 93, median 182 days) treated with locoregional therapy. (b) Transplant-free survival (TFS) by ES grade after locoregional therapy. Kaplan–Meier curves of TFS in patients with hepatocellular carcinoma of high ES grade (n = 16, median 346 days) or low ES grade (n = 107, median 322 days) treated with locoregional therapy.

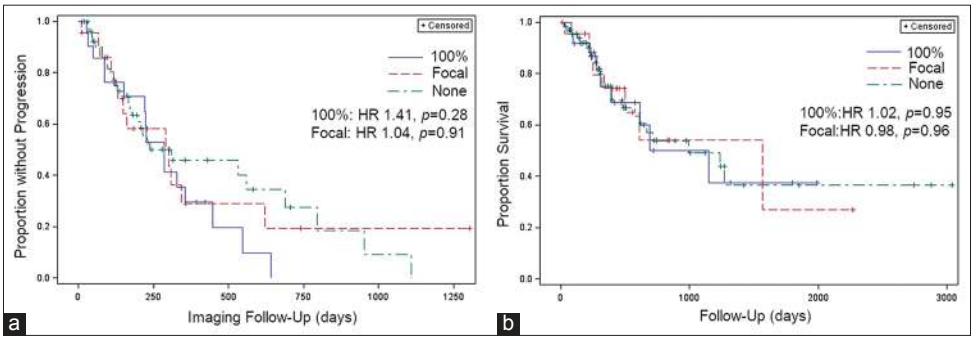

Figure 2: (a) Time to progression by clear-cell cytological composition. Kaplan–Meier curves of time to progression in patients with hepatocellular carcinoma (HCC) comprised 100% (n = 22, median 223 days), focal (n = 22, median 155 days), no (n = 55, median 178 days) clear cells treated with locoregional therapies. (b) Transplant-free survival by clear-cell cytological composition. Kaplan–Meier curves of TFS in patients with HCC comprised 100% (n = 24, median 350 days), focal (n = 23, median 429 days), no (n = 68, median 315 days) clear cells treated with locoregional therapies.

Clear-cell HCC is distinguished from other variants by clear cytoplasm attributed to increased glycogen or lipid contents secondary to metabolic changes.[9] Such morphologic and metabolic changes may result from differential oncogenic process between chronic viral hepatitis and non-viral etiologies of cirrhosis such as alcohol and metabolic syndrome.[20] Furthermore, clear-cell variant of HCC is known to have decreased number of intratumoral arteries in association with fatty changes; this is supportive of its association with atypical enhancement on CT or MRI in this study.[21] A significant association was observed between clear-cell variants and the solid architectural subtype, but its biological and clinical significance remains to be determined in future study.

While poor tumor differentiation is associated with recurrence and poor survival after OLT or surgical resection,[2,3] the impact of clear-cell HCC on prognosis is controversial: Some studies suggest that clear-cell variant is associated with longer overall survival,[6-8] while other studies found similar survival between clear and non-clear-cell variant tumors after surgical resection.[9,10] It is postulated that longer survival in clear-cell variant is associated with relatively low histological grade and capsule formation that are favorable for surgery.[7] However, neither tumor grade nor clear-cell variant of HCC was associated with disease progression or TFS in our cohort after LRT.

Microvascular invasion on explant is strongly correlated with recurrence after OLT or surgical resection.[22,23] In this study, a trend was observed toward a higher hazard of progression in tumors with microvascular invasion but not TFS. The lack of observed association in this study may be due to different methods to acquire HCC specimens: Percutaneous biopsy versus explant or surgical resection. In this cohort, only 13% of tumors were high ES grade and 9.1% demonstrated microvascular invasion on percutaneous core biopsy. In contrast, a study comprised 76% BCLC 0/A patients reported 53% were high ES grade and 51% had microvascular invasion on resection.[24] Thus, the lack of observed association between ES grade and microvascular invasion in the current study with treatment response, TTP, and TFS may

be attributable to the intrinsic limitation of percutaneous biopsy, where high-grade tumors are frequently misclassified as low grade due to sampling error of heterogeneous tumors. Prior studies have demonstrated that percutaneous biopsy underestimates tumor grade and microvascular invasion compared to resection.[25]

This study demonstrates that histological analysis of tumor grade can stratify HCC given its association with aggressive clinical and radiologic features. However, this study also supports the current practice of deferring conventional histological analysis before LRT in tumors diagnosed by imaging criteria[12] given the lack of independent prognostic information imparted by tumor grade and microvascular invasion on percutaneous biopsy. Although the prognostic value of conventional histological analysis before LRT may be limited, immunohistochemistry and genomic sequencing analysis of percutaneous biopsy specimens are under active investigation and percutaneous biopsy may yet prove critical to treatment allocation in the emerging era of precision medicine. Certain genetic mutations involved in Wnt/β-catenin and hypoxia stress response have been associated with objective response after transarterial embolization in primary and metastatic liver tumors.[26] Furthermore, high expression of programmed death ligand-1 on resected HCC specimens is associated with poor tumor differentiation, elevated AFP, microvascular invasion,[24] and poor disease-free survival and overall survival.[27,28]

Our study bears several limitations. First, the retrospective single-institution design renders potential sampling bias in our cohort. Although the majority of tumors in this study demonstrated typical arterial-phase hyperenhancement and washout, a retrospective study of percutaneously biopsied HCC study will be inevitably enriched with HCC with non-diagnostic imaging features. Our cohort consisted of predominantly Caucasian and African-American patients with viral etiologies of HCC, and extrapolation of findings to other populations should be performed with caution. The relatively low number of patients with high ES grade (13%) in this cohort might have increased our susceptibility to a type II error. Similarly, while our study is unique in including BCLC B, C, and D patients, the variability in BCLC stage and LRT modality may have introduced confounding and reduced our ability to observe associations between histological features and outcomes. However, numerous tumor and clinical features, such as tumor size and BCLC stage, were significantly associated with TFS in this study; although a larger study may detect an association between histologic features on percutaneous biopsy on LRT outcomes, it is unlikely that histological features are superior prognostic indicators compared to macroscopic tumor features such as size and portal vein invasion, and clinical factors including BCLC stage. As discussed, studies utilizing percutaneous biopsy specimens are prone to histological misclassification bias due to non-representative tissue sampling of histologically heterogeneous tumors.

CONCLUSION

High histologic grade of HCC on percutaneous biopsy is associated with poor prognostic indicators such as larger tumors, infiltrative appearance, and advanced BCLC stage. The clear-cell variant of HCC was associated with non-viral cirrhosis, and the paucity of arteries in this subtype may underlie the atypical enhancement associated with these tumors. Percutaneous biopsy, therefore, may be used to diagnose HCC, but histological stratification did not provide independent prognostic information. The findings support deferral of percutaneous biopsy and histological assessment of HCC diagnosed by imaging criteria before LRT.

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Conflicts of interest

There are no conflicts of interest.

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SUPPLEMENTAL TABLE

Supplemental Table 1: TTP and TFS for High ES Grade and Clear Cell Variant Stratified by LRT Modality and BCLC Stage.

	TTP		TFS	
	HR	**Significance**	**HR**	**Significance**
High ES grade				
LRT modality				
Y90	-		0.7	*P*=0.78
Ablation	-	-	-	-
TACE	1.5	*P*=0.38	0.8	*P*=0.71
TACE/Ablation	2.5	*P*=0.30	1.5	*P*=0.71
BCLC Stage				
0/A	0.6	*P*=0.65	2.3	*P*=0.45
B	1.1	*P*=0.89	0.5	*P*=0.46
C/D	1.7	*P*=0.37	0.6	*P*=0.51
100% Clear Cell Variant				
LRT modality				
Y90	2.6	*P*=0.45	1.7	*P*=0.52
Ablation	1.4	*P*=0.60	1.2	*P*=0.87
TACE	0.8	*P*=0.58	0.8	*P*=0.66
TACE/Ablation	-	-	-	-
BCLC Stage				
0/A	1.2	*P*=0.72	1.7	*P*=0.54
B	1.0	*P*=0.96	3.2	*P*=0.08
C/D	1.3	*P*=0.63	0.4	*P*=0.17

TTP: Time to progression, TFS : Transplant-free survival, TACE : Transarterial chemoembolization, Y90 : Yttrium-90 radioembolization, BCLC : Barcelona Clinic Liver Cancer, -: no results due to limited sample size, LRT: Locoregional therapy