Original paper

Lymphovascular invasion on explant is associated with presenting tumor characteristics and not direct acting antiviral utilization in hepatitis C candidates undergoing liver transplantation

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Abstract

Aim of the study: Utilization of direct acting antiviral (DAA) therapy in candidates with well-compensated hepatitis C virus (HCV) cirrhosis and hepatocellular carcinoma (HCC) accruing end stage liver disease (MELD) exception points is highly variable among transplant centers based on center location, local organ procurement dynamics, HCV(+) organ availability, and patient preference. The association between DAA utilization prior to transplant and incidence of lymphovascular invasion on explant is unknown.

Material and methods: Retrospective evaluation from 2013-2017 of patients on a liver transplant (LT) waitlist with HCV-related cirrhosis, MELD-Na < 15, and HCC (within T2/Milan criteria). The cohort was divided into the pre-LT DAA treated group and untreated group with clinical/viral demographics collected. Tumor presenting characteristics, locoregional treatments, wait time to LT, dropout rates and explant pathology were compared.

Results: DAAs were used in 44 patients prior to LT (SVR12 of 37/44 [84%]) and 19 left untreated with LT performed in 81% (51/63) of the waitlisted cohort. No significant differences were found between groups with regards to clinical/viral demographics, local-regional therapy (LRT) sessions, or frequency of lymphovascular invasion on explant. The untreated cohort had a higher rate of dropout (6.3% vs. 3.2%) ($p = 0.041$). On subgroup analysis of 51 subjects undergoing LT, AFP > 250 ng/ml ($p = 0.003$) and multifocal HCC (> 1 lesion) ($p = 0.006$) were associated with lymphovascular invasion on explant while DAA therapy was not ($p = 0.578$).

Conclusions: DAA therapy for waitlist active HCV candidates accruing MELD exception points has no deleterious effects on bridging LRT, nor is it associated with increased frequency of lymphovascular invasion on explant. The latter appears driven by tumor related characteristics (AFP and number of lesions) irrespective of DAA utilization prior to LT.

Key words: liver transplantation, hepatocellular carcinoma, lymphovascular invasion, hepatitis C virus infection.

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Introduction

Introduction of highly effective and tolerable direct-acting antivirals (DAA) has improved waitlist mortality and lowered risk of disease progression among chronic hepatitis C virus (HCV) patients awaiting liver transplantation (LT) [1]. Optimal timing of DAA therapy in waitlist active candidates remains a subject of debate. Cost-effective analyses show an advantage for pre-transplant therapy [2, 3]. In con-

trast, a recent study identified higher quality adjusted life-years (QALYs) with DAA therapy after transplant, particularly when deferral maintains access to the expanded pool of HCV(+) donors for otherwise well-compensated HCV-infected cirrhotics with hepatocellular carcinoma (HCC) [4].

Recent literature suggests an association between DAA therapy and the development of *de novo* and recurrent HCC [5-9]. The mechanism for increased risk is hypothesized to be due to alteration in natural killer (NK) cell function and dysregulation of immune surveillance with rapid viral clearance [10]. Initial reports of a link have not been corroborated by more recent investigation [11]. One study reported a lower incidence of HCC with DAA therapy [12] and another identified increased HCC incidence as a function of patient characteristics and lower screening intensity rather than DAA therapy itself [13].

DAA utilization in subjects awaiting LT with low "biologic" model for end stage liver disease (MELD) scores with HCC accruing exception points is highly variable among transplant centers. A recent investigation examined the impact of DAA therapy on HCC recurrence after local-regional therapy (LRT) and waitlist dropout among liver LT candidates with HCC [14]. In LT candidates with HCV and HCC with an initial complete response to LRT, DAA use was not associated with increased risk of HCC recurrence but rather a reduced waitlist dropout due to tumor progression or death. While previous investigation focused on waitlist dropout, little is known about the association of DAA therapy prior to transplant and incidence of lymphovascular invasion on explanted tissue at the time of LT and subsequent outcomes. Histological examination, especially assessment of lymphovascular invasion on pathologic explanted tissue, is important to assess recurrence risk and prognosis after LT [15]. Investigation evaluating microinvasion in patients with surgical resection for HCC found presence associated with lower 5-year survival (24-36% vs. 61-63%) and earlier median recurrence (12 vs. 42.2 months). Invasion of tumor cells into the vasculature is hypothesized to serve as a route for intrahepatic metastasis leading to recurrence [16]. Renzulli *et al.* later evaluated radiographic features of microvascular invasion in HCV-cirrhosis patients with HCC nodules that developed before and after DAA therapy. They found that HCC nodules that developed after DAA therapy had a higher rate of microvascular invasion imaging features (70.7% vs. 33.3%), which was indicative of more aggressive tumor [17]. The aim of this investigation was to examine the incidence of lymphovascular invasion on explant pathology in untreated and DAA treated HCV cirrhotic cohorts with HCC awaiting LT.

Material and methods

After obtaining institutional board approval, we performed a retrospective cohort study (2013-2017) of the University of Arizona College of Medicine-Phoenix LT program identifying consecutive subjects with: HCV related cirrhosis with "biologic" MELD-Na < 15 and HCC presenting within T2/Milan criteria listed for LT. Subjects were excluded from analysis if: biologic MELD-Na > 15 at the time of transplant listing, concomitant etiology for liver disease apart from HCV (concomitant viral, autoimmune, or inherited metabolic diseases) was identified; and if "downstaging" LRT of HCC to T2/Milan criteria was performed prior to listing. Treatment of HCV in waitlist active candidates at our center is determined on a case-by-case basis factoring candidate co-morbidities, baseline liver function, and patient preference. Center heterogeneity in utilization of DAA in this population allowed for identification of 2 cohorts: 1) untreated and 2) DAA treated HCV cirrhotics with HCC listed for transplant. Baseline demographics were collected on untreated and treated cohorts at the time of transplant listing including age, gender, ethnicity, BMI, biologic MELD-Na, as well as HCV genotype and viral load. For the cohort receiving DAA treatment, HCV regimen and sustained virologic response rates (SVR12) were recorded. For the entire cohort, presenting tumor variables – size and number of lesions, alpha-fetoprotein (AFP) – and number of LRT sessions/modalities were tabulated. Response to LRT was defined and tabulated in accordance with the Modified Response Evaluation Criteria in Solid Tumor (mRECIST) for HCC guidelines as applied by a United Network Organ Service (UNOS) certified abdominal radiologist [18]. Outcomes recorded included waitlist dropout or death and wait time to LT when applicable. Explant pathology reports of subjects receiving LT were reviewed for presence of lymphovascular invasion. Significant differences in continuous variables between untreated and treated cohorts were identified by *t*-test; categorical with chi-square test; $p < 0.05$ (SPSS v24.0, Armonk, NY). Subgroup analysis for the cohort undergoing LT was performed using chi-square analysis to identify significant associations with lymphovascular invasion on explant pathology, $p < 0.05$.

Results

Patient population

Sixty-three patients met inclusion and exclusion criteria, 19 untreated and 44 treated with DAA en

route to liver transplant. Of the 19 untreated patients, 10 refused DAA therapy and consented for HCV(+) organ to increase candidate organ competitiveness after informed consent discussion, 6 refused and deferred consideration until after LT, and 3 had unstable financial/insurance coverage and were denied DAA therapy en route to LT. No significant differences existed between untreated and treated cohorts with regards to clinical demographic (Table 1). Untreated cohorts were listed at average "biologic" MELDs of 10.89 ±2.97 compared to the treated cohort at an average of 10.02 ±2.51 ($p = 0.237$). 44 patients treated included 31 with genotype 1, six with genotype 2, and seven with genotype 3. 31 patients with genotype 1 were treated with sofosbuvir/ledipasvir for 12 weeks; 4 patients with genotype 2 were treated with sofosbuvir/ribavirin and 2 with sofosbuvir/velpatasvir for 12 weeks; and 3 patients with genotype 3 were treated with sofosbuvir/velpatasvir for 12 weeks while 4 patients were treated with sofosbuvir/ribavirin for 24 weeks. In subjects receiving ribavirin, subjects were started at 1200 mg in 2 divided doses if > 75 kg or 1000 mg in divided doses if < 75 kg, and dose reduction to 600 mg daily was allowed by treater discretion [19]. With respect to tumor characteristics, the largest tumor size was similar between cohorts; however, the DAA treated cohort had a significantly higher number of candidates with multifocal disease (Table 1).

Response to DAA therapy

Overall SVR12 for the treated group was 37/44 (84%). No significant differences existed between untreated and treated cohorts with respect to genotype (genotype 1: 63% vs. 70%, genotype 2: 16% vs. 14%, genotype 3: 16% vs. 16%, other: 5% vs. 0%; $p = 0.482$), pre-treatment viral load (2.1 million IU/ml vs. 2.74 million IU/ml, $p = 0.652$), or previous treatment history (naive: 58% vs. 36%, relapser: 32% vs. 34%, non-responder: 10% vs. 30%; $p = 0.176$) (Table 2). All treated patients received sofosbuvir-containing regimens for a minimum of 12 weeks. No subjects discontinued therapy while on treatment.

Locoregional therapy treatment and dropout rates

No significant differences were noted between untreated and DAA treated cohorts with respect to locoregional therapy modality which included transarterial chemo or radioembolization (or combination) while waitlist active for transplant (Table 3). There were no significant differences in number of LRT sessions

Table 1. Baseline demographics: Summary of clinical and tumor demographics between untreated and treated subjects with HCV and HCC

Parameter	Untreated pre-transplant ($n = 19$)	Treated pre-transplant ($n = 44$)	P
Age at diagnosis of HCC	60.05 ±5.8	58.89 ±6.25	0.490
Gender			0.532
Male	16 (84%)	34 (77%)	
Female	3 (16%)	10 (23%)	
Ethnicity			0.967
White	13 (68%)	29 (65%)	
Black	1 (5%)	3 (7%)	
Hispanic	5 (27%)	12 (27%)	
BMI	27.31 ±4.13	27.99 ±4.84	0.594
Creatinine	0.85 ±0.23	0.94 ±0.29	0.282
Bilirubin	1.54 ±1.01	1.16 ±0.67	0.142
INR	1.27 ±0.26	1.20 ±0.16	0.168
Sodium	137.89 ±2.54	137.95 ±3.21	0.943
MELD at diagnosis	10.89 ±2.979	10.02 ±2.51	0.237
Number of lesions			0.006
1	11 (58%)	36 (82%)	
2	8 (42%)	4 (9%)	
3	0 (0%)	4 (9%)	
Size of maximal lesion	3.16 ±1.02	2.86 ±1.03	0.294
AFP at diagnosis	184.8 ±202.7	81.33 ±123.01	0.050

Table 2. HCV treatment specifics: 44 out of 63 waitlist active HCV subjects underwent sofosbuvir-based direct acting antiviral therapy with an SVR12 rate of 84%

Parameter	Untreated pre-transplant ($n = 19$)	Treated pre-transplant ($n = 44$)	P
Genotype			0.482
1	12 (63%)	31 (70%)	
2	3 (16%)	6 (14%)	
3	3 (16%)	7 (16%)	
Other	1 (5%)	0 (0%)	
Pretreatment viral load	2178803.05 ±2275685.06	2748875.82 ±5260029.37	0.652
Prior treatment			0.176
Naïve	11 (58%)	16 (36%)	
Relapser	6 (32%)	15 (34%)	
Non-responder	2 (10%)	13 (30%)	
SVR12	Not applicable (NA)	37 (84%)	NA

Table 3. HCC treatment and transplant outcomes: Waitlist dropout due to tumor progression and death was seen in 4 patients in the untreated group and 2 patients in the treated group. Of the remaining 6 of 8 non-transplanted patients in the treated cohort, 2 were delisted due to active substance abuse and 4 remained waitlist active for transplant within T2/Milan criteria

Parameter	Untreated pre-transplant (n = 19)	Treated pre-transplant (n = 44)	P
LRT modalities used			0.104
TACE	12 (63%)	21 (48%)	
TARE	6 (32%)	10 (23%)	
Combination	1 (5%)	13 (29%)	
Number of LRT sessions			0.056
0	1 (5%)	1 (2%)	
1	5 (26%)	20 (45%)	
2	2 (10%)	14 (32%)	
3	7 (37%)	7 (13%)	
4	3 (17%)	1 (2%)	
5	1 (5%)	1 (2%)	
Tumor dropout			0.041
No	15 (23.8%)	42 (66.7%)	
Yes	4 (6.3%)	2 (3.2%)	
Transplanted	15 (79%)	36 (81%)	NS
Lymphovascular invasion on explant	2	4	0.164

TACE – transarterial chemoembolization, TARE – transarterial radioembolization

utilized while waitlist active for transplant. Dropout (progression of HCC beyond transplant criteria) rates were noted to be higher in the untreated cohort (6.3%) compared to the DAA treated cohort (3.2%) ($p = 0.04$).

Transplant rates

Fifty-one of 63 (81%) of subjects underwent LT (36/44 [81%] treated, 15/19 [79%] untreated) at an average of 403.38 ±197.55 days (396.22 ±196.39-treated vs. 420.53 ±206.18-untreated, $p = 0.693$). All 4 of the non-transplanted patients in the untreated cohort died from tumor progression. With regards to the 8 non-transplanted subjects in the treated cohort, 2 had dropped out and died, 2 were de-listed secondary to active substance abuse, and 4 remain waitlist active for transplant within T2/Milan criteria.

Risk factors for lymphovascular invasion

In the 51 subjects (15 from the untreated and 36 from the treated group) undergoing LT there were no differences in incidence of lymphovascular invasion on explant: 2/15 (13.3%) in the untreated cohort

and 4/36 (11.1%) in the treated cohort ($p = 0.164$). On further subgroup analysis stratifying for lymphovascular invasion, pretreatment AFP level > 250 ng/ml ($p = 0.003$) and multifocal HCC (> 1 lesion) ($p = 0.006$) was associated with presence of lymphovascular invasion on explant while DAA exposure was not ($p = 0.578$).

Discussion

Highly effective and tolerable DAA therapy has altered the landscape of HCV therapy even in subjects with decompensated cirrhosis [20]. Patients with advanced fibrosis and associated portal hypertension can experience rapid improvement in liver function parameters [21]. Such dramatic responses have influenced treatment algorithms in waitlist active candidates for LT, with many centers deferring treatment to facilitate access to the pool of HCV(+) donor livers and avoid the phenomenon of placing patients in "MELD purgatory" (improvement in liver parameters with associated reduction in MELD score/priority awaiting transplant) [22].

A number of clinical issues arise in the waitlist active candidate for transplant with HCV viremia, fibrosis, and HCC. Well-compensated patients have lower biologic MELD scores and the position on the waitlist has been historically driven by accrual of points with MELD exception [23]. Recently, a number of conflicting reports have evaluated the association between HCV treatment and incidence of HCC development in subjects with advanced fibrosis [5, 6, 12]. Application of DAA therapy in candidates with well-compensated HCV cirrhosis and HCC accruing MELD exception points is highly variable among transplant centers and often based on center location (local organ procurement dynamics/HCV(+) organ availability) and patient preference.

In this investigation, we examined differences in locoregional therapy, dropout rates, and incidence of lymphovascular invasion on explant in a cohort of otherwise well-compensated subjects with HCV related cirrhosis and HCC exposed and not exposed to DAA therapy. Similar to a previous study [14] we saw a slightly higher rate of HCC dropout in untreated subjects compared to those treated due to increased rates of tumor progression or death. Importantly, we found no significant differences in incidence of lymphovascular invasion between untreated and treated subjects who ultimately underwent LT despite the treated cohort having higher frequency of multifocal disease at the time of transplant listing. On subgroup analysis, we found that presence of lymphovascular invasion on explant was significantly associated with higher AFP

level at HCC diagnosis and multifocal presentation rather than DAA therapy.

Additionally, our study showed lower SVR12 rates (84%) when compared with historical rates seen in HCV patients without HCC. This is consistent with prior data showing that HCV patients with active HCC had higher treatment failure with DAA therapy. In a large study by the Veterans Affairs, Beste *et al.* found that SVR12 was achieved in 91.1% in non-HCC patients but only 74.4% with the presence of HCC [24]. It has been hypothesized that active HCC may serve as a "protected reservoir" for HCV infection, thereby reducing DAA efficacy, though mechanisms of viral evasion in the context of neoplasia need to be explored further [25].

Our study has limitations as it is retrospective, from a single center with a relatively small population. With regards to baseline demographics, we did not assess for the presence of concomitant non-alcoholic steatohepatitis (NASH) or alcohol as etiologies in subjects with HCV; these co-factors may influence HCC biology and response to LRT. Another limitation is that our AFP measurement was cross-sectional at the time of diagnosis of clinical HCC diagnosis. Fluctuations in AFP level, not only with subsequent LRT but also with DAA therapy, may have prognostic value, and this should be the subject of a future prospective study. In addition, the decision to treat with DAA was left at the discretion of the treating hepatologist and, as such, it is plausible that subjects with a longer expected wait time were treated whereas those with higher priority (higher biologic MELD/exception point accrual) were left untreated, thereby introducing a classification bias. Nevertheless, mean MELD at listing was similar between cohorts and the entire cohort was subject to the same organ acceptance practices and regional organ procurement organization (OPO) dynamics. Our results suggest that DAA therapy for waitlist active HCV candidates accruing MELD exception points is efficacious with no deleterious effects on bridging LRT or increase in frequency of lymphovascular invasion on explant. Lymphovascular invasion appears driven by tumor related characteristics (AFP and number of lesions) irrespective of DAA utilization prior to LT.

Disclosure

Authors report no conflict of interest.

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