TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Portal vein encasement predicts neoadjuvant therapy response in liver transplantation for perihilar cholangiocarcinoma protocol

Mamatha Bhat,[1] Matthew Hathcock,[2] Walter K. Kremers,[2] Sarwa Darwish Murad,[1] Grant Schmit,[3] James Martenson,[4] Steven Alberts,[5] Charles B. Rosen,[6] Gregory J. Gores[1] and Julie Heimbach[6]

1 Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA
2 Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA
3 Department of Radiology, Mayo Clinic, Rochester, MN, USA
4 Department of Radiation Oncology, Mayo Clinic, Rochester, MN, USA
5 Department of Oncology, Mayo Clinic, Rochester, MN, USA
6 Division of Transplantation Surgery, Mayo Clinic, Rochester, MN, USA

Keywords
chemotherapy, Cholangiocarcinoma, liver transplantation, portal vein encasement, radiotherapy.

Correspondence
Julie Heimbach MD, Associate Professor of Surgery, Mayo Clinic College of Medicine, 200 First St., SW, Rochester, MN 55905, USA.
Tel: 507-284-2511;
fax: 507-266-2810;
e-mail: heimbach.julie@mayo.edu

Conflicts of interest
The authors have declared no conflicts of interest.

Received: 22 March 2015
Revision requested: 4 May 2015
Accepted: 10 July 2015
Published online: 6 August 2015

doi:10.1111/tri.12640

Summary

Background: Survival and recurrence of cancer after liver transplant (LT) for perihilar cholangiocarcinoma (CCA) following neoadjuvant chemoradiotherapy are strongly correlated with the presence of residual CCA in the liver explant.
Aim: To determine factors predicting response to neoadjuvant therapy using the presence of residual CCA on explant as a surrogate marker.
Methods: Characteristics of 109 patients having undergone LT for cholangiocarcinoma were abstracted, with attention to parameters hypothesized to influence radiation therapy efficacy.
Results: In the multivariable model, the presence of portal vein encasement (OR 11.8; 95% CI: 2.43–57.21; $P = 0.002$) and MELD score (OR 1.13; 95% CI: 1.02–1.26; $P = 0.017$) were predictive of residual macroscopic disease (c-statistics 0.78). Oral capecitabine in addition to standard 5-fluorouracil chemotherapy (OR 0.32, 95% CI: 0.14, 0.71; $P = 0.006$) was independently protective against residual cancer, independent of MELD score.
Conclusions: Portal vein encasement was strongly predictive of residual macroscopic disease. Radial tumor diameter did not have greater predictive value than longitudinal diameter, confirming the appropriateness of current protocol selection criteria. No particular tumor morphology predicted better response. Maintenance oral capecitabine following 5-fluorouracil infusion was independently protective against residual disease. Portal vein encasement as a negative prognostic finding should be taken into account to optimize patient selection and management.

Introduction

Perihilar cholangiocarcinoma (CCA) is a highly aggressive bile duct cancer with limited therapeutic options [1]. Only a small proportion of patients are diagnosed early enough to be candidates for resection. While 5-year survival rates with resection are 20–40% [2–6], many patients present with unresectable disease. Beginning in 1993, a specialized protocol involving neoadjuvant chemoradiotherapy followed by liver transplantation (LT) offered the possibility of cure to a highly selected group of patients with unresectable, yet early stage perihilar CCA [7–9]. This protocol consists of 3 weeks of external beam radiation therapy (EBRT) administered concurrently with 5-fluorouracil, a

radiosensitizing chemotherapeutic agent. This is followed by brachytherapy delivered locally to the biliary tract, oral capecitabine as maintenance chemotherapy, staging laparoscopy to assess for metastases, and for those without metastasis, subsequent LT. Studies have confirmed this protocol to be very effective, with 65–70% recurrence-free survival at 5 years [9–12]. However, approximately 15–20% of patients do develop recurrent CCA post-LT, which is eventually fatal. Residual tumor in the liver explant has been strongly correlated with increased risk of recurrence and decreased survival post-LT [10,13]. It is unclear at this time why certain patients have tumors with complete, or nearly complete response to pre-LT chemoradiation therapy with little to residual tumor in the explant, while others have an incomplete response as manifested by significant burden of residual tumor in the liver explant. In the radiation oncology literature, factors such as tumor volume [14], tissue hypoxia [15], anemia [16], molecular markers on immunohistochemistry [17], and body mass index [18] have been shown to influence response to chemoradiotherapy.

Therefore, given that residual tumor post-radiation therapy is the primary risk factor for disease recurrence following LT, the aim of this study was to identify factors predicting response to neoadjuvant chemoradiation therapy in patients who undergo liver transplantation following completion of the protocol, using the presence of residual CCA in the explant as a surrogate marker for response to therapy. We were particularly interested in whether the tumor diameter and morphology may impact response to radiation therapy. Our current selection criterion excludes those with a radial diameter >3 cm, but includes patients with a longitudinal extension of the tumor along the bile duct to exceed 3 cm. Given that the behavior of CCA can also be of superficial spreading type, which may impact the efficacy of the chemoradiation regimen, we wondered whether including those with a longer stricture was appropriate. Hence, we also wished to assess whether radial diameter is more predictive of residual disease on explant than longitudinal diameter in this protocol. Awareness of predictive factors would enable more targeted treatment among the at-risk patients and better select patients for this protocol, thereby potentially optimizing outcomes following LT.

Patients and methods

Study design and transplant protocol

This was a single-center retrospective study using data from the LT database at Mayo Clinic, Rochester, Minnesota. Patients had undergone an established protocol for LT following neoadjuvant chemoradiation for CCA between January 1993 and December 2012. Standard selection for this protocol is based on diagnosis of unresectable perihilar CCA as reflected by the presence of (i) intraluminal brushings positive for adenocarcinoma or an endoscopic biopsy demonstrating adenocarcinoma or, (ii) radiographic malignant-appearing stricture plus either CA 19-9 > 100 U/ml in the absence of acute bacterial cholangitis, polysomy on fluorescence *in situ* hybridization (FISH, since 2003), or well-defined mass on cross-sectional imaging. In order to try to compare patients with similar initial disease burden, we included only those patients who had an obvious mass on imaging or positive biopsy/brushings [19]. We also performed a subgroup analysis in order to assess whether patients with both a mass and positive cytology were more susceptible to having residual disease on explant.

Standard exclusion criteria for the protocol include extrahepatic disease, previous malignancy (excluding skin or cervical cancer) within 5 years prior, prior abdominal radiotherapy, uncontrolled infection, previous attempt at surgical resection with violation of the tumor plane, or any medical condition precluding transplantation. Vascular encasement and stricture/mass extension along the duct were not contra-indications, although patients with mass with a clear radial diameter of >3 cm were generally excluded.

Patients received neoadjuvant therapy according to our previously published protocol[2]. EBRT was administered to a total dose of 4500 cGy in 30 fractions of 150 cGy twice daily for 3 weeks, with Fluorouracil (5-FU) initially administered at 500 mg/m^2 for the first 3 days, and later changed to continuous infusion of 5-FU given for the duration of EBRT. Radiation fields were designed to treat the known extent of primary tumor, regional lymph nodes in the hilum of the liver and celiac lymph nodes. The resulting radiation therapy fields generally included the right and left hepatic ducts and the common hepatic duct. The common bile duct was included in the radiation field if involved. Transluminal radiation boost was initially given as 2000 cGy over 24 h using low dose-rate brachytherapy, although most recently is given as high-dose brachytherapy of 930–1600 cGy in 1–4 fractions. In a small minority of patients, brachytherapy was not technically feasible, and in these cases, patients received a boost of external radiation therapy of 2000 cGy. This was followed by chemotherapy consisting of oral capecitabine at 2000 mg/m^2 in two divided doses for 2 of every 3 weeks until transplantation. Operative staging with routine biopsy of hepatic artery and peri-choledochal lymph nodes plus any suspicious lesion was performed prior to transplantation, and only those with a negative staging operation remained eligible for LT.

This retrospective chart review study was approved by the Institutional Review Board at Mayo Clinic, Rochester, Minnesota.

Data collection

Routine demographic, clinical, laboratory, radiographic, and pathology data at onset of chemoradiation therapy were collected from a prospectively maintained database. A radiologist specialized in liver malignancies re-read all magnetic resonance images (MR) of patients with mass lesions and described lesions according to morphology (i.e., round-, wedge- or long cylinder-shaped mass). Additionally, radial and longitudinal diameters of the mass were measured. The presence of portal vein encasement was reflected by the portal vein being partially or completely encased, or compressed by tumor. Details pertaining to neoadjuvant timing and therapy were collected, including doses of EBRT, chemotherapy, and brachytherapy. We were particularly interested in those characteristics pertaining to tumor biology and factors that could influence response to radiation therapy. These included hemoglobin at time of radiation therapy as a surrogate for tumor hypoxia, need for blood transfusion, body mass index, presence of cirrhosis, dose of brachytherapy administered, and pre- and post-radiation therapy CA-19-9 values. Pathologic features in the explant were noted, including the presence of macroscopic or microscopic tumor foci, portal vein and hepatic arterial encasement, lymph node and perineural invasion.

Patient population

We specifically included only those patients who had either a hilar mass on imaging or ERCP brushings diagnostic of adenocarcinoma. A perihilar tumor was considered a mass if a well-circumscribed solid lesion extending into the liver parenchyma was seen on cross-sectional imaging.

In order to use residual CCA on explant as a valid study endpoint representative of response to radiation therapy, we attempted to select patients with similar disease burden upon initiation of radiation therapy. Therefore, those patients selected for LT solely on the basis of a malignant-appearing stricture on cholangiogram, with FISH polysomy and/or CA-19-9 level >100 U/ml were excluded.

Outcome definition and statistical analysis

Response to chemoradiation therapy was determined on the basis of residual CCA on the liver explant. The report by a pathologist specialized in liver malignancies was the source of this information. The presence of residual tumor was categorized as no residual tumor, microscopic foci, or macroscopic residual disease. Given that only 15 patients had microscopic disease on explant, and that these patients had survival similar to those with no residual disease, we combined these two groups as a single group and compared them to patients with macroscopic disease on explant.

Recurrence of CCA was diagnosed on the basis of imaging or pathologic evidence of CCA following LT. Patients enrolled in the protocol undergo routine screening for recurrence post-LT with CA 19-9 levels and CT at 4 months and 1, 2, and 3 years post-LT. Recurrence-free survival was determined based on the time from the date of LT to recurrence, death, or date of last follow-up visit. Continuous variables were summarized by means (and standard deviations) or as medians (and interquartile range, or IQR) when appropriate. Categorical variables were summarized by N (% of total).

ANOVA or the chi-square test was used to determine factors at the time of radiation therapy that were predictive of the presence of macroscopic, microscopic CCA, and no residual tumor. Logistic regression analysis was used to assess the independent effect of various parameters of interest on the presence of residual disease, by assessing the group with macroscopic residual disease on explant versus patients with microscopic disease or no residual tumor on the explant. Multivariable model was developed using a stepwise selection process. A P-value of <0.05 was considered statistically significant. Recurrence-free survival was calculated using the Kaplan–Meier method for each of the three groups.

Results

Patient population

A total of 109 patients meeting the criteria of hilar mass or ERCP brushing cytology diagnostic of adenocarcinoma were included in the study. These patients had undergone the standard chemoradiation protocol for cholangiocarcinoma in anticipation of liver transplantation between January 1993 and December 2012. Fifteen patients were excluded, given that they had been selected for liver transplantation on the basis of the other criteria for the cholangiocarcinoma protocol (elevated CA-19-9, FISH polysomy, and malignant-appearing stricture). Demographic and clinical characteristics at the time of starting chemoradiation therapy are listed in Table 1.

Residual Cholangiocarcinoma on explant

The Kaplan–Meier survival curve (Fig. 1a) demonstrates that the groups with no residual disease and microscopic foci had comparable survival curves following LT. Those with no residual disease on explant had an excellent 5-year recurrence-free survival rate of 77% (95% CI of 66–91%), those with microscopic foci had 62% (38–100%) 5-year survival, while those with macroscopic disease had 5-year survival of 39% (26–59%) (Fig. 1b).

Table 1. Demographic and clinical characteristics of patients with perihilar cholangiocarcinoma who underwent liver transplantation, categorized based on residual tumor on explant

Parameters	Macroscopic disease (*N* = 45)	Microscopic foci (*N* = 15)	No residual tumor (*N* = 49)	Total (*N* = 109)	*P*-value
Female	14 (31.1%)	4 (26.7%)	9 (18.4%)	27 (24.8%)	0.35
Age	52.4 (10.1)	51.7 (12.4)	50.8 (10.7)	51.6 (10.6)	0.72
Body mass index	26.4 (4.5)	23.9 (4.1)	25.5 (3.9)	25.6 (4.3)	0.16
Hemoglobin	12.5 (1.4)	13.0 (1.6)	12.8 (1.9)	12.7 (1.7)	0.49
CA 19-9 level prior to Radiation Therapy (IU/ml)*	112 (19, 489)	42 (21, 233)	29 (12.9, 101.5)	68 (16, 233.5)	0.05
CA 19-9 after radiation therapy (IU/ml)*	97 (24, 286)	78 (45, 163)	40 (16, 92)	64 (24, 163)	0.03
Cirrhosis	13 (28.9%)	6 (40.0%)	14 (28.6%)	33 (30.3%)	0.68
Primary sclerosing cholangitis	22 (48.9%)	10 (66.7%)	37 (75.5%)	69 (63.3%)	0.03
Cholecystectomy	15 (33.3%)	5 (33.3%)	14 (28.6%)	34 (31.2%)	0.88
History of weight loss at presentation	30 (66.7%)	7 (46.7%)	29 (59.2%)	66 (60.6%)	0.38
Fluorescence *in situ* hybridization (FISH)					
Missing	12	5	14	31	0.14
Polysomy	11 (33.3%)	7 (70.0%)	20 (57.1%)	38 (48.7%)	
Trisomy	8 (24.2%)	2 (20.0%)	7 (20.0%)	17 (21.8%)	
Creatinine	0.9 (0.2)	0.9 (0.1)	0.9 (0.2)	0.9 (0.2)	0.32
Total bilirubin	2.3 (1.3, 7.8)	2.9 (1.0, 8.0)	1.2 (1.0, 2.8)	1.9 (1.0, 7.0)	0.06
INR	1.1 (0.2)	1.3 (1.2)	1.0 (0.2)	1.1 (0.5)	0.08
MELD score	12 (5.0)	12 (7.5)	10 (4.5)	11 (5.3)	0.32
Portal vein encasement	15 (33.3%)	4 (26.7%)	2 (4.1%)	21 (19.3%)	0.001
Hepatic artery	20 (54.1%)	6 (60.0%)	13 (29.5%)	39 (42.9%)	0.04
Months between timing of External beam radiation therapy to transplant	6.5 (4.1)	6.4 (2.9)	7.4 (5.2)	6.9 (4.5)	0.64
Brachytherapy	43 (95.6%)	14 (93.3%)	47 (95.9%)	104 (95.4%)	0.84
Longitudinal extent of tumor (along ducts); cm					
Not applicable†	13	4	22	39	0.05
0–1	0 (0.0%)	0 (0.0%)	1 (3.7%)	1 (1.4%)	
1–2	7 (21.9%)	3 (27.3%)	11 (40.7%)	21 (30.0%)	
2–3	16 (50.0%)	2 (18.2%)	11 (40.7%)	29 (41.4%)	
>3	9 (28.1%)	6 (54.5%)	4 (14.8%)	19 (27.1%)	
Radial extent of tumor; cm					
Not applicable†	13	4	22	39	0.02
0–1	3 (9.4%)	1 (9.1%)	4 (14.8%)	8 (11.4%)	
1–2	17 (53.1%)	7 (63.6%)	20 (74.1%)	44 (62.9%)	
2–3	10 (31.3%)	2 (18.2%)	3 (11.1%)	15 (21.4%)	
>3	2 (6.3%)	1 (9.1%)	0 (0.0%)	3 (4.3%)	
Chemotherapy					
5-Fluorouracil (FU)	18 (36.7%)	5 (33.3%)	27 (60.0%)	50 (45.9%)	0.05
5-FU with Oral Capecitabine	31 (63.3%)	10 (66.7%)	18 (40.0%)	59 (54.1%)	
Living donor liver transplant	11 (24.4%)	3 (20.0%)	20 (40.8%)	34 (31.2%)	0.16
Explant characteristics					
Lymphatic invasion	4 (8.9%)	0 (0.0%)	0 (0.0%)	4 (3.7%)	0.08
Vascular invasion	5 (11.1%)	0 (0.0%)	0 (0.0%)	5 (4.6%)	0.03
Vascular encasement	2 (4.4%)	0 (0.0%)	0 (0.0%)	2 (1.8%)	0.43
Perineural invasion	20 (44.4%)	5 (33.3%)	0 (0.0%)	25 (22.9%)	
Tumor grade					
0	2 (4.4%)	3 (20.0%)	47 (95.9%)	52 (47.7%)	<0.0001
1	2 (4.4%)	0 (0.0%)	0 (0.0%)	2 (1.8%)	
2	14 (31.1%)	5 (33.3%)	0 (0.0%)	19 (17.4%)	
3	24 (53.3%)	6 (40.0%)	2 (4.1%)	32 (29.4%)	
4	3 (6.7%)	1 (6.7%)	0 (0.0%)	4 (3.7%)	

*Median (IQR provided).

†Cholangiocarcinoma diagnosed on the basis of ERCP brushings typical of adenocarcinoma.

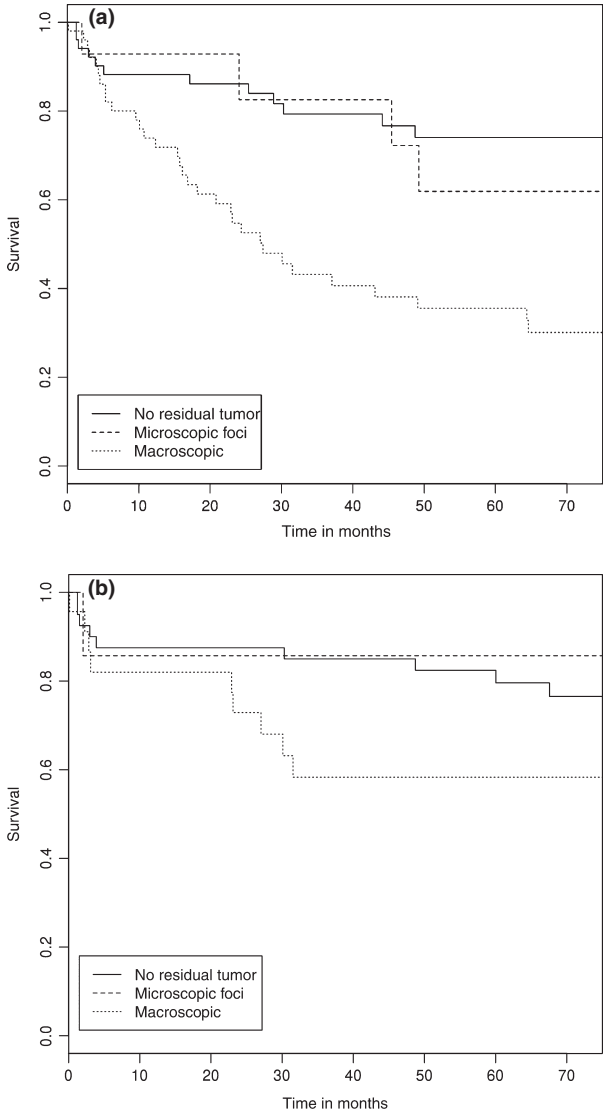

Figure 1 (a) Kaplan–Meier estimate for mortality within each of the outcome groups based on the presence of residual cholangiocarcinoma on liver explant. (b) Kaplan–Meier estimate for recurrence-free survival within each of the outcome groups based on the presence of residual cholangiocarcinoma on liver explant.

Factors predicting response to radiation therapy

Univariate analysis was performed to evaluate the correlation of various demographic and clinical characteristics with the presence of residual CCA on explant (Table 2).

Complete portal vein encasement (present in all 21 cases, with no compression, on imaging prior to chemoradiotherapy), log CA-19-9 pretreatment, and lack of maintenance oral capecitabine strongly correlated with the presence of macroscopic CCA in the explant. Age, BMI, cirrhosis or stage of fibrosis, anemia, differences in brachytherapy dose, and need for transfusion had no predictive value.

The radial extent of the tumor as seen on imaging at diagnosis was slightly more predictive of residual disease than the longitudinal extent of the tumor, but not significantly so. We also evaluated whether any particular tumor morphology, such as round-, wedge-, or long cylinder-shaped mass as categorized by an expert radiologist, was more predictive of response. There was no particular tumor morphology that predicted response (data not shown). Doses of chemoradiation, change of chemotherapy protocol from bolus to continuous 5-FU (data not shown), as well as the time elapsed between administration of chemoradiation therapy and liver transplantation, did not affect the presence of residual disease.

We also performed a subgroup analysis of patients with the presence of both mass and positive cytology, in order to assess whether they represented a group with greater resistance to chemoradiation therapy. We found that increased body mass index ≥30 (OR 1.21; 95% CI 1.02–1.43; $P = 0.03$) and CA-19-9 levels (OR 4.02; 95% CI 1.05–15.42; $P = 0.04$) predicted residual macroscopic disease in this subgroup. A subgroup analysis was also performed for the 75 patients with perihilar CCA mass only, with no preoperative histological/cytological diagnosis. Of these, 34 (45%) had macroscopic residual disease on the explant and 41 (54%) had no residual tumor.

In the multivariable logistic model, we adjusted for age and gender as this is standard practice in clinical studies (Table 3). We found that the presence of portal vein encasement (OR 11.8; 95% CI: 2.43–57.21; $P = 0.002$) and increased MELD score at presentation (OR 1.13; 95% CI: 1.02–1.26; $P = 0.017$) were predictive of the presence of residual macroscopic disease on explant (c-statistics 0.78). PV encasement significantly increased a patient risk of recurrence or death (HR (95%CI): 1.9 (0.93–3.94) P-value = 0.08). We also categorized the patients into low and high CA-19-9 (<100 IU/ml vs. ≥100 IU/ml) categories and discovered that there was no interaction of CA 19-9 with portal vein encasement (P-value > 0.05). Taking maintenance oral capecitabine in addition to standard 5-fluorouracil chemotherapy infusion was protective against the presence of residual cancer on explant (OR 0.32, 95% CI: 0.14–0.71; $P = 0.006$) with c-statistic 0.68.

Median MELD score was not significantly different between patients able to tolerate oral capecitabine versus those in whom it was not able to be administered (Fig. 2a), and the area under the curve for this model was very good at 0.78 (Fig. 2b). Serum bilirubin was tested, but was found not to contribute to the model as effectively as the MELD score in its entirety. This indicated that MELD score was not a confounder as to whether or not patients had received oral capecitabine.

Table 2. Predictors of Macroscopic residual disease on univariate analysis, where explants with microscopic foci were grouped together with those having no residual disease

Parameters	Odds ratio estimate	Lower 95% confidence limit for odds ratio	Upper 95% confidence limit for odds ratio	*C*-statistic	$P > \chi^2$
Gender	1.77	0.74	4.26	0.55	0.20
Age at time of radiation therapy	1.01	0.98	1.05	0.53	0.49
Body mass index	1.07	0.98	1.18	0.58	0.13
Hemoglobin at time of radiation therapy	0.88	0.70	1.11	0.56	0.27
CA 19-9 level prior to radiation therapy (IU/ml)*	1.26	1.02	1.56	0.63	0.03
CA 19-9 after radiation therapy (IU/ml)*	1.23	0.97	1.55	0.61	0.08
Stage of Fibrosis					
1	0.21	0.02	1.88	0.61	0.25
2	0.71	0.10	5.04		0.26
3	0.26	0.04	1.58		0.17
4	0.34	0.05	2.15		0.58
Cirrhosis	0.89	0.39	2.06	0.51	0.79
Primary sclerosing cholangitis	0.35	0.16	0.78	0.62	0.01
Cholecystectomy	1.18	0.52	2.69	0.52	0.69
History of hepatobiliary surgery	1.25	0.39	4.01	0.51	0.70
Weight loss	1.56	0.70	3.44	0.55	0.27
Fluorescence *in situ* hybridization (FISH) polysomy	0.33	0.13	0.85	0.63	0.02
Creatinine	1.39	0.16	12.24	0.51	0.77
Bilirubin	1.05	0.99	1.12	0.61	0.14
Bilirubin levels (reference 1 < bilirubin ≤2 mg/dl)					
Bilirubin >2 mg/dl	0.79	0.30	2.06	0.61	0.44
Bilirubin ≤1 mg/dl	0.33	0.11	0.99		0.03
INR	1.04	0.47	2.28	0.61	0.92
MELD score	1.06	0.99	1.14	0.63	0.12
MELD score ≥20	1.88	0.47	7.41	0.52	0.37
Platelet count	1.00	1.00	1.002	0.53	0.43
Portal vein encasement	5.90	1.96	17.79	0.62	0.002
Hepatic artery encasement	2.11	0.94	4.59	0.59	0.07
External beam radiation therapy	1.00	1.00	1.00	0.50	0.77
Brachytherapy dose	1.00	1.00	1.00	0.50	0.92
Number of months between radiation therapy and liver transplant	0.96	0.88	1.05	0.56	0.42
Capecitabine	0.37	0.17	0.82	0.62	0.01
Presence of hilar mass	2.57	1.16	5.68	0.62	0.02
Longitudinal extent of mass	1.21	0.97	1.53	0.61	0.10
Radial extent of mass	z	1.00	2.18	0.60	0.051

Discussion

While initial results in treating perihilar cholangiocarcinoma with LT alone were associated with very poor outcomes due to a high recurrence rate and subsequently high mortality rate, the combination of neoadjuvant chemoradiotherapy followed by LT for patients with unresectable perihilar CCA has been quite effective, with 5-year disease-free survival rates of 65–70% [10]. The availability of LT for selected patients with unresectable perihilar CCA has resulted in cure for patients with a previously bleak outlook. Neoadjuvant chemoradiation therapy appears to be efficacious, often with complete tumor destruction as evidenced by lack of residual CCA in the explant [12]. However, there is a subset of patients that appears not to respond to chemoradiotherapy, as reflected by bulky residual macroscopic disease in the liver explant. The presence of macroscopic CCA tumor strongly correlates with tumor recurrence and decreased survival following LT [13]. Identifying factors that may predict response to chemoradiation therapy is imperative to improve patient selection, especially given the current climate of liver allograft shortage.

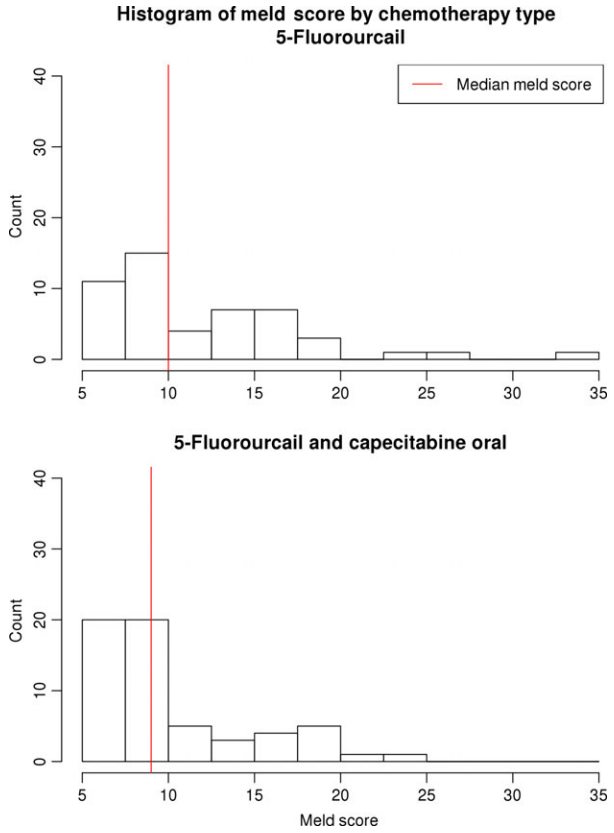

Figure 2 Distribution of MELD score by chemotherapy regimen type, with 5-fluorouracil infusion only versus 5-fluorouracil followed by oral capecitabine.

Table 3. Predictors of Macroscopic residual disease on explant, multivariable models. Portal vein encasement, MELD score, and lack of maintenance therapy with capecitabine following standard chemoradiation are all significant predictors of residual disease

Parameter	Effect	95% CI	*P*-value	*C*-Statistic
Model A				
Age	1.01	0.97, 1.05	0.66	0.78
Female	2.33	0.87, 6.38	0.12	
Portal vein encasement present	11.8	2.43–57.21	0.002	
MELD score	1.13	1.02 –1.26	0.017	
Model B				
Age	1.02	0.98, 1.06	0.33	0.68
Female	2.00	0.79, 5.19	0.15	
5-Fluorouracil with capecitabine Oral	0.32	0.14, 0.71	0.006	

If we could accurately predict who will respond completely to neoadjuvant therapy, we could potentially include patients with large tumors, whom we are currently excluding. Conversely, we may exclude those we are currently treating who are determined to be at high risk of failure, or potentially offer this group additional neoadjuvant therapy such as higher dose of radiation or additional chemotherapy with associated increased toxicity justified by increased risk of recurrence.

In our current study, we found that the presence of portal vein encasement by tumor was the most significant predictor of residual CCA. It is most likely that portal vein encasement reflects more aggressive tumor biology, although other factors (i.e., impact of a large, high-flow vessel within tumor mass on radiation efficacy) may contribute. Additionally, a higher MELD score also predicted significant residual tumor in the explant. This more likely reflects the impact of a more advanced or aggressive tumor rather than an intrinsic resistance to therapy for patients with more advanced liver disease. Nonetheless, patients with high MELD scores are more ill and more immunocompromised; these patients therefore may not be able to provide a good response against the tumor.

We also performed a subgroup analysis of a higher risk patient subpopulation with mass and positive cytology at presentation. This analysis revealed that increased BMI may affect response to chemoradiation therapy, as reflected by greater disease burden. It may be that increased BMI leads to more difficulty in targeting the dose or impacts dose delivery, or that increased body size has some other impact on response to the radiation. Additionally, a higher CA-19-9 value prior to radiation also predicted poor response to radiation therapy in the subgroup analysis, which most likely represents more advanced disease upon therapy initiation.

We also had a radiologist expert in liver malignancies review all MR/CT scans of those patients with CCA tumors, so that exact longitudinal and radial diameters could be measured. CCA tumors most often have hazy, ill-defined borders due to a surrounding desmoplastic reaction. Additionally, once patients have had stent placement, tumor extension along the bile duct is more difficult to interpret. This renders radiologic size determination very difficult and results in variable interpretation. We therefore had a single radiologist review all scans so as to make the interpretation uniform. However, longitudinal and radial measurements were not found to be predictive of radiation response. In other words, tumors <3 cm all had equal chances to respond, thereby confirming the appropriateness of current size criterion for this liver transplant protocol. In addition to looking at radial and longitudinal measurements, we also assessed whether any particular tumor morphology was more predictive (i.e., round-, wedge-, or long cylinder-shaped mass) and found no correlation.

In terms of protective factors, having taken oral chemotherapy as maintenance after standard chemotherapy with 5-FU infusion was strongly predictive of no residual cancer in the explant. All patients are intended to receive maintenance chemotherapy, although at times the dose must be reduced or held due to ongoing infection or poor tolerance of therapy (e.g., exacerbation of underlying colitis in patients with PSC, refractory cholangitis). Therefore, it is possible that this protective effect of oral chemotherapy is simply due to patients with less aggressive tumors being less ill and better able to tolerate oral chemotherapy, rather than representing a true protective effect of the chemotherapy. We attempted to control for this by adjusting for MELD score at initiation of therapy. We found that the protective effect of capecitabine was independent of MELD score; however, we recognize that potential for selection bias remains.

In the radiation therapy literature, tissue hypoxia and anemia have been associated with resistance to radiation therapy [15,20]. In addition to hemoglobin level prior to chemoradiation therapy, we looked at BMI, presence of cirrhosis, radiation therapy dosing, brachytherapy dosing and CA-19-9 levels before and after radiation therapy. None of these parameters were predictive of radiation therapy efficacy, as reflected by residual CCA on explant.

Although the Mayo Clinic patient series in the cholangiocarcinoma liver transplant protocol is the largest in the literature, it is admittedly small from the statistical perspective. Additionally, the unique CCA morphology and dimensions due to its obscured perimeter are an impediment to accurate radiologic interpretation. The presence of stents in many patients may have also affected the radiology reading. Nonetheless, this is the practical reality when it comes to evaluation of patients with CCA, and the extensive experience at our center with this protocol comes with valuable lessons. Finally, as previously noted, determining the impact of neoadjuvant therapy is limited by the difficulty in precisely determining the disease burden and tumor biology prior to the initiation of therapy.

In summary, portal vein encasement and an increased MELD score significantly predicted lack of response to radiation therapy in the CCA liver transplant protocol, as reflected by residual CCA on liver explant. Maintenance oral chemotherapy following chemoradiation as a bridge to liver transplantation protected against the presence of residual cancer. Radial and longitudinal tumor diameters were strongly correlated to each other, but one measure did not have greater predictive value than the other in terms of response to radiation therapy. This finding further confirms the adequacy of the current standard candidate selection policy for the CCA liver transplant protocol. It seems that each patient's individual tumor biology is the strongest predictor of response to radiation therapy, along with maintenance chemotherapy. Having identified those patients with a lesser likelihood of responding to radiation therapy, the question arises whether they should receive more aggressive therapy prior to LT. The alternative is that such patients be excluded as potential LT recipients, given that the disadvantages of aggressive recurrent disease following LT far outweigh benefits. These concerns must be addressed, given the number of patients who die on the LT list. An additional avenue is to consider adjuvant therapy following LT in those patients with residual tumor on the explant. Future efforts will need to be directed toward deciphering what constitutes less versus more aggressive CCA tumor biology at the molecular level.

Authorship

MB, GJG, CBR and JH: designed the study. MB, GS, JM, SA and JH: performed the study. MB, SDM, GS, JM, SA and JH: collected data. MH and WK: analyzed data. MB and JH: wrote the paper.

Funding

The authors have declared no funding.

References

1. Gores GJ. Cholangiocarcinoma: current concepts and insights. *Hepatology* 2003; **37**: 961.
2. Rea DJ, Heimbach JK, Rosen CB, *et al.* Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. *Ann Surg* 2005; **242**: 451; discussion 8–61.
3. Su CH, Tsay SH, Wu CC, *et al.* Factors influencing postoperative morbidity, mortality, and survival after resection for hilar cholangiocarcinoma. *Ann Surg* 1996; **223**: 384.
4. Rea DJ, Munoz-Juarez M, Farnell MB, *et al.* Major hepatic resection for hilar cholangiocarcinoma: analysis of 46 patients. *Arch Surg* 2004; **139**: 514; discussion 23–5.
5. Jarnagin WR, Fong Y, DeMatteo RP, *et al.* Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 2001; **234**: 507; discussion 17–9.
6. DeOliveira ML, Cunningham SC, Cameron JL, *et al.* Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; **245**: 755.
7. Heimbach JK, Gores GJ, Haddock MG, *et al.* Liver transplantation for unresectable perihilar cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 201.
8. Heimbach JK, Gores GJ, Nagorney DM, Rosen CB. Liver transplantation for perihilar cholangiocarcinoma after aggressive neoadjuvant therapy: a new paradigm for liver and biliary malignancies? *Surgery* 2006; **140**: 331.
9. De Vreede I, Steers JL, Burch PA, *et al.* Prolonged disease-free survival after orthotopic liver transplantation plus

adjuvant chemoirradiation for cholangiocarcinoma. *Liver Transpl* 2000; **6**: 309.
10. Darwish Murad S, Kim WR, Harnois DM, *et al.* Efficacy of neoadjuvant chemoradiation, followed by liver transplantation, for perihilar cholangiocarcinoma at 12 US centers. *Gastroenterology* 2012; **143**: 88.e3; quiz e14.
11. Rea DJ, Rosen CB, Nagorney DM, Heimbach JK, Gores GJ. Transplantation for cholangiocarcinoma: when and for whom? *Surg Oncol Clin N Am* 2009; **18**: 325.
12. Heimbach JK, Gores GJ, Haddock MG, *et al.* Predictors of disease recurrence following neoadjuvant chemoradiotherapy and liver transplantation for unresectable perihilar cholangiocarcinoma. *Transplantation* 2006; **82**: 1703.
13. Darwish Murad S, Kim WR, Therneau T, *et al.* Predictors of pretransplant dropout and posttransplant recurrence in patients with perihilar cholangiocarcinoma. *Hepatology* 2012; **56**: 972–81.
14. Dubben HH, Thames HD, Beck-Bornholdt HP. Tumor volume: a basic and specific response predictor in radiotherapy. *Radiother Oncol* 1998; **47**: 167–74.
15. Harrison LB, Chadha M, Hill RJ, Hu K, Shasha D. Impact of tumor hypoxia and anemia on radiation therapy outcomes. *Oncologist* 2002; **7**: 492–508.
16. Macdonald G, Hurman DC. Influence of anaemia in patients with head and neck cancer receiving adjuvant postoperative radiotherapy in the Grampian region. *Clin Oncol (R Coll Radiol)* 2004; **16**: 63–70.
17. Zlobec I, Steele R, Nigam N, Compton CC. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. *Clin Cancer Res* 2005; **11**: 5440–3.
18. Huang PY, Wang CT, Cao KJ, *et al.* Pretreatment body mass index as an independent prognostic factor in patients with locoregionally advanced nasopharyngeal carcinoma treated with chemoradiotherapy: findings from a randomised trial. *Eur J Cancer* 2013; **49**: 1923–31.
19. Rosen CB, Darwish Murad S, Heimbach JK, Nyberg SL, Nagorney DM, Gores GJ. Neoadjuvant therapy and liver transplantation for hilar cholangiocarcinoma: is pretreatment pathological confirmation of diagnosis necessary? *J Am Coll Surg* 2012; **215**: 31; discussion 8–40.
20. Harrison L, Blackwell K. Hypoxia and anemia: factors in decreased sensitivity to radiation therapy and chemotherapy? *Oncologist* 2004; **9**(Suppl 5): 31–40.