ORIGINAL ARTICLE

Vascular invasion and survival after liver transplantation for hepatocellular carcinoma: a study from the European Liver Transplant Registry

Hans-Christian Pommergaard[1], Andreas A. Rostved[1], René Adam[2], Lau C. Thygesen[3], Mauro Salizzoni[4], Miguel A. Gómez Bravo[5], Daniel Cherqui[2], Franco Filipponi[6], Karim Boudjema[7], Vincenzo Mazzaferro[8], Olivier Soubrane[9], Juan C. García-Valdecasas[10], Joan F. Prous[11], Antonio D. Pinna[12], John O'Grady[13], Vincent Karam[2], Christophe Duvoux[14], Allan Rasmussen[1] for the European Liver and Intestine Transplant Association (ELITA)

[1]Department of Surgical Gastroenterology and Transplantation, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [2]Department of Hepatobiliary Surgery, Cancer and Transplantation, AP-HP, Hôpital Universitaire Paul Brousse, Inserm U 935, Univ Paris-Sud, Villejuif, France, [3]National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark, [4]Liver Transplant Center and General Surgery, A.O.U. Città della Salute e della Scienza di Torino, Molinette Hospital, Turin, Italy, [5]Department of Surgery – Liver Transplant Unit, Hospital Virgen Del Rocio, Sevilla, Spain, [6]Hepatobiliary Surgery and Liver Transplantation Unit, University of Pisa Medical School Hospital, 56124, Pisa, Italy, [7]Service de Chirurgie Hépatobiliaire et Digestive, Hôpital Pontchaillou, Centre Hospitalier Université de Rennes 1, Rennes, France, [8]University of Milan and Division of Gastrointestinal Surgery and Liver Transplantataion, Istituto Nazionale Tumori, Fondazione IRCCS, Milan, Italy, [9]Department of HPB Surgery and Liver Transplant, Beaujon Hospital, Clichy, University Denis Diderot, Paris, France, [10]Hepatobiliopancreatic & Transplant Surgery, ICMDiM, Hospital Clínic, [11]Unitat de Cirurgia Hepato-bilio-pancreàtica, Hospital Universitari de Bellvitge, Barcelona, Spain, [12]General Surgery and Transplant Division, S. Orsola Hospital, University of Bologna, Bologna, Italy, [13]Institute of Liver Studies, King's College Hospital, London, United Kingdom, and [14]Department of Hepatology and Liver Transplant Unit, Henri Mondor Hospital, Paris Est University (UPEC), Créteil, France

Abstract

Background: Studies suggest that vascular invasion may be a superior prognostic marker compared with traditional selection criteria, e.g. Milan criteria. This study aimed to investigate the prognostic value of micro and macrovascular invasion in a large database material.

Methods: Patients liver transplanted for HCC and cirrhosis registered in the European Liver Transplant Registry (ELTR) database were included. The association between the Milan criteria, Up-to-seven criteria and vascular invasion with overall survival and HCC specific survival was investigated with univariate and multivariate Cox regression analyses.

Results: Of 23,124 patients transplanted for HCC, 9324 had cirrhosis and data on explant pathology. Patients without microvascular invasion, regardless of number and size of HCC nodules, had a five-year overall survival of 73.2%, which was comparable with patients inside both Milan and Up-to-seven criteria. Patients without macrovascular invasion had an only marginally reduced survival of 70.7% after five years. Patients outside both Milan and Up-to-seven criteria without micro or macrovascular invasion still had a five-year overall survival of 65.8%.

Conclusion: Vascular invasion as a prognostic indicator remains superior to criteria based on size and number of nodules. With continuously improving imaging studies, microvascular invasion may be used for selecting patients for transplantation in the future.

Received 16 November 2017; accepted 3 March 2018

Correspondence

Hans-Christian Pommergaard, Department of Surgical Gastroenterology and Transplantation, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100, Copenhagen, Denmark. E-mail: HCPommergaard@gmail.com

Introduction

In 1996, the Milan criteria were proposed for selecting patients with hepatocellular carcinoma (HCC) for liver transplantation[1] and have remained the gold standard since.[2,3] The Milan criteria are based on the assumption that survival for liver transplantation for HCC should be comparable with survival in non-malignant indications.[3]

Being a strong predictor of poor prognosis, macrovascular invasion is an absolute contraindication for transplantation.[3,4] The Milan and Up-to-seven criteria are based on size and number of HCC nodules, which are not independently prognostic factors when adjusted for micro and macrovascular invasion.[5] Among patients within criteria for transplantation, microvascular invasion is a strong predictor of survival.[6–9] Moreover, patients without microvascular invasion despite having advanced tumors outside transplantation criteria, have a prognosis comparable to that of patients within criteria.[10] Together, this suggests that standard criteria for selecting patients for transplantation based on size and number of HCC nodules, e.g. Milan criteria, may rule out patients with a good prognosis due to the absence of microvascular invasion.

Selection of patients with HCC for transplantation is based on preoperative imaging. These modalities may under-stage patients, however, rarely due to missed macrovascular invasion.[11] With emerging imaging techniques, microvascular invasion may be detected prior to transplantation in future patients.[12,13]

Earlier investigations have shown the importance of vascular invasion as a prognostic marker.[5,7,9,11,14] However, these were primarily smaller single center studies. Therefore, the aim of the present study was to investigate, on a large scale, the impact of microvascular and macrovascular invasion on survival in patients who were liver transplanted for HCC using the European Liver Transplant Registry (ELTR) database. Thus, representing general clinical practice in Europe.

Methods

This study was reported according to the STROBE guideline.[15] A protocol was registered at clinicaltrials.org with ID NCT02995096 prior to performing the statistical analyses.

This study was register-based with prospectively recorded data from the ELTR database, which comprises data from 172 liver transplantation centers in Europe, reporting pre-transplant and follow-up data. All patients are managed and followed up locally. The database contains information on donor, recipient, locoregional treatments before transplantation, immunosuppression, pathology from the explanted liver, underlying liver disease, presence of cirrhosis in addition to HCC, time of death, and cause of death.

All patients included were registered in ELTR from 1990 to November 2016 and transplanted due to HCC. Patients transplanted for HCC without cirrhosis and patients without data on explant pathology were excluded.

The outcomes were overall survival and HCC specific survival, five and 10 years after transplantation. The exposure variables were based on explant pathology. These included vascular invasion, number of HCC nodules and maximum size of HCC nodules. Vascular invasion was defined as microvascular or macrovascular invasion according to information in the ELTR database. These represent the presence of tumor cells inside smaller and larger vessels, respectively. The local pathology departments defined the specific definitions of microvascular and macrovascular invasion. The Milan criteria were defined as no extrahepatic disease, no macrovascular invasion, one nodule of maximum 5 cm in diameter or 1–3 nodules, each with a maximum diameter of 3 cm^1. The Up-to-seven criteria also include no extrahepatic disease or macrovascular invasion. In addition, the sum of number of nodules and size of the largest nodule in cm may be up to seven.[10]

All patients with any data on explant pathology were selected (data on number of nodules, size of largest nodule or vascular invasion). Due to missing data in some variables, a multiple imputation model with fully conditional specification and five imputations was used with following variables: number of nodules (0% missing), size of largest nodule (5.4% missing), vascular invasion (20.2% missing), time on waiting list (19.4% missing), cirrhosis (0% missing), age (0.1% missing), gender (0% missing), MELD-score (26.4% missing), and transplantation center (0% missing). Distribution and mean values for variables were comparable before and after imputation.

Univariate and multivariate Cox regression were used to evaluate the association between transplantation criteria, vascular invasion and survival, as well as HCC specific survival, and were reported as hazards ratios (HR) with 95% confidence intervals (CI). Multivariate models included gender, age, center, time on waiting list, number of nodules, size of largest nodule, vascular invasion (micro, macro or none), and MELD-score, as these were considered possible predictors of survival. Variables already included in exposure variable (e.g. size and number for Milan criteria) were excluded. Proportional hazards assumption was checked for all covariates using log minus log plots with natural logarithm of follow-up time. Five and 10 year overall and HCC specific survival were calculated as cumulative survival with 95% CI using Kaplan Meier statistics. IBM SPSS statistic version 23 was used. Statistical significance was defined as P-value ≤ 0.05.

Results

Description of patients

Of 23,124 patients in the database, 9560 had any data on explant pathology. Of these, 9324 had cirrhosis in addition to HCC and were included. Patients with and without data on pathology were comparable with respect to gender, age, volume of transplant

center, cirrhosis, time on waiting list and MELD-score. However, patients with data were primarily transplanted in the late part of the investigated period. Description of the 7439 patients (data prior to imputation) who had information about vascular invasion is shown in Table 1. Macrovascular invasion was found in 3.1% and microvascular in 17.8% of the patients. Moreover, 59.9% and 75.7% were inside Milan and Up-to-seven criteria, respectively. 67.2% outside Milan criteria and 59.6% outside Up-to-seven criteria had no vascular invasion. No difference in MELD score relative to vascular invasion was observed. The median follow-up was 23 months (range 0–289) and 94.9% had one transplantation. The majority of patients were transplanted after 2006 (90.3%). Three or more nodules were found in 19.3% and in 11.8%, the largest nodule was more than 50 mm.

Tumor characteristics and survival

As shown in Table 2, the five-year overall survival for being inside the Milan and Up-to-seven criteria were 75.0% and 73.3%, respectively. The more inclusive Up-to-seven criteria provided a correspondingly reduced survival (Fig. 1). Interestingly, patients without microvascular invasion, regardless of nodule size and number, had a five-year survival of 73.2%, comparable to that of the Up-to-seven criteria. Compared to patients without vascular invasion, patients with vascular invasion had a poorer prognosis with macrovascular invasion representing the worst (Fig. 2).

Assuming that microvascular invasion may not be known preoperatively, patients without macrovascular invasion (including those with microvascular invasion), regardless of nodule size and number, had a only marginally reduced survival of 70.7% compared with the Milan and Up-to-seven criteria (Table 2). Macrovascular invasion showed the highest HR for survival, with an acceptable prognosis for patients without invasion and a poor prognosis for patients with invasion (Table 2/Fig. 2). As shown in Table 2, a similar pattern for analyses with HCC specific survival was seen. Lastly, the analyses repeated without imputed data yielded comparable results.

Vascular invasion and survival in patients inside and outside Milan and Up-to-seven criteria

For all included patients, the five-year overall survival rates were 75.0%, 66.3% and 57.7% for within Milan criteria, within Up-to-seven criteria outside Milan criteria, and outside both, respectively. Interestingly, patients without vascular invasion who were outside both criteria still had a five-year overall survival of 65.8%, which was comparable to that of patients outside Milan within Up-to-seven criteria (Table 3/Fig. 3). In addition, patients without macrovascular invasion (including those with microvascular invasion) who were outside both criteria still had a five-year overall survival of 60.6%.

Regardless of vascular invasion, survival was lower for patients outside the two criteria compared with being inside. This suggested that other factors besides vascular invasion influence prognosis. However, being inside both criteria, the absence of vascular invasion only benefits marginally with respect to survival.

Similar patterns were found for the analyses with HCC specific survival (results not shown). In addition, analyses without imputed data yielded comparable results.

Discussion

The present study showed that vascular invasion is an important prognostic factor for patients liver transplanted for HCC. Patients without vascular invasion, regardless of size and number of nodules, had a survival comparable to that of patients within Milan and Up-to-seven criteria. In addition, patients outside these criteria still had a fair prognosis in the absence of vascular invasion, especially in the absence of microvascular invasion. Therefore, if microvascular invasion could be used to select patients, the ones with a good prognosis excluded by the conventional criteria, may be offered transplantation.

Previous studies support the findings of the present study, that vascular invasion is an important prognostic factor.[5,7–9,14] In a study of 479 patients, only microvascular invasion, macrovascular invasion and tumor grade remained significant in the

Table 1 Patients characteristics depending on vascular invasion

	Total	No vascular invasion	Macrovascular invasion	Microvascular invasion
Age (mean (SD))	56.8 (8.6)	56.8 (8.6)	56.5 (9.3)	56.6 (8.4)
Male gender (n)	6339 (85.2%)	5005 (85.0%)	196 (84.8%)	1138 (86.1%)
Transplanted after 2006 (n)	6720 (90.3%)	5337 (90.7%)	212 (91.8%)	1171 (88.5%)
Outside Milan criteria (n)	2894 (40.1%)	1946 (34.3%)	–	717 (54.8%)
Outside Up to seven criteria (n)	1717 (24.3%)	1023 (18.4%)	–	463 (35.8%)
> 3 nodules (n)	1540 (20.7%)	1030 (17.5%)	74 (32%)	436 (33%)
Max size of nodule > 50 mm (n)	760 (10.7%)	494 (8.9%)	67 (29%)	199 (15.4%)
MELD-score (mean (SD))	13.1 (6.1)	13.1 (6.0)	12.8 (5.8)	13.1 (6.1)
Total number of patients (n)	7439	5885 (79.1%)	231 (3.1%)	1323 (17.8%)

n: number of patients, SD: Standard deviation, MELD: Model for End-stage Liver Disease.

Table 2 Overall and HCC specific survival depending on Milan criteria, Up-to-seven criteria and vascular invasion

		Survival [95% CI]		Hazard ratio [95% CI]	Adjusted hazard ratio [95% CI]	HCC specific survival [95% CI]		Hazard ratio [95% CI]	Adjusted hazard ratio [95% CI]
		5 year	10 year	5 year survival	5 year survival	5 year	10 year	5 year HCC specific survival	5 year HCC specific survival
Milan criteria	Inside	75.0% [73.6–76.4]	60.0% [57.1–62.9]	1.64 [1.50–1.80]	1.54 [1.48–1.60]	95.2% [94.4–96.0]	92.4% [90.6–94.2]	2.76 [2.23–3.41]	2.74 [2.50–3.00]
	Outside	60.8% [58.6–63.0]	47.9% [44.6–51.2]			88.5% [86.8–90.2]	85.6% [83.4–87.8]		
Up to seven criteria	Inside	73.3% [71.9–74.7]	58.9% [56.4–61.4]	1.72 [1.66–1.80]	1.61 [1.54–1.67]	94.8% [94.0–95.6]	92.4% [90.4–94.0]	3.09 [2.51–3.79]	2.46 [2.24–2.70]
	Outside	57.7% [55.0–60.4]	43.8% [39.3–48.3]			85.8% [83.6–88.0]	81.6% [78.5–84.7]		
Macrovascular invasion	No	70.7% [71.9–69.5]	56.7% [54.5–58.9]	2.52 [2.34–2.72]	2.25 [2.08–2.43]	93.2% [92.4–94.0]	90.6% [89.2–92.0]	4.81 [3.49–6.64]	4.03 [3.50–4.64]
	Yes	39.6% [32.5–46.7]	12.2% [0.0–24.9]			74.6% [67.3–81.9]	62.4% [47.3–77.5]		
Microvascular invasion	No	73.2% [71.8–74.6]	59.2% [56.6–61.8]	1.60 [1.44–1.77]	1.53 [1.46–1.60]	94.8% [94.0–95.6]	91.9% [90.3–93.5]	2.92 [2.33–3.66]	2.58 [2.34–2.85]
	Yes	58.8% [55.7–61.9]	46.2% [41.5–50.9]			86.3% [83.9–88.7]	84.8% [82.1–87.5]		

95% CI: 95% confidence interval.
Adjusted analyses included the variables: gender, age, center, time on waiting list, number of nodules, size of largest nodule, vascular invasion (micro, macro or none), and MELD-score.
Variables already included in exposure variable (e.g. size and number for Milan criteria) were excluded.

multivariate model including number of nodules, size of largest nodule and sum of nodule diameters.[5] In addition, Mazzaferro *et al*. showed that patients outside the Up-to-seven criteria had a five-year survival of 64% without microvascular invasion and only 33% with microvascular invasion.[10] Compared to the present study, the prognosis for patients with microvascular invasion was even worse, but similar for those without microvascular invasion.

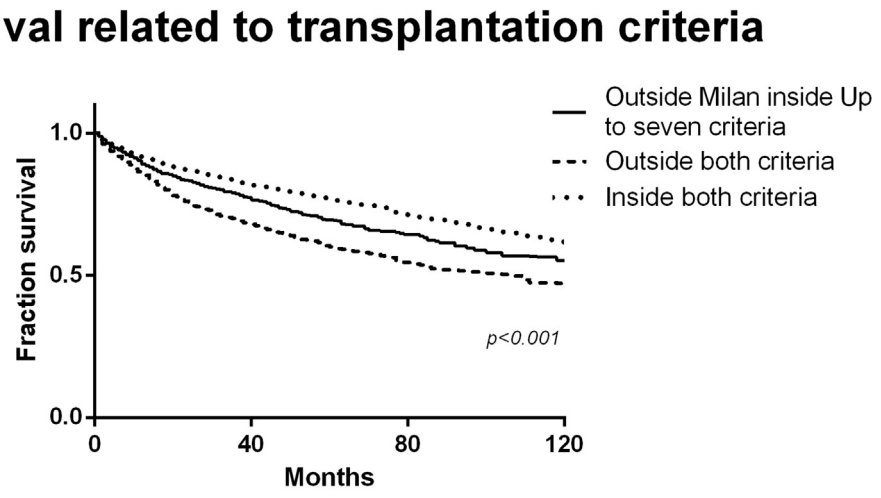
To our knowledge, this study is the largest study to date on vascular invasion in patients liver transplanted for HCC and cirrhosis with many transplantation centers involved. Since the Milan criteria have been generally accepted in Europe, it may be presumed that the majority of patients were comparable and selected based on the same criteria. With a wide inclusion of patients, the results reflect a true picture of general clinical practice of liver transplantation for HCC in Europe.

The results were based on explant pathology data, which does not necessarily compare well to pre-operative imaging. Thus, the results cannot be directly implemented into clinical practice and cannot be used to select patients for transplantation. Based on a study from 2006, preoperative CT may under-stage patients in as much as 43% of cases.[11] However, primarily due to under-staging of size and number of nodules and rarely due to missed macrovascular invasion. In fact, only 5.1% of patients were under-staged due to macrovascular invasion not seen pre-operatively.[11] Patients with macrovascular invasion (3.1%) in the present study may represent under-staging since macrovascular invasion is generally considered an absolute contraindication for transplantation.[3] For some patients, disease may have progressed since the last CT-scan, stressing the importance of repeat CT-scan before transplantation, especially for patients with rapid growing tumors. However, it cannot be excluded that some patient, especially younger, may have underwent transplantation despite being outside criteria. In addition, there was no general definition of micro and macrovascular invasion, which therefore may differ between centers,[16] which is a further limitation of the study. The clinical practice may have changed over the relatively long study period, which may have influenced the results. In addition, data was based on the patients actually transplanted. Thus, the results may not apply to all patients evaluated for transplantation. Included patients are likely to have less severe disease, since transplantation criteria have already been applied. Furthermore, data on cause of death, which form the basis of the outcome HCC specific survival rate may suffer from reporting bias, and therefore may underestimate the rate. There was a substantial amount of missing data primarily due to that data on explant pathology were not incorporated into the ELTR questionnaire until 2007–2008. In fact, only 41.3% of patients in ELTR had any data on pathology. In addition, data on vascular invasion were missing for 20% of the included patients. Thus, the investigated patients may represent a selected sample. Lastly, as in all observational studies, there is a risk of confounding in which case vascular invasion in itself may not be causal factor. However,

Table 3 Overall survival for inside and outside criteria depending on vascular invasion

	5 year survival [95% CI]			
	Inside both (n=5662)	**Outside Milan inside up to seven (n=1319)**	**Outside both (n=2317)**	**All patients (n=9298)**
Macrovascular invasion	–	–	39.6% [32.5–46.7]	39.6% [32.5–46.7]
No macrovascular invasion[a]	74.1% [72.3–75.9]	66.3% [63.0–69.6]	60.6% [57.9–63.3]	70.7% [69.5–71.9]
Microvascular invasion	69.0% [64.9–73.1]	51.2% [43.2–59.2]	47.8% [42.3–53.3]	58.8% [55.7–61.9]
No vascular invasion	76.3% [74.7–77.9]	70.5% [67.0–74.0]	65.8% [62.5–69.1]	73.2% [71.8–74.6]
All patients	75.0% [73.6–76.4]	66.3% [63.0–69.6]	57.7% [55.0–60.4]	69.4% [68.2–70.6]

95% CI: 95% confidence interval.
[a] Includes patients with microvascular invasion and patients without vascular invasion.

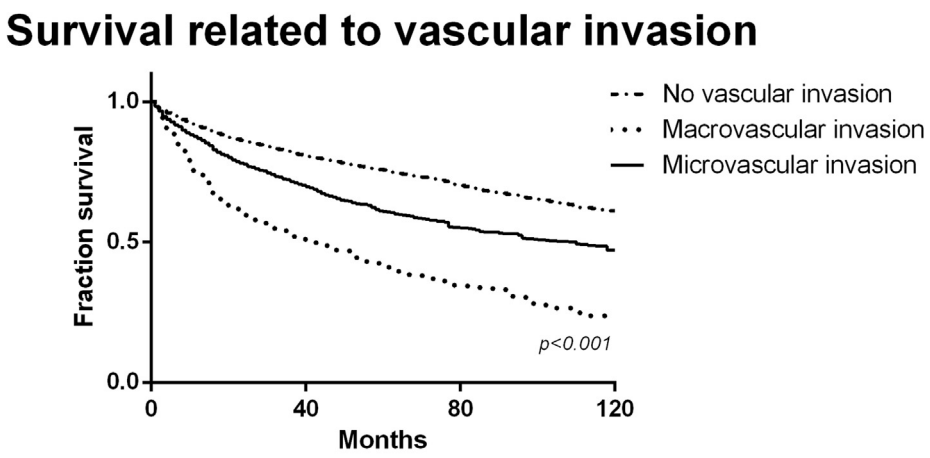


Follow-up time		40 months	80 months	120 months
Outside Milan inside Up to seven	*Numbers at risk*	473	186	58
	Survival (%)	73.5	61.8	55.0
Outside both	*Numbers at risk*	688	251	54
	Survival (%)	64.9	51.7	43.8
Inside both	*Numbers at risk*	2026	783	193
	Survival (%)	79.1	69.2	60.0

Figure 1 Survival related to transplantation criteria

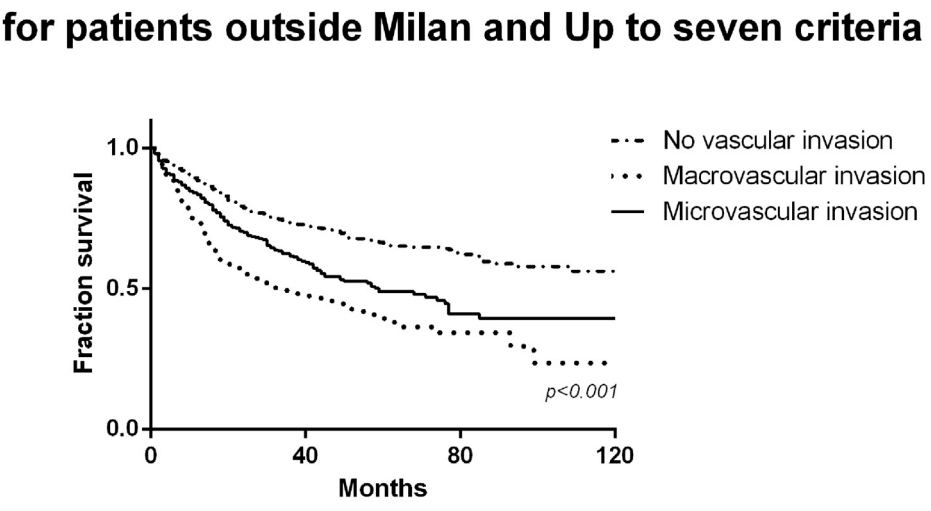
the estimates for vascular invasion did not change in the adjusted analyses and there were comparable MELD-scores between the groups with and without vascular invasion. Therefore, it may be assumed that the groups were comparable and the increased mortality observed related to vascular invasion was due to a difference in tumor aggressiveness. This was supported by comparable findings between overall survival and HCC specific survival estimates.

With novel imaging techniques, preoperative identification of features representing microvascular invasion may allow even better selection of patients. In addition, possible under-staging will likely be less common with contemporary imaging with increasing diagnostic accuracy.[17–19] Using 18 F-FDG PET, microvascular invasion may be accurately predicted preoperatively.[12] Patients with no signs of microvascular invasion based on preoperative scanning with advanced tumors outside Milan criteria had comparable survival to patients inside to Milan criteria. This modality had high positive and negative predicting values to identify microvascular invasion in pathology data. In addition, a non-invasive CT-based radiogenomic marker accurately predicted microvascular invasion and was highly associated with survival.[13] In fact, patients within Milan criteria and radiogenomic signs of microvascular invasion had a median overall survival of 69 months compared with >147 months in patients within Milan criteria and no signs of microvascular invasion. Therefore, should a patient outside criteria be offered transplantation, we believe there should no indirect signs of microvascular invasion using these two modalities. However,



Follow-up time		40 months	80 months	120 months
No vascular invasion	*Numbers at risk*	2558	1006	242
	Survival (%)	77.7	67.9	59.2
Macrovascular invasion	*Numbers at risk*	80	23	2
	Survival (%)	48.7	30.0	12.2
Microvascular invasion	*Numbers at risk*	549	192	43
	Survival (%)	67.2	52.8	46.2

Figure 2 Survival related to vascular invasion



Follow-up time		40 months	80 months	120 months
No vascular invasion	*Numbers at risk*	454	181	48
	Survival (%)	71.5	61.2	52.7
Macrovascular invasion	*Numbers at risk*	80	23	2
	Survival (%)	48.7	30.0	12.2
Microvascular invasion	*Numbers at risk*	154	51	39
	Survival (%)	57.2	41.3	40.2

Figure 3 Survival for patients outside Milan and Up to seven criteria

these results need confirmation in prospective studies before implementing them into clinical practice.

In addition to preoperative imaging, alpha-fetoprotein (AFP) may be used as a prognostic variable and is correlated with vascular invasion.[20–23] When used in conjunction with size and number of HCC nodules, AFP improved selection of patients compared with the Milan criteria in a study with validated results.[22] Patients outside Milan criteria with a low AFP had a lower risk of recurrence and may therefore be eligible for transplantation. Thus, AFP, size and number of nodules together with other biomarkers may be used with novel imaging techniques to predicts microvascular invasion and improve future selection of patients for transplantation of HCC. Unfortunately, AFP was not registered in the ELTR. Therefore, the prognostic value of AFP and correlation to vascular invasion could not be investigated in the present study. Additional biomarkers such as des-gamma-carboxy prothrombin (DCP) and allelic imbalance in microsatellites may also be considered. However, these are less extensively investigated compared with AFP.[23]

Furthermore, locoregional treatment while on the waiting list for transplantation, such as radiofrequency ablation or transarterial chemoembolization, was not investigated in the present study. However, response to such treatment may be a surrogate marker of tumor aggressiveness.[24,25] Thus, a good response leads to improved recurrence free survival.[24] Therefore, response to locoregional treatment may, in addition to microvascular invasion, be used to select patients for transplantation.[25]

Some have suggested the use of preoperative biopsy to obtain the HCC diagnosis.[26] In addition, preoperative biopsy may allow for pathological classification of tumor aggressiveness. However, preoperative biopsy is discouraged by most due risk of tumor seeding in approximately 3% of the cases.[27] Tumor biopsy may be relevant especially for small lesions (<2 cm) where imaging may be inconclusive.[28] However, for these lesions the false negative rate for tumor biopsy is 30%.[29] Moreover, tumor biopsy has a sensitivity of only 12.5% for identification of microvascular invasion.[13] Thus, the use of tumor biopsy does not seems to be a valuable option for preoperative determination of prognostic factors. In addition, with current transplantation criteria preoperative biopsy may have limited clinical consequences.

In conclusion, this study showed that vascular invasion is an important prognostic factor for patients liver transplanted for HCC. With continuously improving imaging studies, microvascular invasion may be used to together with others factors to select patients for transplantation in the future.

Acknowledgements

We thank the ELTR for supplying data for the present study. Thanks to all the centers who contribute to the ELTR. The Organ Sharing Organizations the French ABM (Sami Djabbour and Alain Jolly), the Dutch NTS (Cynthia Konijn), the Eurotransplant Foundation (Marieke Van Meel and Erwin de Vries), the Spanish ONT (Gloria de la Rosa), and the UK–Ireland NHSBT (Mike Chilton and Julia Micciche) are acknowledged for the data cross-check and sharing with the ELTR.

Disclosure/Funding

ELTR received financial support from Astellas Pharma Europe Ltd., Novartis Pharma S.A.S., Institut Georges Lopez S.A.S. and ELITA (European Liver and Intestine Transplant Association). The authors of this manuscript have no conflicts of interest to disclose.

Conflict of interest

None declared.

References

1. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F *et al*. (1996) Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 334: 693–699.
2. Mazzaferro V, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R *et al*. (2011) Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 17(Suppl. 2):S44–S57.
3. Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A *et al*. (2012) Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 13:e11–22.
4. Zavaglia C, De Carlis L, Alberti AB, Minola E, Belli LS, Slim AO *et al*. (2005) Predictors of long-term survival after liver transplantation for hepatocellular carcinoma. *Am J Gastroenterol* 100:2708–2716.
5. D'Amico F, Schwartz M, Vitale A, Tabrizian P, Roayaie S, Thung S *et al*. (2009) Predicting recurrence after liver transplantation in patients with hepatocellular carcinoma exceeding the up-to-seven criteria. *Liver Transplant* 15:1278–1287.
6. Lohe F, Angele MK, Rentsch M, Graeb C, Gerbes A, Lohrs U *et al*. (2007) Multifocal manifestation does not affect vascular invasion of hepatocellular carcinoma: implications for patient selection in liver transplantation. *Clin Transplant* 21:696–701.
7. Parfitt JR, Marotta P, Alghamdi M, Wall W, Khakhar A, Suskin NG *et al*. (2007) Recurrent hepatocellular carcinoma after transplantation: use of a pathological score on explanted livers to predict recurrence. *Liver Transplant* 13:543–551.
8. Shah SA, Tan JC, McGilvray ID, Cattral MS, Levy GA, Greig PD *et al*. (2007) Does microvascular invasion affect outcomes after liver transplantation for HCC? A histopathological analysis of 155 consecutive explants. *J Gastrointest Surg* 11:464–471.
9. Iguchi T, Shirabe K, Aishima S, Wang H, Fujita N, Ninomiya M *et al*. (2015) New pathologic stratification of microvascular invasion in hepatocellular carcinoma: predicting prognosis after living-donor liver transplantation. *Transplantation* 99:1236–1242.
10. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L *et al*. (2009) Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 10:35–43.
11. Shah SA, Tan JC, McGilvray ID, Cattral MS, Cleary SP, Levy GA *et al*. (2006) Accuracy of staging as a predictor for recurrence after liver transplantation for hepatocellular carcinoma. *Transplantation* 81: 1633–1639.
12. Kornberg A, Freesmeyer M, Barthel E, Jandt K, Katenkamp K, Steenbeck J *et al*. (2009) 18F-FDG-uptake of hepatocellular carcinoma on PET predicts microvascular tumor invasion in liver transplant patients. *Am J Transplant* 9:592–600.

13. Banerjee S, Wang DS, Kim HJ, Sirlin CB, Chan MG, Korn RL *et al*. (2015) A computed tomography radiogenomic biomarker predicts microvascular invasion and clinical outcomes in hepatocellular carcinoma. *Hepatology* 62:792–800.
14. Klintmalm GB. (1998) Liver transplantation for hepatocellular carcinoma: a registry report of the impact of tumor characteristics on outcome. *Ann Surg* 228:479–490.
15. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP *et al*. (2008) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 61:344–349.
16. Rodriguez-Peralvarez M, Luong TV, Andreana L, Meyer T, Dhillon AP, Burroughs AK. (2013) A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. *Ann Surg Oncol* 20:325–339.
17. Sun H, Song T. (2015) Hepatocellular carcinoma: advances in diagnostic imaging. *Drug Discov Ther* 9:310–318.
18. Sahani D, Saini S, Pena C, Nichols S, Prasad SR, Hahn PF *et al*. (2002) Using multidetector CT for preoperative vascular evaluation of liver neoplasms: technique and results. *AJR Am J Roentgenol* 179:53–59.
19. Choi BI, Lee JM. (2010) Advancement in HCC imaging: diagnosis, staging and treatment efficacy assessments: imaging diagnosis and staging of hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci* 17: 369–373.
20. Lai Q, Nicolini D, Inostroza Nunez M, Iesari S, Goffette P, Agostini A *et al*. (2016) A novel prognostic index in patients with hepatocellular cancer waiting for liver transplantation: time-Radiological-response-Alpha-fetoprotein-INflammation (TRAIN) score. *Ann Surg* 264:787–796.
21. Fujioka M, Nakashima Y, Nakashima O, Kojiro M. (2001) Immunohistologic study on the expressions of alpha-fetoprotein and protein induced by vitamin K absence or antagonist II in surgically resected small hepatocellular carcinoma. *Hepatology* 34:1128–1134.
22. Duvoux C, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T *et al*. (2012) Liver transplantation for hepatocellular carcinoma: a model including alpha-fetoprotein improves the performance of Milan criteria. *Gastroenterology* 143:e914–985, 986-994 e983; quiz.
23. Pommergaard HC, Burcharth J, Rosenberg J, Rasmussen A. (2016) Serologic and molecular biomarkers for recurrence of hepatocellular carcinoma after liver transplantation: a systematic review and meta-analysis. *Transplant Rev (Orlando)*. 30:171–177.
24. Otto G, Herber S, Heise M, Lohse AW, Monch C, Bittinger F *et al*. (2006) Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transplant* 12:1260–1267.
25. Mazzaferro V. (2016) Squaring the circle of selection and allocation in liver transplantation for HCC: an adaptive approach. *Hepatology* 63: 1707–1717.
26. Wang P, Meng ZQ, Chen Z, Lin JH, Ping B, Wang LF *et al*. (2008) Diagnostic value and complications of fine needle aspiration for primary liver cancer and its influence on the treatment outcome-a study based on 3011 patients in China. *Eur J Surg Oncol* 34:541–546.
27. Silva MA, Hegab B, Hyde C, Guo B, Buckels JA, Mirza DF. (2008) Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut* 57: 1592–1596.
28. Bolondi L, Gaiani S, Celli N, Golfieri R, Grigioni WF, Leoni S *et al*. (2005) Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. *Hepatology* 42:27–34.
29. Forner A, Vilana R, Ayuso C, Bianchi L, Sole M, Ayuso JR *et al*. (2008) Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology* 47:97–104.