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LIVER TRANSPLANTATION FOR "VERY EARLY" INTRAHEPATIC CHOLANGIOCARCINOMA. INTERNATIONAL RETROSPECTIVE STUDY SUPPORTING A PROSPECTIVE ASSESSMENT

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List of abbreviations:

iCCA Intrahepatic Cholangiocarcinoma
HCC Hepatocellular Carcinoma
LT Liver transplantation
AFP Alpha-fetoprotein
WL Waiting list
TACE Transarterial chemoembolization

RFA   Radiofrequency ablation
PEI   Percutaneous ethanol injection
PSC   Primary sclerosing cholangitis
PBC   Primary biliary cirrhosis
AIH   Autoimmune hepatitis

ABSTRACT

The presence of an intrahepatic cholangiocarcinoma (iCCA) on a cirrhotic liver is a contraindication for liver transplantation (LT) in most centers worldwide. Recent investigations have shown that *"very early"* iCCA (single tumors ≤2 cm) may have acceptable results after LT. This study further evaluates this finding in a larger international multicentre cohort. The study group was composed of those patients that were transplanted for hepatocellular carcinoma (HCC) or decompensated cirrhosis and were found to have an iCCA at explant pathology. Patients were divided in those with *"very early"* iCCA and those with *"advanced"* disease (single tumor >2 cm or multifocal disease). Between January 2000 and December 2013, 81 patients were found to have an iCCA at explant; 33 had separate nodules of iCCA and HCC and 48 had only iCCA (study group). Within the study group, 15/48 (31%) constituted the *"very early"* iCCA group and 33/48 (69%) the *"advanced"* group. There were no significant differences between groups in the preoperative characteristics. At explant, the median size of the largest tumor was larger in the *"advanced"* group [3.1 (2.5 − 4.4) vs. 1.6 (1.5 − 1.8)]. After a median follow-up of 35 (13.5 − 76.4) months, the 1-, 3- and 5-years cumulative risk of recurrence was 7%, 18% and 18% in the *very early iCCA* group vs. 30%, 47% and 61% in the *advanced iCCA group*, p=0.01. The 1-, 3- and 5-years actuarial survival was 93%, 84% and 65% in the *very early iCCA* group vs. 79%, 50% and 45% in the *advanced iCCA group,* p=0.02. Conclusion: Cirrhotic patients with *very early* iCCA may become

candidates for LT. A prospective multicenter clinical trial is needed to further confirm these results.

INTRODUCTION

Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary tumor of the liver (1). The incidence of this disease in cirrhotic patients is growing and the medical community is now recognizing iCCA in patients with liver disease more often (2-3). Hepatocellular carcinoma (HCC) is the most frequent liver cancer and liver transplantation (LT) is the best treatment option in selected patients with early disease, as it removes both the cancer and the underlying main risk factor (cirrhotic liver) for the development of new tumors (4). Initial results of LT for HCC were dismal, but after refinements in the selection criteria, the results have become more than acceptable (5).

On the other hand, iCCA is a formal contraindication for LT in most transplant centers worldwide due to historical very poor results (6-7). Contrary to what has happened with HCC, few studies have attempted to select cirrhotic patients with iCCA for LT. In a recent multicenter study from Spain we were able to demonstrate – with a limited cohort of patients – that results of LT for patients with *very early* iCCA (single tumors ≤2 cm) at explant pathology examination could be acceptable (5-year survival 73%) (8-9). The main problems with that study were that the number of patients was limited and certainly needed further validation and confirmation by another and/or a larger cohort of patients.

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Therefore, the aim of this multicenter international study was to ascertain if a subgroup of LT recipients with and iCCA diagnosis at pathological examination of the explant present an acceptable survival after LT.

MATERIAL AND METHODS

Ethics approval for this study was obtained from the Research Ethics Board of the different participating centers.

Study design

A retrospective cohort international multicenter study was designed and 17 major transplant centers accepted to participate. All patients included in the study should have been diagnosed with cirrhosis and received a first liver transplant. The indication for LT could have been HCC suspicion, or end stage liver disease without pretransplant radiologic recognition of a hepatic nodule; incidental tumors detected only on pathology examination were also included in the present study. The study group was composed of patients found to have only iCCA at the explant (iCCA group) and those with an iCCA and an HCC (in different nodules) at the explant (iCCA + HCC group). Patients with mixed iCCA + HCC (in the same nodule) were excluded from the current study. Patients in each group were also classified according to the explant pathology characteristics of the tumors: *very early iCCA group* (single tumor ≤2 cm) and *advanced iCCA group* (single tumor >2 cm or multiple tumors).

Preoperative Tumor Biomarkers

All patients had alpha fetoprotein (AFP) values at the time of tumor diagnosis and prior to LT. Not all centers collected CA19.9 data in these patients so it was registered when it was available.

Incidental Tumors

Patients transplanted without pretransplant radiologic recognition of a hepatic nodule and found to have an iCCA at explant pathology examination were considered as incidentals. Patients suspected to have an HCC, but diagnosed with iCCA at pathology examination were registered as non incidentals.

Preoperative Tumor Treatment

Patients with an expected waiting list (WL) time of over 6 months could have been treated with transarterial chemoembolization (TACE), radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI) (4) as a bridge to LT. Treatment was not an exclusion criteria.

Liver Transplantation Criteria

In most transplant programs, enlistment for transplantation is limited to patients within the Milan criteria (5). However, at some centers the criteria are expanded to UCSF or other definitions (10). As the present study was an exploratory analysis, 4 patients beyond UCSF criteria were also included in the study cohort.

Histopathologic Analysis

Due to the retrospective nature of the study each center had their own pathologist analyzing the explant. This fact may lead to a bias, as the number and size of tumors may have been understaged. To confirm the iCCA component diagnosis, all centers were required to submit images of the microscopic evaluation of the tumor together with the

rest of the data. An independent pathologist with more than 10 years in liver pathology at the University of Genève (LRB) had to confirm that all tumors were iCCA. Since retrieval of slides for independent validation was not feasible in all cases, we performed a secondary analysis including only those patients in whom the iCCA diagnosis was confirmed. The diagnosis of iCCA was based on morphology according to standard criteria (11). Tumor characteristics in liver explants, such as tumor size, number of tumors, grade of differentiation (11), presence of satellite lesions and presence or absence of vascular invasion, were retrospectively retrieved.

Postoperative Surveillance

All patients included in the study were followed up according to each center protocol. Once tumor recurrence was diagnosed, complete tumor staging was performed and treatment decision individualized.

Statistical Analysis

Data were expressed as means and standard deviation or as median and interquartile range when a non normal distribution of data was identified. Students´ t test was used for numerical variables. A nonparametric test (Mann Whitney U) was used for numerical variables when an abnormal distribution was identified. Chi square test with Fisher's correction was employed for categorical variables. Patient survival rates were estimated with the Kaplan Meier method and compared with the log rank test. All variables were dichotomized to perform a uni and multivariate analysis of recurrence using Cox regression. Uni and multivariate analysis was performed as an exploratory analysis and only on variables with clinical significance. Variables with p value of <0.20 in univariate analysis were tested in the multivariate Cox proportional hazard model to

select prognostic variables using a step by step approach. Only cases were all data was available were included in the model. Statistical analyses were performed with SPSS 20.0 (SPSS, Inc., Chicago, IL). A p value <0.05 was considered statistically significant. All data were prospectively collected and retrospectively analyzed. The median follow up was 35 (13.5 – 76.4) months. Follow up was carried out until July 2015.

RESULTS

Study population

Between January 2000 and December 2013, 25.016 LT were performed at the 17 transplant centers participating in the current study. Diagnosis at the time of transplant indication included HCC, or suspicion of it, in 7503 (30%). Sixty five of these patients (0.9%) were diagnosed of an iCCA on pathology examination. Additionally, during the same study period, 16 patients were found to have an incidental iCCA on pathology study. Finally, the study group comprised 81 patients who were found to have an iCCA on pathology examination. Of these, 48 had only iCCA at pathology explant and comprised the iCCA group, while 33 had both iCCA and an HCC at the explant and comprised the iCCA + HCC group.

iCCA Group

Patients in the iCCA group were divided according to the pathological characteristics of the tumors at the explant in *very early iCCA group* (single tumors ≤2 cm) (15/48, 31.3%) and the *advanced iCCA group* (single tumors >2 cm or multiple tumors) (33/48, 68.7%).

Preoperative characteristics of patients

All data on preoperative characteristics are summarized in Table 1. There were no differences in the baseline characteristics between both groups. All patients in the *very early iCCA group* and 80% in the *advanced iCCA group* had preoperative tumors within Milan criteria. Most patients received treatment as a bridge to transplant. CA19.9 level was available in only 13 patients with a median value of 54 ng/mL (18.9 – 169.4).

Explant pathologic findings

All pathologic findings analyzed are described in Table 1. Seven patients (46.7%) in the *very early iCCA group* and 8 (24.2%) patients in the *advanced iCCA group* were transplanted for decompensated cirrhosis and were found to have an incidental tumor at explant pathology. Although it did not reach statistical significance, microvascular invasion was observed more frequently in the *advanced iCCA* group. Tumor necrosis was found more frequently in the *advanced iCCA group* even though there was no statistical significance. Twenty patients (60.6%) in the *advanced iCCA group* had tumors that fulfilled the Milan criteria at pathology and 30.3% (10 patients) were outside UCSF criteria.

Patient Outcomes

Tumor recurrence (Table 1)

The median follow up was 57.3 (23.4 – 104.5) months for the *very early iCCA* group and 24.7 (12.7 – 63.4) months for the *advanced iCCA group*, p=0.04. During follow up, tumor recurrence was observed in 2/15 patients (13.3%) in the *very early iCCA* group vs. 18/33 (54.5%) in the *advanced iCCA group*, p=0.006. The median time to recurrence in the *advanced iCCA group* was 10.5 (4.2 – 22.6) months while the 2 patients that recurred in the *very early iCCA* group did so at 5.8 and 31 months post transplant. One , 3 and 5

year cumulative risk of recurrence was 7%, 18% and 18% in the *very early iCCA* group vs. 30%, 47% and 61% in the *advanced iCCA group*, p=0.01 (Figure 1).

Tumor differentiation was available in 36/48 (75%) of the patients. Among the patients in which tumor differentiation was not available, recurrence was diagnosed in 33.3% of the cases. There were 4 patients within the *very early iCCA* group that did not have an available tumor differentiation but none presented tumor recurrence. Among the 36 patients with iCCA and available tumor differentiation, tumor recurrence increased with worst tumor differentiation (28.6% in well differentiated vs. 39.1% in moderately differentiated and 83.3% in poorly differentiated) even though it did not reach statistical significance (p=0.2). Within the a*dvanced iCCA* group 5 patients had well differentiated tumors and 2/5 presented recurrence.

Survival

At the end of follow up 5/15 (33.3%) of the patients in the *very early iCCA* group had died vs. 20/33 (60.6%) of the patients in the *advanced iCCA group*, p=0.08. The 2 patients with recurrence in the *very early iCCA* group died; the other 3 patients died of cerebrovascular accidents (n=2) and respiratory complications (n=1). Within the *advanced iCCA group,* most patients died due to tumor recurrence. The 1 , 3 and 5 years actuarial survival was 93%, 84% and 65% in the *very early iCCA* group vs. 79%, 50% and 45% in the *advanced iCCA group,* p=0.02 (Figure 2).

Incidental Tumors

More patients (46.7%) had been transplanted without pretransplant radiologic recognition of a suspicious hepatic nodule in the *very early iCCA* group in contrast with 25% in the *advanced iCCA* group. One of the recurrences in the very early iCCA group was

incidental (recurred at month 32 after LT), while the other was not. The 1 , 3 and 5 year

actuarial survival was 100%, 100% and 80% in the incidentals vs. 88%, 70% and 53% in

the non incidentals, p=0.1. Similarly, the incidence of tumor recurrence was also similar

between groups (incidentals versus non incidentals) within the *advanced iCCA* group

(62.5% vs. 54.2%, p=0.7) as was the 1 , 3 and 5 year actuarial survival (63%, 38% and

38% vs. 83%, 52% and 46%, p=0.4).

Factors associated with tumor recurrence

Table 3 exposes the factors that were associated with tumor recurrence within the iCCA

group. In the univariate analysis the risk of tumor recurrence in those patients with

advanced iCCA was 5.2 folds higher than in patients with *very early* iCCA. Factors

associated with tumor recurrence at multivariate analysis were the presence of

microvascular invasion and poor differentiation of the tumor. As tumor differentiation

appeared as a strong predictor of outcome, we analyzed the actuarial survival of those

patients within the *advanced iCCA* group according to tumor differentiation. Those

patients without a poorly differentiated tumor had a 5 year survival of 45% compared to

17% of those with poorly differentiated tumors, p=0.2.

Subgroup analysis of patients with advanced iCCA

Taking into account the results of our multivariate analysis we decided to perform a sub

analysis of patients with not poorly differentiated tumors ≤3 cm. Patients in the *advanced*

iCCA group were divided into an intermediate stage (n=6) (single tumors 2.1   3 cm, not

poorly differentiated) and advanced stage (n=27) (all other tumors). Both groups were

compared to the *very early* iCCA group. The 1 , 3 and 5 year actuarial survival was

82%, 61% and 61% in the intermediate stage compared to 55%, 47% and 42% in the advanced stage, p=0.03 (Supplemental Figure).

iCCA + HCC Group

Within those patients that were diagnosed of both an iCCA and a HCC at the explant pathology (n=33), patients were divided in those in which the iCCA was *very early* (single ≤2 cm) (14/33, 42.4%) and those in which the iCCA was *advanced* (single tumor >2 cm or multiple tumors) (19/33, 57.6%). There were no significant differences in the explant pathological findings regarding the HCC at the explant (Table 2). The recurrence rate was higher in the *advanced* iCCA and HCC group. The 1-, 3- and 5- year cumulative risk of recurrence was 7%, 23% and 23% in the *very early* iCCA and HCC group vs. 32%, 61% and 61% in the *advanced* iCCA and HCC group, p=0.04 (Figure 3a). The 1-, 3- and 5-years actuarial survival was 93%, 84% and 84% in the *very early* iCCA and HCC group vs. 78%, 49% and 43% in the *advanced* iCCA and HCC group, p=0.04 (Figure 3b). Within these patients, the cause of death was tumor recurrence in all cases.

Explant pathology external review

All centers were asked to submit photographs of their slides in which an iCCA component could be identified. Finally we were able to collect the slides from 55/81 patients (67.9%). The reason for not being able to submit the remaining slides varied but in most cases was due to impossibility of the different centers' for accessing the slides. Of these 55 patients, an iCCA was identified in all cases except one, in which the external review classified the tumor as an HCC. Of the 54 patients, 34 (63%) were within the iCCA group and 20 (37%) within the iCCA + HCC group.

iCCA group (n=34)

Within these patients, 13 had a *very early* iCCA and 21 an *advanced* iCCA. The median follow up was 36.4 (22.6 – 90.8) months for the *very early* iCCA group vs. 16.1 (7.5 – 40.3) months for the *advanced* iCCA group, p=0.01. The 1 , 3 and 5 year actuarial survival was 92%, 81% and 58% for the *very early* iCCA group and 71%, 34% and 34% for the *advanced* iCCA group, p=0.01. Significant differences were also observed in the cumulative risk of recurrence, 8%, 12% and 12% in the *very early* iCCA group vs. 33%, 60% and 77% in the *advanced* iCCA group, p=0.006.

iCCA + HCC group (n=20)

Within these patients, 8 had a *very early* iCCA and 12 an *advanced* iCCA. There were no statistical significant differences in the 1 , 3 and 5 year actuarial survival between the *very early* iCCA group and the *advanced* iCCA group, even though it was worst in the latter – 88%, 73% and 73% vs. 91%, 53% and 42%, respectively, p=0.3. There were also no statistical significant differences in the cumulative risk of malignancy recurrence (it could be either iCCA or HCC), 12%, 37% and 37% in the *very early* iCCA group vs. 27%, 63% and 73% in the *advanced* iCCA group, p=0.3.

DISCUSSION

This large multicenter international retrospective study in a cohort of patients from 17 large institutions worldwide has confirmed that patients who are diagnosed of a *very early* iCCA at explant pathology after LT have an acceptable 5 year survival and a low recurrence rate. These results have an important impact on considering cirrhotic patients with *very early* iCCA as candidates for LT.

The present study was able to identify 81 patients that were found to have an iCCA at pathological explant after LT. Most of these patients had lesions that were

misdiagnosed – in the preoperative setting – as being HCC, but some patients were transplanted for decompensated cirrhosis and the tumors were incidentally found. We were able to identify 48 patients that had only iCCA at explant, representing by far, the largest cohort of patients with this characteristics published to date (6, 8, 12 16). Also, we have been able to almost duplicate the previous number of patients in the *very early* iCCA group from the previous Spanish multicenter experience (8), adding value to the current work.

Our results demonstrate that the recurrence rate of patients with *very early iCCA* is much lower than that of patients with *advanced iCCA*. Indeed, a 13% recurrence rate is within the standards that are recommended for patients transplanted for malignancies (mostly HCC) (5, 17). Compared to a previous multicenter Spanish study (8) the recurrence rate is higher as in that study patients experienced no recurrence. This is probably a random effect because of the reduced sample size in the prior study and the fact that whatever the stage at transplant, recurrence will never be zero. Also, if we would sum both cohorts the recurrence rate for patients with *very early* iCCA would be around 9%.

The most salient result of the present study is the confirmation that that patients in the *"very early"* iCCA group can benefit from a survival that exceeds 60%. Compared to the results of the previous multicenter study, the 5 year survival is lower in the present series (65% vs. 73%) but still an acceptable survival for LT (17 18). This result argues in favour of considering patients with these tumors for LT. It is important to realize that the cohort of patients analyzed would not likely have been eligible for any other curative treatment in case of confident diagnosis prior to LT. iCCA patients are excluded from LT

in most centers, and surgical resection and ablation have a limited applicability and efficacy. In the current study there were 3 patients in the *very early* iCCA group that died from causes unrelated with the tumor; accounting for the decrease in the 5 year survival. It is important to emphasize though, that the number of patients at risk is small when analyzing the 5 year survival and therefore, it is prudent to state that these patients achieved an encouraging 3 and 5 year survival. Certainly larger studies and/or prospective investigations will be needed to confirm these findings.

When compared to other treatment options for iCCA the results of LT are better. The 5 year survival after liver resection, the best accepted treatment option for patients with iCCA, range from 15% to 45% (19 21). These studies did not analyze specifically patients with *very early* iCCA. On the other hand a recent study from Japan, showed that resection in patients with *very early* iCCA had a 5 year survival rate of 82% reinforcing the concept of "good outcomes" in patients with initial stages of iCCA (22). In the present series, although the median MELD scores were low, the different participating centers decided to include the patients in the waiting list for LT presumably due to impossibility of offering liver resection (tumor location and/or portal hypertension). Certainly, if a cirrhotic patient is diagnosed of an iCCA and there is no contraindication for a liver resection, the first treatment option would be to offer resection (23). Other treatment options like RFA may be adequate for patients with these lesions and some small studies have shown a 5 year survival rate between 15 30%, lower than that achieved in our series (24 26). Nevertheless, there is no recommendation on the use of RFA in cirrhotic patients with iCCA and further research studying the effects of these treatments on cholangiocarcinoma need to be undertaken (23).

In the present study we decided to include patients with iCCA and HCC in separate nodules, but not include those patients with pure mixed HCC CC (Goodman type II) (27). This decision was made because patients with HCC CC will be difficult to diagnose preoperatively (even with a biopsy). Moreover, in a previous study (with a small sample of patients) the results of LT in patients diagnosed with mixed HCC CC at the explant were more than acceptable (5 year survival, 78%) (8). On the other hand, others have shown worst results with these tumors (14, 16). Those patients with HCC and a concomitant *very early* iCCA had a better 5 year survival (84%) than those in which the concomitant iCCA was *advanced* (43%). Even though from a clinical perspective this has less value, it reinforces the concept that patients with *very early* iCCA may achieve an acceptable 5 year survival after LT. Finally, due to the retrospective nature of this study and the non standardized explant pathology assessment (17 different transplant centers worldwide), we decide to include in the study an expert liver cancer pathologist to review all slides to confirm the diagnosis. Unfortunately, not all cases could be reviewed, but the majority were. Only one case had not the iCCA diagnosis confirmed. When restricting the analysis to those patients with external pathology the results achieved were not modified, thus reinforcing that *very early* iCCA have excellent results after LT.

What are the next steps in these investigations? To extend the indication of LT to cirrhotic patients with *very early iCCA* these results need to be validated in a prospective clinical trial. The most important challenge when conducting this clinical trial would be to be able to diagnose an iCCA at a very early stage preoperatively. Due to this difficulty in diagnosis, 7 patients in the *very early* iCCA group had incidentally found tumors. The 5 year survival of these patients was better than those with non incidental tumors even

though not statistically significant. The recurrence rate was similar and causes of death were mostly not due to tumor recurrence. Due to the small number of patients, currently this result has a difficult interpretation. In the recent years, there have been some studies that have identified characteristics radiological features of these tumors that will help to make this diagnosis (28). Nevertheless, a biopsy of the tumor will be needed to confirm the diagnosis (29). The main problem will be that in some patients due to their clinical status with major liver impairment and others due to the location of their tumor this biopsy will be challenging. For that reason, further investigations for non invasive diagnosis of iCCA would be welcome (26). The other issue that needs to be investigated is if the aggressiveness of patients with a primary diagnosis of iCCA is different of that of patients that were diagnosed of an HCC and were found to have an iCCA at the explant. This information will need to be obtained in a prospective clinical trial.

Tumor differentiation and the presence of microvascular invasion were found to be risk factors for the development of tumor recurrence. These factors are similar to those found in LT for HCC and similar to previous studies on iCCA (9, 19 21). It has been reported that the presence of positive lymph nodes and lymphovascular invasion is a risk factor for tumor recurrence in patients that are resected for iCCA (30). In the current study the information available on these two factors was scarce. These patients were not thought to have an iCCA so most likely an extensive regional lymphadenectomy was not performed at the time of transplant. It would certainly be interesting in future studies to investigate the need of an extensive portal lymphadenectomy in the case of LT for iCCA, and its impact on prognosis. Such intervention was in place in the early days of LT and was abandoned due to the poor impact in decision making if restrictive criteria were in

place. Size below 2 cm did not appear as predictive because microscopic vascular invasion and poor differentiation degree seemed to be linked to larger size and thus, size by itself was not powerful enough. When conducting a prospective clinical trial, these tumors will likely need to be biopsied to confirm the diagnosis and information on tumor differentiation may then be available and be used as supplementary information (31). However, with the information available in the current study, it is unlikely that expansion of size and number can be done at this point. Hence, size below 2 cm would be the cut off for a prospective validation trial. Nevertheless, with the information available from the current study, if a patient is included in a prospective trial with a not poorly differentiated tumor <2cm and while waiting the tumor grows, it would seem adequate to accept growth to 3 cm before the patient is excluded and drops out. However, the limited number of cases in the intermediate category of the supplemental figure prevents a robust opinion in that regard.

This study has several limitations. Even though the number of patients analyzed in this study is larger than previous series, it is still modest, especially in the group of interest (*very early* iCCA); 47% were incidentals. Secondly, we cannot confirm that the pathological diagnosis of iCCA in the different centers is accurate as we were only able to do an external review in ~70%. Nevertheless, all centers involved are large institutions with expert pathologists and the probability of an inaccurate diagnosis is probably low. Finally, this is a retrospective study with all the limitations of such study design.

In summary, this study has been able to demonstrate – in a larger cohort of patients than at the first attempt – that patients with single ≤2 cm iCCA at pathology examination after LT achieve good 5 year survival and have a low recurrence rate.

Cirrhotic patients with *very early* iCCA may become candidates for LT. A prospective multicenter clinical trial is needed to further confirm these results.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1. Cumulative risk of recurrence of patients in the *very early* vs. *advanced* iCCA groups.

Figure 2. Actuarial patient survival in the *very early* vs. *advanced* iCCA groups.

Figure 3a. Cumulative risk of recurrence of patients in the very early iCCA + HCC group vs. the advanced iCCA + HCC group.
Figure 3b. Actuarial patient survival of patients in the very early iCCA + HCC group vs. the advanced iCCA + HCC group.

Table 1. Demographics, preoperative characteristics, pathologic findings and outcome of patients in the study group

Demographics	iCCa Single tumor ≤2 cm n= 15	iCCa Single tumor >2 cm or multiple tumors n= 33	p
Median age (years)	58.5 (56 – 65)	60 (53 – 64)	0.9
Sex			0.4
Male	13 (86.7%)	25 (75.8%)	
Female	2 (13.3%)	8 (24.2%)	
Cause of cirrhosis			0.9
HCV	2 (13.3%)	7 (21.2%)	
HBV	5 (33.3%)	9 (27.3%)	
Alcoholic disease	4 (26.7%)	9 (27.3%)	
PSC/PBC/AIH	3 (20%)	6 (18.2%)	
Others	1 (6.7%)	2 (6%)	
Median real MELD score at the time of inclusion on the WL	15.5 (8.8 – 26)	12.5 (9 – 22.5)	0.8
Median time on the WL (months)	10.6 (6 – 23.7)	6 (1.7 – 13.3)	0.1
Preoperative tumor characteristics	**n=8**	**n=26**	
Median number of nodules	1 (1-2)	1 (1 – 2)	0.6
Uninodular (%)	7 (87.5%)	18 (69.2%)	0.5
Multinodular (%)	1 (12.5%)	7 (27%)	0.5
Median size of the larger nodule (cm)	1.9 (1.5 – 2.7)	2.8 (2 – 3.5)	0.3
Within Milan criteria (%)	8 (100%)	21 (80.8%)	0.2
Out of UCSF criteria (%)	0	3 (11.5%)	0.3
Median AFP value at diagnosis (ng/mL)	3 (2.3 – 24)	6 (3.7 – 10.6)	0.3
Median AFP value at the time of LT (ng/mL)	7.9 (2 – 46)	5.6 (3.5 – 11)	0.9
Preoperative tumor treatment	7 (87.5%)	18 (69.2%)	0.3
Types of tumor treatment			0.9
TACE	3 (42.9%)	9 (50%)	
Ablation	2 (28.6%)	6 (33.4%)	
TACE + Ablation	1 (14.3%)	2 (11.1%)	
Others	1 (14.3%)	1 (5.5%)	
Pathology findings	**n=15**	**n=33**	
Incidental tumors (%)	7 (46.7%)	8 (24.2%)	0.2
Median number of nodules	1	1 (1 – 2)	0.01
Uninodular (%)	15 (100%)	20 (60.6%)	0.004
Median size of the largest nodule (cm)	1.6 (1.5 – 1.8)	3.1 (2.5 – 4.4)	<0.001
Microvascular invasion	2 (13.3%)	9 (27.3%)	0.3
Macrovascular invasion	1 (6.7%)	1 (3%)	0.5
Tumor necrosis*	3 (23.1%)	10 (52.4%)	0.2
0-30%	2 (66.7%)	1 (10%)	
30-60%	-	1 (10%)	
60-90%	-	6 (60%)	
100%	1 (33.3%)	2 (20%)	
Tumor differentiation			0.3
Well-differentiated	2 (13.3%)	5 (15.2%)	
Mod-differentiated	9 (60%)	14 (42.4%)	
Poorly-differentiated	0	6 (18.2%)	
Not available	4 (26.7%)	8 (24.2%)	
Within Milan criteria (%)	15 (100%)	20 (60.6%)	0.004
Within UCSF criteria (%)	15 (100%)	23 (69.7%)	0.02
Out of UCSF criteria (%)	0	10 (30.3%)	0.02
Outcome			

Tumor recurrence (%)	2 (13.3%)	17 (51.5%)	0.006
Location of recurrence			0.8
Hepatic	-	3 (17.6%)	
Extra-hepatic	1 (50%)	8 (47%)	
Both hepatic and extra-hepatic	1 (50%)	6 (35.4%)	
Overall mortality (%)	5 (33.3%)	20 (60.6%)	0.08
Causes of mortality			0.2
Tumor recurrence	2 (40%)	15 (75%)	
Recurrence of primary liver disease	-	1 (5%)	
Others	3 (60%)	4 (20%)	

* Tumor necrosis data was not available in 14/48 (29.2%) patients

Table 2. Explant pathological characteristics and outcomes of patients in the iCCA and HCC group.

Demographics	iCCa + HCC iCCa Single tumor ≤2cm n=14	iCCa + HCC iCCa Single tumor >2 cm or multiple tumors n=19	p
Pathology findings			
Median number of HCC nodules	2 (1 – 4.3)	2 (1 – 3)	0.8
HCC - Uninodular (%)	4 (28.6%)	6 (31.6%)	0.8
HCC - Multinodular (%)	10 (71.4%)	13 (68.4%)	0.8
Median size of the largest HCC nodule (cm)	2.6 (1.7 – 3.5)	2.5 (1.5 – 3.8)	0.7
Microvascular invasion	8 (57.1%)	7 (36.8%)	0.2
HCC - Within Milan criteria (%)	7 (50%)	13 (68.4%)	0.3
HCC - Within UCSF criteria (%)	9 (64.3%)	14 (73.7%)	0.6
HCC - Out of UCSF criteria (%)	5 (35.7%)	5 (26.3%)	0.6
Outcome			
Tumor recurrence (%)	3 (21.4%)	11 (57.9%)	0.03
Location of recurrence			0.08
Hepatic	-	5 (45.5%)	
Extra-hepatic	3 (100%)	2 (18.2%)	
Both hepatic and extra-hepatic	-	4 (36.4%)	
Overall mortality (%)	2 (14.3%)	10 (52.6%)	0.03

Table 3. Risk factors for tumor recurrence in the iCCA group . Uni- and multivariate analysis (n=48).

| Risk Factors	Univariate Analysis			Multivariate Analysis*		
	HR	CI 95%	*p*	HR	CI 95%	*p*
Cause of cirrhosis	0.9	0.6 – 1.4	0.7			
Bridging therapies	1	0.4 – 2.5	0.9			
Incidental tumor	0.8	0.3 – 2.2	0.7			
Tumor size at pathology	1.2	1.1 – 1.4	0.001			
Advanced iCCA group	5.2	1.2 – 22.4	0.03			
Microvascular invasion	3.5	1.4 – 8.5	0.006	4.7	1.6-13.8	0.005
Out of UCSF criteria	4.0	1.6 – 10	0.003			
Poor differentiation	3.8	1.3 – 11.2	0.01	6.1	1.9-20.2	0.003

* Multivariate analysis in performed on 36 patients in which tumor differentiation is available
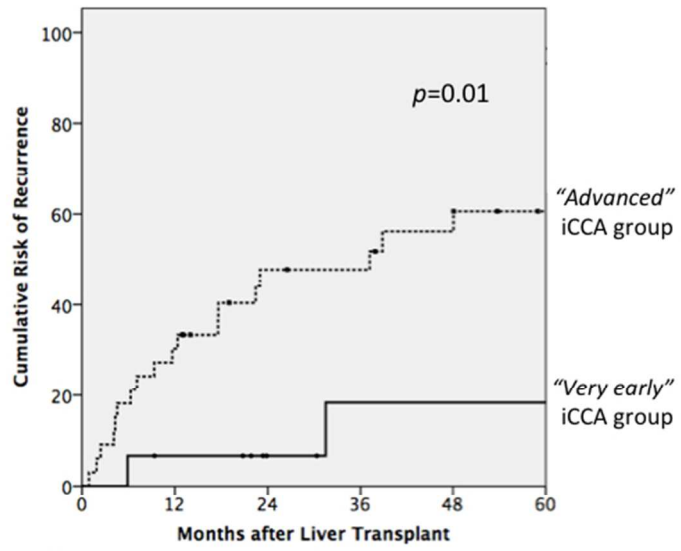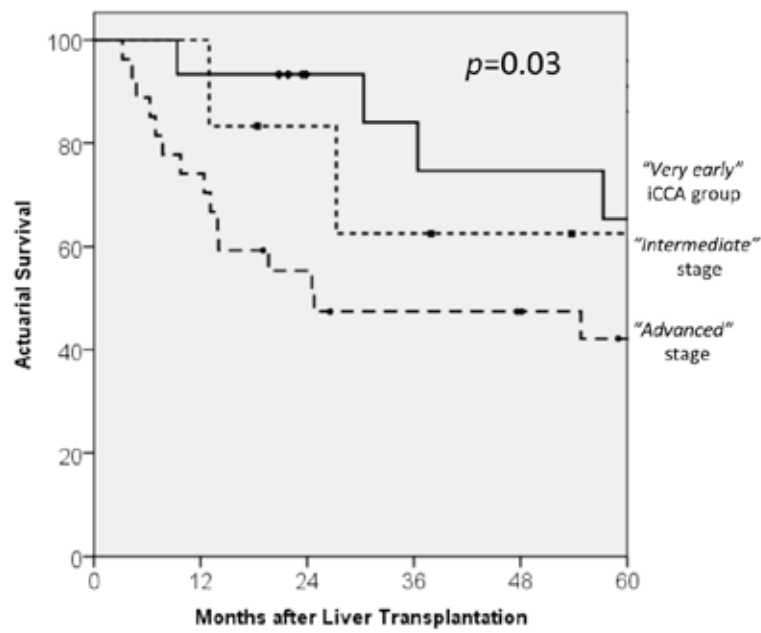
Patients at risk

"Very Early"	15	13	9	7	7	7
"Advanced"	33	23	14	13	10	6

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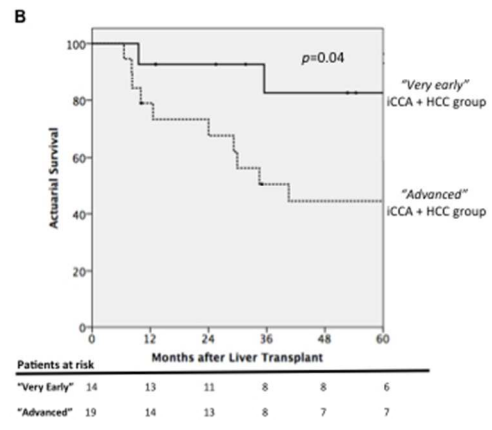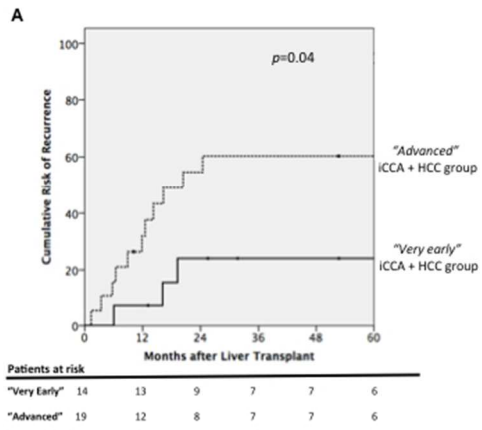
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Supplemental Figure. Actuarial patient survival in the very early vs. the intermediate and advanced stage iCCA.

Patients at risk

"Very Early"	15	14	10	9	8	7
"Intermediate"	6	6	4	3	2	1
"Advanced"	27	20	14	11	10	7