Locoregional treatments before liver transplantation for hepatocellular carcinoma a study from the European Liver Transplant Registry

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Running title

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Key words

Hepatocellular carcinoma; HCC; Liver transplantation; Ltx; locoregional treatment; pretreatment

Abbreviations

Liver transplantation (Ltx)

Hepatocellular carcinoma (HCC)

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European Liver Transplant Registry (ELTR)

Radiofrequency ablation (RFA)

Transarterial chemoembolization (TACE)

Hazards ratio (HR)

95% confidence intervals (CI)

Model for End-Stage Liver Disease (MELD)

Conflict of interest statement

There are no conflicts of interest associated with the study for any of the authors.

Abstract

Introduction

Locoregional treatment while on the waiting list for liver transplantation (Ltx) for hepatocellular carcinoma (HCC) has been shown to improve survival. However, the effect of treatment type has not been investigated. We investigate the effect of locoregional treatment type on survival after Ltx for HCC.

Methods

We investigated patients registered in the European Liver Transplant Registry (ELTR) database using multivariate Cox regression survival analysis.

Results

Information on locoregional therapy was registered for 4,978 of 23,124 patients and was associated with improved overall survival (hazard ratio (HR) 0.84 [0.73-0.96]) and HCC-specific survival (HR 0.76

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[0.59-0.98]). Radiofrequency ablation (RFA) was the one monotherapy associated with improved overall survival (HR 0.51 [0.40-0.65]). In addition, the combination of RFA and transarterial chemoembolization (TACE) also improved survival (HR 0.74 [0.55-0.99]).

Conclusion

Adjusting for factors related to prognosis, disease severity and tumor aggressiveness, RFA was highly beneficial for overall and HCC-specific survival. The effect may represent a selection of patients with favorable tumor biology; however, the treatment may be effective per se by halting tumor progression. Clinicaltrials.gov number: NCT02995096.

Introduction

In patients awaiting liver transplantation (Ltx) for hepatocellular carcinoma (HCC), disease burden may progress beyond transplantation criteria while on the waiting list. Model for End-Stage Liver Disease (MELD) exception points were introduced to alleviate dropouts due to tumor progression [1]. In addition, locoregional treatments may be used to prevent progression during waiting time [2-4] or to downstage patients initially outside transplantation criteria [5, 6].

Response to locoregional treatment is correlated with improved recurrence-free survival after Ltx, [7] suggesting it to be a surrogate marker of tumor aggressiveness that may be used to select patients with acceptable outcome after Ltx [8]. This is supported by studies where patients downstaged from being outside transplantation criteria have similar survival to patients inside criteria [5, 6].

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Locoregional treatments may halt tumor progression. However, an induction of the immune system may also explain the improved prognosis [9-11]. Investigating this, smaller observational studies have not shown convincing evidence of benefit from locoregional treatments [12-16]. Conversely, intention-to-treat studies following patients from listing and including dropouts suggested a benefit [5, 6].

Whether locoregional treatments are beneficial or merely mirror selection of patients with favorable tumor biology has not been fully elucidated. While the present study, with only data on transplanted patients, does not account for dropouts, the large sample size representing general clinical practice may elucidate important factors such as the importance of the type of locoregional treatment.

The aim was to investigate the impact of locoregional treatment including types of treatment before liver transplantation for HCC on survival and HCC-specific survival in a large cohort from the European Liver Transplant Registry (ELTR).

Methods

Reporting of this study complied with the guidelines laid out in the STROBE statement [17]. Prior to performing data analyses, a protocol was registered at clinicaltrials.gov (ID NCT02995096). This was a retrospective cohort study using register-based data recorded in the ELTR database. This database is comprised of information from 172 liver transplantation centers across Europe, each reporting local data pre- and post-transplantation in patients undergoing Ltx. No data were recorded for patients who dropped out from the waiting list. 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. Data are electronically reported to the central ELTR database from each center.

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We included patients registered for transplant in ELTR from 1990 to November 2016 due to HCC. Participants with information on locoregional treatments were included. Locoregional treatment was defined as localized treatment for the HCC tumor(s) in order to downstage or prevent progression outside criteria while on the waiting list.

The outcomes were five-year overall survival and HCC-specific survival after transplantation. Exposures were locoregional treatment (yes/no), locoregional treatment types (radiofrequency ablation (RFA) as monotherapy, transarterial chemoembolization (TACE) as monotherapy, resection as monotherapy, other treatment as monotherapy, RFA + TACE, RFA + TACE + other, other combinations) and number of locoregional treatments (0, 1, 2, 3 or more)

We used a multiple imputation model with fully conditional specification and five imputations due to missing data on covariates. The model included number of nodules, size of largest nodule, vascular invasion, time on waiting list, cirrhosis, age, gender, and MELD-score. Before and after imputation, the distribution and mean values for variables were comparable. All analyses were repeated with 50 imputations instead of five, which did not change the magnitude of the estimates.

Univariate Cox regression models were used to evaluate association between locoregional treatment and five-year survival as well as five-year HCC-specific survival from the time of Ltx. These were reported as hazards ratios (HR) and 95% confidence intervals (CI). Outcome data and follow-up time for these analyses were censored after five years. To test the independent effect of locoregional treatment, multivariate models were done for each exposure variable and adjusted for the plausible confounders of gender, age, time on waiting list, number of nodules, maximum size of nodules, vascular invasion (micro, macro or none), cirrhosis and MELD-score. We checked the proportional hazards assumption for covariates with log-minus-log plots with the natural logarithm of follow-up time. For the continuous variables (time on waiting list, number of nodules, maximum size of nodules, MELD-score), the linear effect was evaluated by including the second order polynomial of the variables in the model. No deviation from linearity was found. To test for a change

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in effect over time, we included an interaction term between the locoregional treatment yes/no variable and a categorical variable with four periods (1990-1996, 1997-2003, 2004-2009 and 2010-2016) in the multivariate COX regression model. Five-year overall and HCC-specific survival were analyzed as cumulative survival with a 95% CI using Kaplan Meier statistics, and groups were compared with a log-rank test. The Mann-Whitney U-test was used to analyze continuous data that was not normally distributed, and a Chi-square test or Fisher's exact test was used to evaluate nominal data. Using the Reserve-Kaplan Meier method, we reported the median follow-up time with interquartile range (IQR) [18]. We used IBM SPSS Statistics version 23, with statistical significance defined as $p \leq 0.05$.

Results

Patient characteristics (Table 1)

From 23,124 patients transplanted for HCC in the ELTR, 4,978 patient records had data on locoregional treatments. Patients excluded due to missing data on locoregional treatments were comparable to the included patients with respect to age, gender, number of nodules, size of largest nodule, vascular invasion, MELD-score and cirrhosis (Supplementary Table 1). The majority (71.8%) of the included patients received locoregional treatment and 85.2% of these received just one treatment. Only 1.5% received three or more treatments. TACE was most common treatment (59.1%), followed by RFA (18.0%).

Time on waiting list was longer for patients receiving locoregional treatment (median 118 vs. 49 days, $p<0.001$, Mann-Whitney U-test). This difference remained significant when stratified by size of largest nodule (> 5 cm and $\leq$ 5 cm). In addition, fewer patients had vascular invasion ($p=0.003$, Fisher's exact test), and the average MELD-score was lower in the locoregional treatment group

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(median 10.1 vs 12.2, p>0.001 Mann-Whitney). However, the groups were comparable with regards to being inside the Milan criteria (40.6 vs. 42.7%, p=0.20, Chi-square test).

Locoregional treatment (Table 2 and 3)

The five-year overall survival rate was 69.7% [67.7-71.7] for patients receiving locoregional treatment compared with 65.8% [62.5-69.1] for patients not receiving treatment (p< 0.001, Log-rank test). Locoregional treatment was significantly associated with improved prognosis for five-year overall and HCC-specific survival in univariate analyses and the estimates remained largely unchanged and significant in multivariate analyses (Tables 2 and 3). We found no significant interaction between the year of transplantation and locoregional treatment, suggesting that the effects were comparable throughout the study period.

Locoregional treatment types

The five-year overall survival rate was 80.9% [77.3-84.7] for patients treated with RFA, 67.6% [65.1-70.2] for TACE treatment and 51.3% [40.5-62.1] for resection compared with 65.8% [62.5-69.1] for patients not receiving locoregional treatment. Regarding treatment type (Tables 2 and 3), RFA had the strongest association with improved overall survival, both in univariate and multivariate analyses (HR 0.51 [0.40-0.65]). The effect was even stronger for HCC-specific survival (HR 0.43 [0.26-0.69]). In contrast, TACE was not significant in multivariate analysis (HR 0.89 [0.77-1.03]). However, the combination of RFA and TACE also improved survival (HR 0.74 [0.55-0.99]). Conversely, resection was associated with reduced overall survival (HR 1.37 [1.02-1.83]). The remaining treatments were not significant for five-year overall survival in multivariate analysis.

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The differences in survival between types of locoregional treatment are shown in Figure 1. The median follow-up time was 26 months (IQR, 7-60 months) with no treatment; 44 months (IQR 16-60 months) with RFA; 38 months (IQR, 13-60 months) with TACE, 35 months (IQR, 12-60 months) with resection; and 33 months (IQR, 11-60 months) for other treatments, including combination treatments.

Number of treatments

In univariate analysis, two treatments showed stronger association with improved survival than one treatment. However, three or more treatments showed no association. These estimates remained unchanged in multivariate analysis (Tables 2 and 3).

Subgroup analyses

More than five nodules or size of largest nodule > 3 cm

Patients with more than five nodules, or whose largest nodule was > 3 cm were evaluated in a subgroup analysis, since RFA treatment is normally not considered suitable for these patients. In a multivariate model of overall five-year survival, locoregional treatment remained significant (0.78 [0.65-0.94]). Evaluated based on type of treatment, RFA remained significantly associated with improved survival, with a HR of 0.54 [0.39-0.77]. However, TACE was now also significantly associated with improved survival (HR 0.81 [0.67-0.98]). Furthermore, the combination of RFA and TACE was associated with improved survival (HR 0.60 [0.39-0.93]). There was no association for resected patients (HR 1.05 [0.69-1-60]).

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Cirrhosis vs. non-cirrhosis

The distribution of types of locoregional treatment was significantly different between patients with and without cirrhosis ($p<0.001$, Chi-square test). More patients with cirrhosis received TACE alone (43.0 vs. 29.4%), fewer patients with cirrhosis received resection (3.2 vs. 7.2%), and fewer patients with cirrhosis received RFA alone (12.2 vs. 28.1%). In a multivariate model for overall five-year survival for the subgroup of patients with cirrhosis, the effect of locoregional treatment (HR 0.86 [0.75-0.99]) was comparable to that of the whole group. Similarly, RFA was associated with improved survival (HR 0.52 [0.40-0.67]), TACE was not (HR 0.91 [0.78-1.05]), and resection was associated with diminished survival (HR 1.48 [1.09-2.00]).

Vascular invasion

The effect of locoregional treatment was stronger for patients with micro- or macrovascular invasion (HR 0.71 [0.55-0.92]) compared to patients with no vascular invasion, where no effect was observed (HR 0.88 [0.75-1.05]). Regarding type of treatment, both RFA (HR 0.54 [0.32-0.91]) and TACE (HR 0.69 [0.52-0.92]) were associated with improved survival for patients with micro- or macrovascular invasion.

Discussion

Adjusted for disease severity, the present study showed that locoregional treatment with RFA was associated with improved overall and disease-specific survival. To a lesser extent, the combination of RFA and TACE was also beneficial. Subgroup analyses showed that the effect was stronger for patients with vascular invasion. Moreover, patients with extensive disease still experienced improved outcomes from RFA.

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Previous studies have shown that locoregional treatment may successfully downstage patients to be inside Milan criteria and improve survival following liver transplantation [5, 6, 16, 19, 20]. Two intention-to-treat studies have been conducted in which patients were followed since listing for transplantation [5, 6]. In one study, 118 patients scheduled for downstaging treatment with TACE/ RFA were compared with 488 patients within the Milan T2 criteria at the time of listing [5]. Downstaging was successful in 65.3% of the cases and 54.2% received Ltx. The five-year survival rate was 56.1% for all patients receiving downstaging treatment vs. 63.3% for patients meeting the T2 Milan criteria (p=0.29). Among the patients proceeding to Ltx, the five-year survival rate was 77.8% in the downstaging groups and 81% in the Milan T2 group (p=0.69). In 40.6% of patients receiving downstaging, there was no residual tumor in explant pathology. In another study, 129 patients inside Milan criteria were compared with 48 patients outside the criteria receiving downstaging treatment; the results showed comparable transplantation rates and intention-to-treat survival between the groups [6].

A direct effect of the locoregional treatments may be induction of the immune system to produce a response toward the tumor, causing a reduction in tumor size, as well as halting progression. In one study, RFA induced stimulation of natural killer cells, demonstrating a benefit to recurrence-free survival among patients with the largest response [11]. Moreover, in another study, the antigen-specific response from T-cells was enhanced in 31 patients with HCC receiving locoregional treatments [10]. Lastly, at least one study has shown that the extent of necrosis induced by locoregional treatment was associated with cell-mediated tumor rejection and clinical response [9]. In a clinical study, 101 patients randomized to TACE or transarterial embolization (TAE) had comparable response, progression free survival and overall survival. Thus, questioning the additional benefit of local chemotherapy for these patients besides the effect induces by ischemia [21].

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Surprisingly, patients in a downstaging study had extremely low rates of microvascular invasion in explant pathology (1.6% versus 18.6% for all patients receiving locoregional treatment in the present study) [5]. Moreover, in the present study, vascular invasion was less common among patients receiving locoregional treatment. Thus, while intention-to-treat studies suggest an effect of locoregional treatments per se [5, 6], these treatments may also select patients with favorable tumor biology. Patients with progression-free waiting time after TACE had a lower risk of recurrence, possibly related to less aggressive tumors [7]. This may be illustrated by the longer time on the waiting list for patients receiving locoregional treatment in the present study. Thus, one may argue that transplantation should be limited to patients without progression after locoregional treatment. In fact, selection on this basis may be superior to selecting based on the Milan criteria [7], as those guidelines may exclude patients outside criteria with favorable tumor biology. This is supported by comparable outcomes between downstaged patients and patients inside Milan criteria [5, 6]. Correspondingly, we show that an indirect measure of tumor aggressiveness (vascular invasion) may be a superior prognostic marker compared with the Milan criteria [22].

Our results suggest that resection was associated with reduced overall survival compared with no treatment. Randomized controlled trials comparing RFA and resection as primary treatment for patients with small HCC [23] and HCC with Milan criteria [24] agree that resection is the superior treatment. Therefore, despite adjusting for tumor characteristics, our results may be a result of selection bias, where patients with large tumors, and thus a worse prognosis, are limited to surgical resection.

This is the first study to compare RFA, TACE and other locoregional treatment types on a large scale, and to show that RFA may be superior. It is a pan-European study, with many centers reflecting general clinical practice with a broad external validity. An important limitation is that we could not account for patients dropping out from the waiting list; thus, only transplanted patients were evaluated. While approximately 7% will drop out while on the waiting list due to tumor progression

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[4], the included patients may represent a selective sample, presumably including more patients with favorable tumor biology. With longer time on waiting list and less vascular invasion, the locoregional treatment group may have a higher proportion of patients with favorable tumor biology and, thus, good response to treatment or stable disease, allowing subsequent transplantation. Unfortunately, response to locoregional treatment was not included in the ELTR, posing another limitation. With a considerable amount of missing data, we had information on locoregional treatments for only 4,978 of 23,124 patients transplanted for HCC in the study period. This was a result of locoregional data not being included in the ELTR questionnaire before 2007-2008. Thus, these data may not reflect the true picture of the whole population; however, the two populations were comparable with respect to prognostic variables. Lastly, despite adjusting for indirect measures of tumor aggressiveness and tumor load (size, number and vascular invasion), the benefit from RFA may be a product of residual confounding resulting from patients treated with RFA having less severe disease prior to treatment.

Studies have shown that response to locoregional treatment is an important factors for prognosis [7, 25]. Thus, as proposed by Mazzaferro [8], future selection criteria may be based on response to locoregional treatments. In other words, all patients with HCC on a waiting list for Ltx should be treated with locoregional treatments followed by a suitable observation period regardless of tumor size and number. Transplant priority should be given to patients based not only on conventional criteria, but also on the response to locoregional treatment [8]. The results of the present paper support this notion.

In conclusion, we show that locoregional treatments with RFA were beneficial for both overall and HCC-specific survival. After adjusting for variables related to tumor aggressiveness, treatment with RFA was still highly effective. These results may represent a selection of patients with favorable tumor biology who responded to locoregional treatments and subsequently received Ltx; however, intention-to-treat studies suggest an effect of locoregional treatments per se. Thus, induction of the

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immune system by locoregional treatments, rather than, or in addition to, patient selection, may explain these observations.

Figure legends

Figure 1: Overall survival for different types of locoregional treatment

RFA: Radiofrequency ablation, TACE: Transarterial chemoembolization

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Patient characteristics and missing data

	No locoregional treatment (n=1406)	**Locoregional treatment (n= 3572)**	**Missing data, n (%)**
Recipient age, median (range)	55 (0-78)	58 (0-77)	2 (0%)
Male gender, n (%)	1151 (81.9%)	3030 (84.8%)	1 (0%)
AB0 matching, n (%)			75 (1.5%)
Identical	1185 (85.8%)	3305 (93.8%)	
Compatible	177 (12.8%)	188 (5.3%)	
Non-compatible	19 (1.4%)	29 (0.8%)	
Number of transplantations, n (%)			0 (0%)
1	1354 (96.3%)	3378 (94.6%)	
2	47 (3.3%)	186 (5.2%)	
3 or more	5 (0.4%)	8 (0.2%)	
Number of locoregional treatments, n (%)			0 (0%)
1		3042 (85.2%)	
2		477 (13.4%)	
3 or more		53 (1.5%)	
Type of locoregional treatment, n (%)			0 (0%)
RFA as monotherapy		643 (18%)	
TACE as monotherapy		2110 (59.1%)	
Resection as monotherapy		169 (4.7%)	
Other as monotherapy		120 (3.4%)	
RFA+ TACE		280 (7.8%)	
RFA + TACE + other		49 (1.4%)	
Other combination		201 (5.6%)	

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Days on waitinglist, median (range)	49 (0-8492)	118 (0-3991)	777 (15.6%)
Transplanted after 2006, n (%)	1126 (80.1%)	2949 (82.6%)	0 (0%)
Outside Milan criteria, n (%)	531 (42.7%)	1143 (40.6%)	919 (18.5%)
More than 3 nodules, n (%)	197 (14.9%)	561 (17.6%)	471 (9.5%)
Size of largest nodules > 5 cm, n (%)	230 (17.8%)	328 (10.6%)	590 (11.9%)
Vascular invasion, n (%)			849 (17.1%)
None	933 (74.9%)	2264 (78.5%)	
Macrovascular	59 (4.7%)	83 (2.9%)	
Microvascular	254 (20.4%)	536 (18.6%)	
MELD score, median (range)	12.1 (6.4-49.6)	10.1 (6.4-42.8)	1519 (30.5%)
Cirrose, n (%)			0 (0%)
Non-cirrose	42 (3%)	179 (5%)	
Cirrose	1358 (96.6%)	3385 (94.8%)	
Fibrolamellar	6 (0.4%)	8 (0.2%)	

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	Overall 5 year survival Hazard ratio [95% CI]	**HCC specific 5 year survival Hazard ratio [95% CI]**
	HR [CI 95%]	*HR [CI 95%]*
Age (10 year increase)	1.10 [1.02-1.18]	0.92 [0.82-1.04]
Gender (Male)	0.90 [0.77-1.06]	0.88 [0.78-1.00]
Time on waiting list (30 days increase)	1.00 [1.00-1.01]	1.00 [0.99-1.01]
More than 3 nodules	1.61 [1.51-1.71]	2.59 [2.33-2.87]
Size of largest nodule > 5 cm	2.00 [1.88-2.14]	2.99 [2.68-3.33]
Vascular invasion		
No vascular invasion	Ref.	Ref.
Macrovascular invasion	2.66 [2.04-3.47]	8.87 [7.64-10.30]
Microvascular invasion	1.57 [1.36-1.82]	3.16 [2.86-3.49]
MELD-score	1.04 [1.03-1.05]	1.01 [1.00-1.02]
Cirrose		
Non-cirrosis	Ref.	Ref.
Cirrosis	0.86 [0.67-1.11]	0.59 [0.50-0.69]
Fibrolamellar	2.10 [0.91-4.86]	3.02 [1.86-4.92]
Locoregional treatment (yes/no)	0.82 [0.72-0.94]	0.73 [0.66-0.80]
Locoregional treatments		
No treatment	Ref.	Ref.
RFA as monotherapy	0.47 [0.37-0.60]	0.35 [0.29-0.43]
TACE as monotherapy	0.90 [0.78-1.03]	0.78 [0.70-0.86]
Resection as monotherapy	1.34 [1.00-1.78]	1.24 [0.99-1.55]
Other as monotherapy	1.19 [0.85-1.67]	1.36 [1.07-1.72]
RFA+ TACE	0.76 [0.57-1.02]	0.74 [0.60-0.92]
RFA + TACE + other	1.03 [0.58-1.82]	1.45[1.00-2.09]
Other combination	0.69 [0.48-0.99]	0.64 [0.48-0.84]

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Number of locoregional treatments		
0	Ref.	Ref.
1	0.83 [0.73-0.95]	0.73 [0.65-0.80]
2	0.72 [0.57-0.91]	0.70 [0.58-0.84]
3 or more	1.17 [0.69-2.00]	1.44 [1.00-2.08]

Univariate COX-regression analyses

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Multivariate COX-regression analysis

	Overall 5 year survival Hazard ratio [95% CI]	**HCC specific 5 year survival Hazard ratio [95% CI]**
Locoregional treatment (yes/no)	0.84 [0.73-0.96]	0.76 [0.59-0.98]
Locoregional treatments		
No treatment	Ref.	Ref.
RFA as monotherapy	0.51 [0.40-0.65]	0.43 [0.26-0.69]
TACE as monotherapy	0.89 [0.77-1.03]	0.79 [0.60-1.04]
Resection as monotherapy	1.37 [1.02-1.83]	1.22 [0.70-2.14]
Other as monotherapy	1.20 [0.85-1.69]	1.50 [0.83-2.70]
RFA+ TACE	0.74 [0.55-0.99]	0.65 [0.38-1.11]
RFA + TACE + other	0.98 [0.55-1.75]	1.35 [0.54-3.35]
Other combination	0.71 [0.49-1.02]	0.66 [0.33-1.33]
Number of locoregional treatments		
0	Ref.	Ref.
1	0.85 [0.74-0.98]	0.77 [0.59-1.00]
2	0.71 [0.56-0.91]	0.66 [0.42-1.03]
3 or more	1.11 [0.65-1.91]	1.35 [0.54-3.35]

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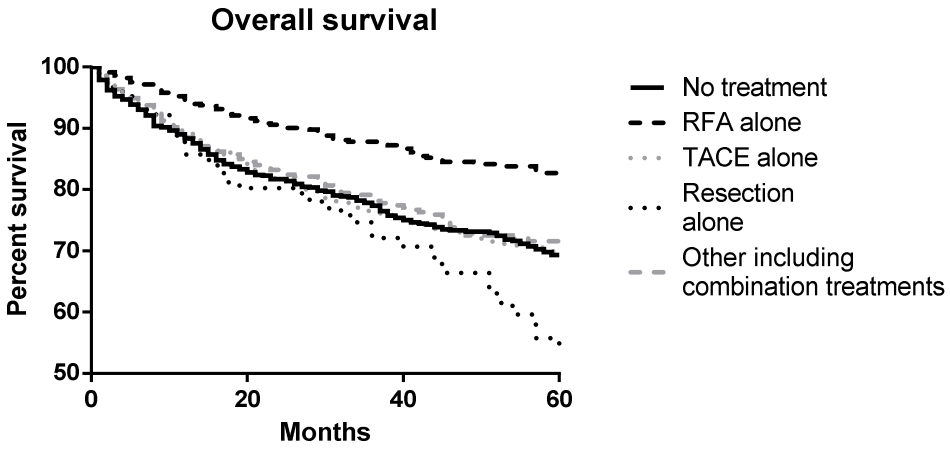
Figure 1: Overall survival for types of locoregional treatment



Follow-up time		20 months	40 months	60 months
No treatment	*Numbers at risk*	626	413	290
	Survival (%)	78.6	71.2	65.8
RFA alone	*Numbers at risk*	417	302	199
	Survival (%)	90.1	85.2	80.9
TACE alone	*Numbers at risk*	1153	743	500
	Survival (%)	80.5	72.4	67.6
Resection alone	*Numbers at risk*	84	50	28
	Survival (%)	74.4	65.4	51.3
Other including combination treatments	*Numbers at risk*	427	257	175
	Survival (%)	80.3	72.7	65.5

RFA: Radiofrequency ablation, TACE: Transarterial chemoembolization

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