Int J Clin Exp Med 2016;9(6):10354-10363
www.ijcem.com /ISSN:1940-5901/IJCEM0024065

Original Article

Percutaneous cryoablation versus hepatectomy for hepatocellular carcinoma treatment

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Received January 14, 2016; Accepted April 6, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Background: Percutaneous cryoablation has been increasingly utilized for hepatocellular carcinoma (HCC) treatment. Long-term percutaneous cryoablation results have not been well described, especially in comparison with surgical resection. Methods: One-hundred patients with 1-3 HCC tumors <5 cm in diameter from Zhangjiagang Hospital Affiliated to Soochow University and Huai'an First People's Hospital, Nanjing Medical University were enrolled into this study. Among them, 49 patients were treated by cryoablation and 51 were treated by hepatectomy. Follow-up examinations were performed for the first four weeks after treatment, then every three months for the first 24 months and every six months thereafter. Results: One-, two- and three-year overall survival rates were 96.1%, 88.2% and 62.7% for hepatectomy, respectively, and 89.8%, 77.5% and 51.0% for cryoablation, respectively (P=0.179). One-, two- and three-year disease-free survival rates were 94.1%, 62.7% and 25.4% after hepatectomy, respectively, and 85.7%, 51.0% and 18.3% after cryoablation, respectively (P=0.185). Subgroup analyses indicate that patients with tumors ≤3 cm had significantly better survival rates compared with patients with tumors >3 cm (P=0.003). Neither patients with ≤3 cm tumors nor patients with >3 cm tumors exhibited survival differences when treated with either resection or cryoablation (P=0.095 and P=0.282, respectively). Patients with single tumors exhibited significantly better survival rates than patients with multiple tumors (P<0.001). No significant differences were observed in the survival rates of either single or multiple tumor patients treated with resection or cryoablation (P=0.851 and P=0.780, respectively). Tumor diameter, number and Child-Pugh scores were significant prognostic factors for overall survival. Conclusion: Percutaneous cryoablation and hepatectomy were equally effective HCC treatments.

Keywords: Hepatocellular carcinoma, percutaneous cryoablation, hepatectomy

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and is responsible for 500,000 annual global deaths [1]. Fortunately, recent advancements in early detection strategies have increased the number of small HCC tumors amenable to curative treatment [2].

Surgical resection is considered the first-line therapeutic option for most HCC patients. Nevertheless, stringent inclusion criteria for the selection of surgical candidates results in the limited role of surgery in HCC treatment [3, 4]. Liver transplantation is a potentially effective option in some cases [5]. However, the feasibility of this treatment strategy is restricted by organ donor shortages. Due to these restrictions, various nonsurgical therapies have been developed; and local ablation therapy is a notable example. A number of local ablation therapies have been developed during the past decade including percutaneous ethanol injection (PEI), percutaneous acetic acid injection (PAI), radiofrequency ablation (RFA), microwave coagulation therapy (MCT) and cryoablation [6-9]. Many of these techniques have exhibited competitive efficacy with surgical resection for the treatment of small HCC tumors [10-13]. Local ablation therapy may have higher recurrence rates and lower disease-free survival rates when compared with surgical resection. However, this is balanced by the relatively low invasiveness of local ablation procedures, shorter hospital stays for local ablation patients, and an extremely low associated rate of mortality [14]. These benefits have led to the growing importance of local ablation in the treatment of patients with HCC, especially among poor surgical candidates and patients with recurrent HCC [14]. A recent study has suggested that local ablation could become the first choice for small HCC tumor treatment [14].

Cryoablation uses low temperatures and repeated freezing and thawing to destroy tumor cells *via* cellular dehydration, protein denaturation and microcirculatory failure [15]. Current cryoablation techniques are based on the cyclic application of low temperatures through a probe inserted into the tumor [16]. Recent technical improvements including the emergence of new generation argon-helium based cryoablation equipment with thinner probes have significantly increased the clinical potential of cryoablation therapy for HCC [17]. Furthermore, combining cryoablation with ultrasound (US) guidance or computed tomography (CT) monitoring has been reported as a feasible, safe and effective tumor treatment technique [18, 19]. Good results have been reported in using cryoablation to treat lung, kidney and prostate tumors [20]. However, despite the wide adoption of cryoablation in the treatment of various cancer types, few studies have investigated the use of percutaneous cryoablation for HCC treatment [21, 22].

Recent studies investigating the advantages of different local ablation therapies for HCC tumors have compared cryoablation and RFA [17, 23]. Cryoablation has several unique advantages over RFA including larger ablative zones, clearer and discernible treatment margins, less pain, and stronger ectopic tumor suppression effects [17, 23]. Studies have reported similar outcomes for liver cancer patients treated with cryoablation, RFA and surgical resection [24-27]. Furthermore, studies have reported superior local tumor control when using cryoablation for the treatment of HCC tumors <5 cm [27]. These reports indicate that further investigation and development of cryoablation therapy for HCC tumors could significantly improve the outlook of HCC patients with tumors <5 cm.

A growing body of evidence suggests that cryoablation may be a valuable therapeutic tool for HCC treatment, but few studies have investigated the long-term outcomes of cryoablation treatment in HCC patients, or compared these outcomes with the outcomes of patients treated by hepatectomy. Therefore, to determine the feasibility and safety of percutaneous cryoablation for HCC treatment, we conducted a retrospective cohort study to compare the treatment outcomes of HCC patients treated *via* percutaneous cryoablation with the outcomes of HCC patients treated *via* liver resection.

Patients and methods

Patient recruitment

From October 2010 to September 2012, patients were recruited from Zhangjiagang Hospital Affiliated to Soochow University and Huai'an First People's Hospital, Nanjing Medical University. One hundred two patients with chronic liver disease and HCC were treated *via* cryoablation at the Zhangjiagang Hospital Affiliated to Soochow University, while 85 patients with HCC were treated *via* liver resection at Huai'an First People's Hospital, Nanjing Medical University. Treatment decisions were made by a multidisciplinary team of doctors from different departments including surgeons, radiologists and oncologists. No study specific treatment selection criteria were used when making treatment decisions in this study. The treatment group selection is described below.

One hundred patients were included in this retrospective study according to the following criteria:

1. no prior HCC treatment; 2. three or less tumors; 3. all tumors smaller than 5 cm in diameter; 4. no radiologic evidence of invasion into major portal/hepatic vein branches; 5. no evidence of severe coagulopathy (i.e. prolonged prothrombin time >5 seconds); 6. no evidence of severe thrombocytopenia (i.e. platelet count <40 × 10^9/L); 7. no history of encephalopathy, ascites refractory to diuretics or variceal bleeding.

Ethics

This study was approved by the Ethics Committee of Zhangjiagang Hospital Affiliated to Soochow University and the Ethics Committee of Huai'an First People's Hospital, Nanjing Medical University. Written informed consent was obtained from each participant.

Table 1. Patient characteristics

Variables	Resection, *n* (%)	Cryoablation, *n* (%)	*P*
Gender			0.727
Male	41 (80)	38 (78)	
Female	10 (20)	11 (22)	
Age			0.729
≤65 years	38 (75)	35 (71)	
>65 years	13 (25)	14 (29)	
Chronic liver disease			0.450
Viral HBV	42 (82)	43 (88)	
Not viral HBV	9 (18)	6 (12)	
Child-Pugh class			0.001
A	45 (88)	26 (53)	
B	6 (12)	23 (47)	
Tumors			0.003
Single	43 (84)	28 (57)	
Multiple	8 (16)	21 (43)	
Size			0.410
≤3 cm	14 (27)	10 (20)	
>3 cm, ≤5 cm	37 (53)	39 (80)	
Serum AFP level			0.410
≤20 ng/dl	26 (51)	29 (59)	
>20 ng/dl	25 (49)	20 (41)	

HBV, Hepatitis B Virus; AFP, alpha-fetoprotein.

HCC diagnosis

HCC diagnosis was based on a combination of clinical examinations with imaging and/or histological findings [4]. All patients completed a standard set of liver function and other clinical tests prior to treatment including bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), creatinine and albumin levels, prothrombin time, blood count, chest x-rays, liver US, and abdominal triple phase CT and/or contrast-enhanced magnetic resonance imaging (MRI).

Resection group selection

Fifty-one HCC patients who received treatments via surgical resection were included in this study. The characteristics of patients submitted to surgery are reported in **Table 1**. Surgical resection was considered the treatment of choice for patients with Child-Pugh class A cirrhosis and single HCC tumors. Resection was also performed in selected patients with multiple HCC tumors or with Child-Pugh class B cirrhosis.

Resection group treatment

All surgeries were performed under general anesthesia using a right subcostal incision with a midline extension. An anatomical partial hepatectomy was performed with a resection margin ≥1 cm over the tumor. Negative resection margins were confirmed *via* histology. Examination of surgical specimens confirmed the presence of liver fibrosis in all patients.

Cryoablation group selection

Forty-nine patients treated *via* cryoablation were included in this study. The characteristics of patients treated *via* cryoablation are reported in **Table 1**. Percutaneous cryoablation was considered the treatment of choice for patients with Child-Pugh class B cirrhosis or patients with multiple tumors. Patients with Child-Pugh class A cirrhosis and single tumors were treated with cryoablation. Ablative therapy was indicated in these patients, because the tumor location would otherwise require major hepatic resection or refusal of surgery. None of the patients included in the study had any general surgical contraindications.

Cryoablation group treatment

Percutaneous cryoablation was performed as previously reported [27]. Cryoablation procedures were conducted using a 4- or 8-cryoprobe cryosurgery system with 2- or 3-mm diameter superconducting cryoprobes (Endocare Inc., Irvine, CA, USA) and the sheath-and-guidewire technique. A 16-slice spiral CT was used for guidance, localization, and intraoperative real-time monitoring of ablation procedures. The location, number and size of cryoprobes were determined according to the "2 to 1" principle to assure the placement of cryoprobes within 1 cm of the tumor edge [28]. Probes were never placed with more than a 2-cm interval between the probes. Generally, no more than three cryoprobes were used during a single cryoablation procedure. Large tumors were divided into two target regions, and treatment was administered *via* two cryoablation procedures that were conducted with an interval of one week. During each treatment session, the cryosurgery system was activated after the placement of all probes. Cryoprobe temperature was first decreased to -150°C for 15 minutes and elevated to 40°C for 10 minutes. This temperature cycle was performed twice, the cryoprobes were withdrawn, and a

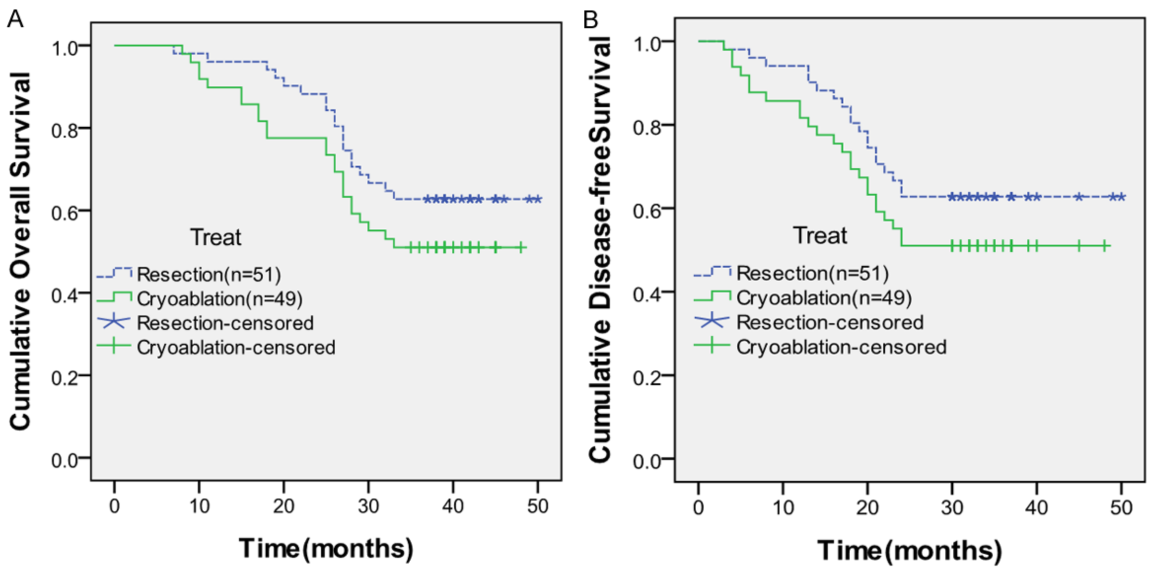

Figure 1. A. Overall survival curves for patients treated with hepatectomy or cryoablation. No significant differences were observed in the overall survival rates of the two groups from the date of hepatectomy or cryoablation (log-rank test, *P*=0.179). B. Disease-free survival curves for patients treated with hepatectomy or cryoablation. No significant differences were observed in the overall survival rates of the two groups from the date of hepatectomy or cryoablation (log-rank test, *P*=0.185).

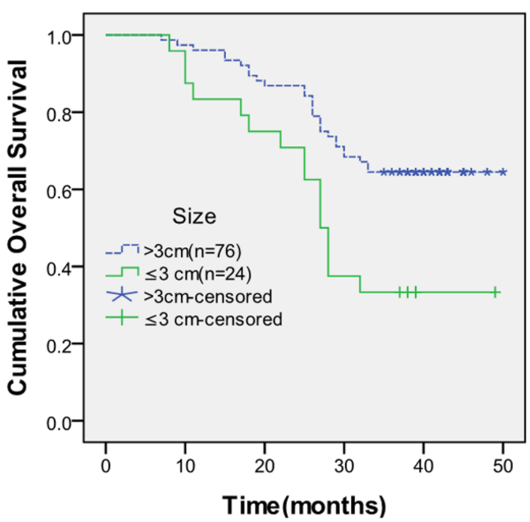

Figure 2. Overall survival curves for patients with different tumor sizes. Patient survival was significantly better with tumors ≤3 cm than with tumors >3 cm (log-rank test, *P*=0.003).

hemostatic gelatin sponge was tamped into the sheath to stop patient bleeding and fill the sinuses. Finally, the sheath was removed.

Treatment response was evaluated using CT or MRI after four weeks. Complete tumor response was defined as the absence of arterial enhancement within or at the periphery of all treated tumors [29]. HCC tumors that exhibited an incomplete response were reevaluated for a second cryoablation session.

Follow-up

All patients received a triple-phase contrast-enhanced spiral CT four weeks after treatment, and every three months for the next two years. MRI was conducted if CT results were inconclusive, which concerns any potential residual, recurrent or metastatic lesions. Blood tests including liver function and serum alpha-fetoprotein (AFP) tests were performed at each follow-up visit. After two years, follow-up visits were conducted once every six months.

All patients with intrahepatic tumor recurrence were evaluated for new treatment with ablative therapies, transarterial chemoembolization or surgery; and an appropriate follow-up treatment was administered relative to the severity of liver dysfunction and tumor stage.

Statistical analysis

Data were expressed as means ± SD. Statistical analyses were carried out using SPSS version 16.0 (SPSS, Chicago, IL, USA). Categorical variables were compared using chi-square test,

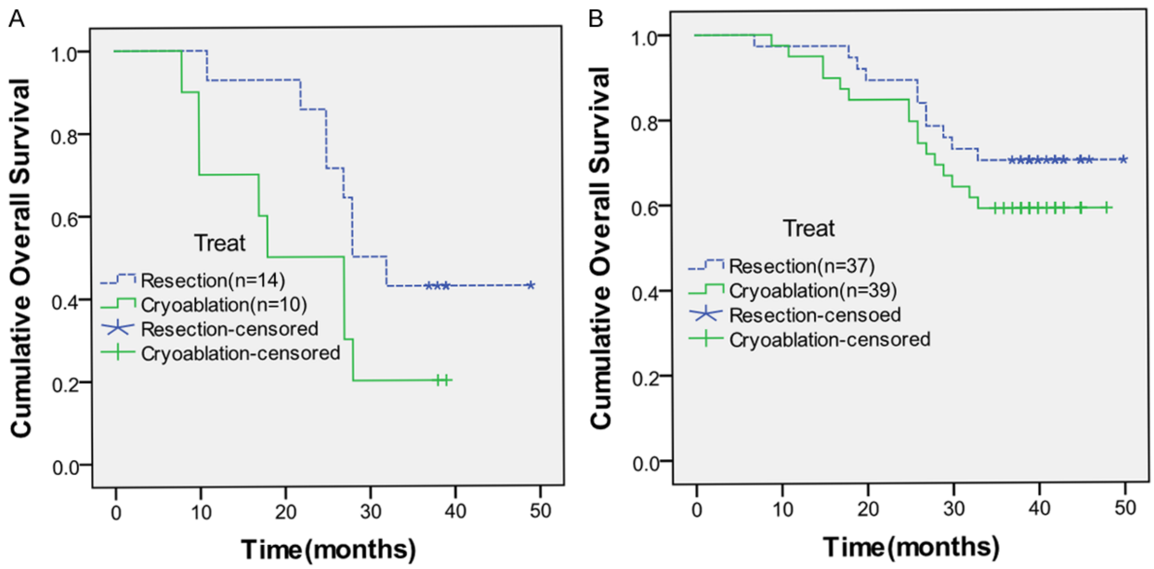

Figure 3. A. Overall survival curves for patients with tumors ≤3 cm treated with hepatectomy or cryoablation. No significant differences were observed in the overall survival rates of the two groups from the date of hepatectomy or cryoablation (log-rank test, *P*=0.095). B. Overall survival curves for patients with tumors >3 cm treated with hepatectomy or cryoablation. No significant differences were observed in the overall survival rates of the two groups from the date of hepatectomy or cryoablation (log-rank test, *P*=0.282).

while Student's *t*-test was used for continuous variables. Overall survival and disease-free survival analyses were carried out using the Kaplan-Meier method. Comparisons between different groups were carried out using log-rank test. Multivariate analyses for overall survival were carried out using the Cox's regression model. *P*<0.05 was considered significant.

Results

Resection group

No operative mortality was observed. Mean follow-up time for resection patients was 34.9 months with a range of 4.0 to 58.0 months. Median survival time was 36.0 months with one-, two- and three-year survival rates of 96.1%, 88.2% and 62.7%, respectively. Median disease-free survival time was 32 months with one-, two- and three-year disease-free survival rates of 94.1%, 62.7% and 25.4%, respectively.

Cryoablation group

No cryoablation procedure-related deaths occurred during the study. In the cryoablation group, mean follow-up duration was 31.1 months with a range of 8.0 to 48.0 months. Complete tumor necrosis was obtained in 49 single and multiple treatment patients including 90.0% of patients with tumors ≤3 cm and 87.1% of patients with tumors >3 cm. The rate of complete tumor necrosis was not significantly different when patients with tumors ≤3 cm were compared with patients with tumors >3 cm (*P*=0.766). Median survival time was 36.0 months with one-, two- and three-year survival rates of 89.8%, 77.5% and 51.0%, respectively. Median disease-free survival was 31.0 months in the cryoablation group with one-, two- and three-year disease-free survival rates of 85.7%, 51.0% and 18.3%, respectively.

Resection and cryoablation group comparisons

No significant differences were observed between the resection and cryoablation groups in terms of overall survival (**Figure 1A**; log-rank test, *P*=0.179). Additionally, no significant differences in disease-free survival were observed between the two groups (**Figure 1B**; log-rank test, *P*=0.185).

Subgroup analysis indicated that the survival rate was significantly better among patients with tumors ≤3 cm (**Figure 2**; log-rank test, *P*=0.003). No significant differences were observed in survival rates of patients with tumors ≤3 or >3 cm treated with resection or

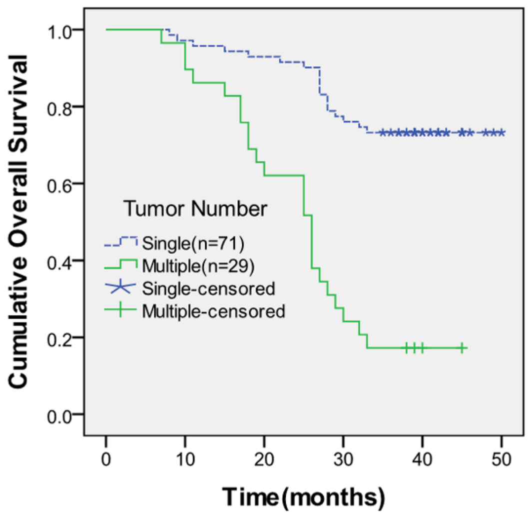

Figure 4. Overall survival curves for patients with different numbers of tumors. The survival of patients with single tumors was significantly better than the survival of patients with multiple tumors (log-rank test, $P<0.001$).

cryoablation (**Figure 3A** and **3B**; log-rank test, $P=0.095$ and $P=0.282$, respectively). Patients with single tumors exhibited significantly better survival rates compared with multiple tumor patients (**Figure 4**; log rank test, $P<0.001$). No significant differences were observed between the survival rates of single- or multiple-tumor patients treated with resection or cryoablation (**Figure 5A** and **5B**; log-rank test, $P=0.851$ and $P=0.780$, respectively).

Multivariate Cox proportional hazards regression analyses indicated that the number of tumors, tumor diameter, and Child-Pugh class were significant prognostic factors of overall survival (**Table 2**). This was true for both the cryoablation and resection treatment groups.

Among the patients examined in this study, Child-Pugh class A patients with single tumors ≤3 cm in size had significantly better overall survival (**Table 2**).

Discussion

It has been suggested that patients with small, solitary tumors and well-preserved liver function are the most suitable candidates for surgical resection [30]. Partial hepatectomy is considered the gold standard therapy for patients with resectable HCC, normal liver function and good general condition [31]. Liver transplantation is the most beneficial approach for individuals who are not good candidates for resection; however, donor shortages greatly limit the implementation of this treatment strategy [5]. Percutaneous ablation is a frequently used treatment strategy for patients who are poor candidates for surgical resection; however, its effectiveness is often limited by tumor size and localization [30]. For instance, according to the HCC management guidelines of the Barcelona Clinic Liver Cancer Group and Liver Cancer Study Group of Japan, RFA is not indicated for tumors >3 cm [32, 33]. Cryoablation may be a superior treatment choice in some cases where RFA is not indicated. The primary advantage of cryoablation over other ablation modalities is the precision that can be attained in the size and shape of the ablation zone. With current technologies, cryoablation is capable of producing significantly larger zones of ablation with comparable associated risks when compared with other ablation modalities [22, 26].

The treatment of a malignant tumor *via* cryoablation was first attempted in 1850 by directly applying frozen saline to a large ulcerative tumor, resulting in decreased size, pain and bleeding of the target tumor [34]. In 1961, the first modern cryoablation system was introduced, which passed liquid nitrogen through a trocar-shaped probe to target areas under open laparotomy [35]. Cryoablation produces ice crystals of consistent size and shape, and reliably causes cell death within the cryolesion [36]. Cryotherapy is believed to kill cells by several mechanisms including intracellular ice formation, solute-solvent shifts that dehydrate and rupture cells, and small-vessel obliteration with resulting hypoxia [37]. Various animal experiments and clinical trials have demonstrated the safety and effectiveness of cryoablation for the treatment of malignant tumors [38, 39]. However, this technology was traditionally limited to intraoperative use in the clinic, because the large probes that were required to deliver the therapy could cause serious bleeding when removed [40]. Nonetheless, successful ablation of HCC tumors ≤5 cm in diameter has been demonstrated using percutaneous cryotherapy [41]. The cryoablation system used in the present study destroys tumor cells with an argon and helium gas probe and a freezing/thawing cycle of -150°C and 40°C.

Imaging technologies have improved the accuracy of percutaneous ablation therapy. The most common guidance methods are currently

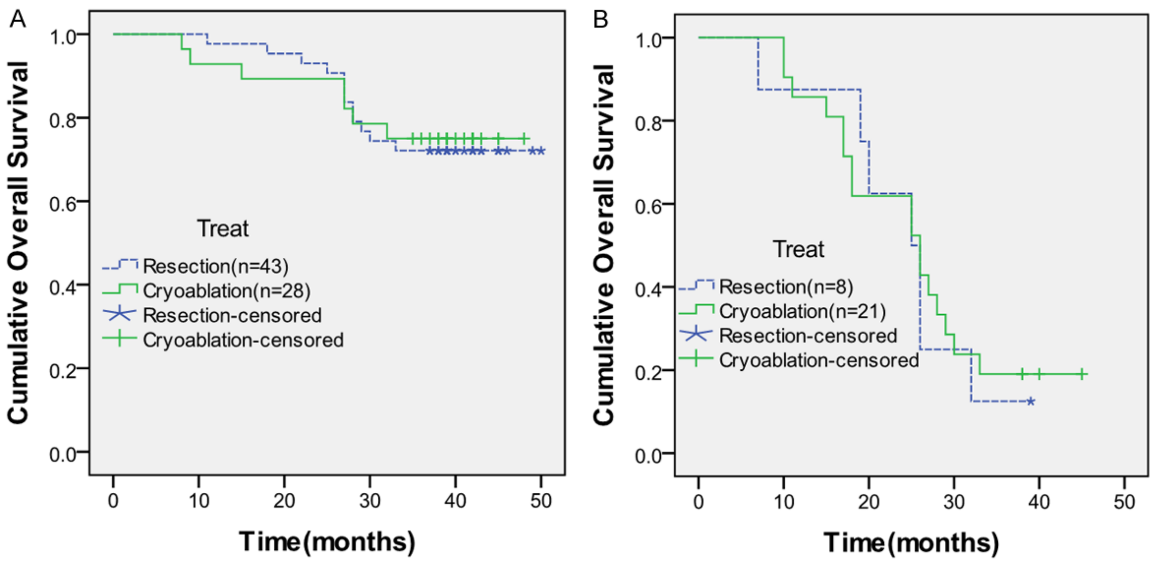

Figure 5. A. Overall survival curves for patients with single tumors treated with hepatectomy or cryoablation. No significant differences were observed in the overall survival rates of the two groups from the date of hepatectomy or cryoablation (log-rank test, *P*=0.851). B. Overall survival curves for patients with multiple tumors treated with hepatectomy or cryoablation. No significant differences were observed in the overall survival rates of the two groups from the date of hepatectomy or cryoablation (log-rank test, *P*=0.780).

Table 2. Multivariate analysis of prognostic factors for overall post-treatment survival

Factor	Regression coefficient	Standard error	*P* value
Age (≤65 years *vs*. >65 years)	0.190	0.366	0.604
Gender (Male *vs*. Female)	-0.089	0.378	0.815
Child-Pugh class (A *vs*. B)	-0.816	0.373	0.029
Number of tumors (single *vs*. Multiple)	-1.604	0.361	0.000
Tumor size (>3 cm, ≤5 cm *vs*. ≤3 cm)	-1.390	0.362	0.000
Chronic liver disease (Non-viral *vs*. Viral HBV)	0.714	0.501	0.155
AFP level (>20 ng/dl *vs*. ≤20 ng/dl)	-0.002	0.318	0.994

HBV, Hepatitis B Virus; AFP, alpha-fetoprotein.

US, MRI and CT [19, 42]. Although MRI may offer the best guidance, the expense of this technology and the requirement for special non-metal surgical instruments limit its use. CT offers the second best guidance. It is affordable and does not require specialized surgical instruments, compared to MRI. CT provides images of significant higher resolution than US and clearly shows organ structures during probe insertion [43]. Therefore, we chose CT as the guidance method for the present study.

In this study, therapeutic efficacy of percutaneous cryoablation was compared with hepatectomy. Patients were enrolled in the study if they had fewer than three tumors, and all tumors were <5 cm in diameter. No significant differences were observed overall or in disease-free survival between the two groups. To the best of our knowledge, this is the largest prospective, multi-center study to compare these two treatment modalities for HCC tumors.

We demonstrated that percutaneous cryoablation is a reliable and safe method for HCC tumor treatment. When either percutaneous cryoablation or hepatectomy was used for treatment, overall survival and disease-free survival rates were the same for patients with fewer than three tumors with a maximum diameter <5 cm. CT was used to guide cryoprobes during all cryoablation procedures conducted during the present study, which greatly improved the precision of probe localization, reduced damage to normal liver tissue, reduced damage to liver vasculature, and enhanced the safety of the therapy. Additionally, we observed that the survival of patients with tumors ≤3 cm in diameter or single tumors was significantly better when compared with patients that had tumors >3 cm or multiple tumors. This final observation was consistent with previous reports [44, 45].

In conclusion, this study demonstrates that percutaneous cryoablation is as effective as hepatectomy for HCC treatment. This approach is best suited for patients with tumors <5 cm in maximum diameter. With recent improvements in technology and associated advances in both cryoprobe and guidance technologies, percutaneous cryoablation should be strongly considered when determining the most appropriate treatment modality for HCC.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial or not-profit sectors.

Disclosure of conflict of interest

None.

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