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Percutaneous comprehensive cryoablation for metastatic esophageal cancer after failure of radical surgery ☆

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ABSTRACT

Esophageal cancer is common in China. There is a lack of treatment strategies for metastatic esophageal cancer (MEC) after radical surgery on the primary tumor. Cryoablation is an attractive option because tumor necrosis can be safely induced in a minimally invasive manner. This study assessed its therapeutic effect in MEC after failure of radical surgery. One hundred and forty patients met the inclusion criteria from May, 2003 to March, 2011. Comprehensive cryotherapy of multiple metastases was performed on 105 patients; 35 received chemotherapy. No severe complications occurred during or after cryoablation. Overall survival (OS) was assessed according to therapeutic protocol, pathologic type, treatment timing and number of procedures. The OS of patients who received comprehensive cryoablation (44 ± 20 months) was significantly longer than that of those who underwent chemotherapy (23 ± 24 months; $P = 0.0006$). In the cryotherapy group, the OS for squamous cell carcinoma (45 ± 19 months) was longer than that for adenocarcinoma (33 ± 18 months; $P = 0.0435$); the OS for timely cryoablation (46 ± 19 months) was longer than that for delayed cryoablation (33 ± 20 months; $P = 0.0193$); the OS for multiple cryoablation (50 ± 17 months) was longer than that for single cryoablation (37 ± 20 months; $P = 0.0172$); and the OS for cryo-immunotherapy (56 ± 17 months) was longer than that for cryoablation alone (39 ± 19 months; $P = 0.0011$). Thus, comprehensive cryotherapy may have advantages over chemotherapy in the treatment of MEC and, in patients with squamous cell carcinoma, supplementary immunotherapy and timely and multiple cryoablation may be associated with a better prognosis.

Introduction

Esophageal cancer is one of the most common cancers in China; about 250,000 new patients receive this initial diagnosis every year, which is half of all esophageal cancer cases worldwide [21]. In early stage cancers with mucosal involvement, surgery has been the main treatment of choice [15], with a median survival of about 15–18 months, corresponding to a 5-year survival rate of less than 20% [19]. Although radical surgery, radiotherapy and chemotherapy all can be used in the treatment of early stage esophageal cancer, many patients suffer tumor recurrence or distal metastases. Palliative strategies, such as endoscopic treatment [38], photodynamic therapy and cryotherapy, chemotherapy [13], radiotherapy [14], targeted drugs [3,18,36,39] and immunotherapy [24,44], can also be used in patients with metastatic esophageal cancer (MEC), though the outcomes in such cases are considerably worse. Cryosurgery, which causes tumor cell necrosis by formation of an ice ball, has potential for use in MEC. It is relatively safe because tumors can be located precisely by imaging methods such as computed tomography (CT) or magnetic resonance imaging, and there is little trauma. Esophageal cancer can metastasize to the neck and thyroid, lung and mediastinum, liver, abdominal wall or peripheral stomach, and cryosurgery can used to ablate tumors in these locations [7,37]. With comprehensive cryoablation, overall survival (OS) can be significantly extended, as demonstrated for patients with metastatic pulmonary [27], breast [28], pancreatic [9] and hepatic [25] cancer and mesothelioma [8]. In this study, 140 patients with MEC after radical surgery were enrolled for comprehensive cryoablation and the therapeutic effect was investigated retrospectively. The value of comprehensive cryoablation and

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supplementary dendritic cell (DC) and cytokine-induced killer (CIK) cell immunotherapy were also examined.

Materials and methods

Ethics

The study protocol received ethical approval from the Regional Ethics Committee of Guangzhou Fuda Cancer Hospital. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

Patient selection

Between May, 2003 and March, 2011, 352 patients came to our hospital for further treatment following the finding of distant metastases of esophageal cancer after primary resection. One hundred and forty patients met our inclusion criteria and were enrolled in the study. Surgery and chemotherapy were deemed unsuitable in any of the following situations: multifocal disease; patient refused to undergo surgery or chemotherapy or was seeking further treatment after failure of chemotherapy; severe complications (i.e. hypertension, ascites); or advanced age. Ideal patients for comprehensive cryoablation are those with: Karnofsky performance status score $\geqslant 70$; platelet count $\geqslant 80 \times 10^9$/l; white blood cell count $\geqslant 3 \times 10^9$/l; neutrophil count $\geqslant 2 \times 10^9$/l; hemoglobin $\geqslant 90$ g/l; prothrombin time international normalized ratio $\geqslant 1.5$; hepatic tumor not obviously invading the gallbladder, diaphragm or large vessels; absence of level 3 hypertension, severe coronary disease, myelosuppression, respiratory disease and acute or chronic infection; and adequate hepatic function (bilirubin <30 μM, aminotransferase <60 U/l and Child–Pugh score A or B) and renal function (serum creatinine <130 μM, serum urea <10 mM).

The diagnosis of MEC was confirmed by findings on previous surgery, imaging techniques (including CT, positron emission tomography [PET]-CT and biochemical markers [29]). Among the 140 patients, 96 had one metastasis and 44 had two; 280 masses were found in total, ranging from 1.8 to 5 cm in diameter. Using Child–Pugh score to assess the severity of cirrhosis, 25 patients were class A and 18 were class B. Until July of 2013, all patients have passed away and received their final treatment in our hospital.

Stent implantation

In patients with compression of the esophagus, either a self-expanding covered metal stent (Ultraflex; Boston Scientific, Natick, MA) or a self-expanding covered silicone stent (Polyflex; Boston Scientific) was inserted. Stent implantation was performed before neck or thyroid cryoablation by a gastroenterologist who was well trained in interventional endoscopy. The exact position of the compression site was marked on the patient's skin and the stent was inserted under radioscopic guidance.

Cryoablation

Comprehensive cryotherapy was performed on 105 patients who chose this treatment. Obvious masses on PET-CT were completely cryoablated during one session. Generally, the following protocols were preferred. (1) Each procedure comprised two freeze/thaw cycles accomplished using an argon gas-based cryosurgical unit (Endocare, Irvine, CA, USA), each reaching a temperature of −180 °C at the tip of the probe. (2) For masses of diameter 1.8–3 cm, one cryoprobe (3 mm in diameter) was used under CT (usually for pulmonary tumors) or ultrasonographic (usually for tumors other than in the lung) guidance; for masses of diameter 3–5 cm, two or three cryoprobes were used. (3) Avoiding injury to vital nerves, blood vessels, the bile duct and the intestine, as much of the tumor as possible was ablated. (4) The duration of freezing was dependent on the achievement of an ice ball, visible as a hypoechoic region on CT or ultrasonography, and a margin of at least 1 cm of normal tissue was frozen circumferentially around the tumor. (5) The maximal freezing time was 15 min, followed by natural thawing for 5 min; this cycle was then repeated. (6) The tracts formed were sealed with fibrin glue immediately after removal of the cryoprobes to ensure hemostasis.

Immunotherapy and chemotherapy

Twenty-eight patients opted for immunotherapy (adoptive transfer of DCs and CIK cells performed four times). DCs/CIK cells were generated according to previously published protocols under "Good Manufacturing Practice" conditions [20,26] and the timing of treatment was compatible with the cryosurgery. One day before drawing blood, 150 μg recombinant human granulocyte–macrophage colony-stimulating factor (rhGM-CSF, Peprotech, Rocky Hill, NJ) was injected to mobilize the white blood cells into the blood. Using Ficoll–Hypaque density centrifugation, we harvested peripheral blood mononuclear cells (PBMCs) from peripheral blood samples (80 ml) collected from the 28 patients 2 days before cryosurgery.

For DC culture, PBMCs were resuspended in "DC" medium (X-VIVO 15 [Lonza, Basel, Switzerland], 25 ng/ml recombinant human interleukin [rhIL]-4 [Peprotech] and 30 ng/ml rhGM-CSF [Peprotech]) at a concentration of $1–2 \times 10^6$/ml. The cells were then allowed to adhere in two plastic flasks (T75; Corning Costa, Cambridge, MA), each containing 50 ml DC medium and approximately 10^8 cells. After overnight culture at 37 °C with 5% CO_2, the suspended cells were transferred to two fresh flasks. The cells sticking to the initial two flasks were continuously cultured in DC medium and a small amount of fresh medium was added daily to the cultures.

For culture of CIK cells, the non-adherent cells from the DC culture were suspended in "CIK" medium (X-VIVO 15 [Lonza], 1000 U/ml IL-2 [Peprotech], 2.5 μg/ml monoclonal antibody to CD3 [OKT-3; Jansen-Kyowa, Tokyo, Japan], 25 μg/ml phytohemagglutinin [Peprotech] and 1000 U/ml recombinant human interferon-γ [Peprotech]). The CIK cells were allowed to grow and then continuously passaged. At approximately 7 days of culture, the CIK cells were passaged to fourteen T225 flasks. Cells adhering to the flasks were removed with a cell spatula, centrifuged and resuspended in "DC–CIK" medium (X-VIVO 15 [Lonza], 400 U/ml IL-2 and 0.5 μg/ml monoclonal antibody to CD3). All DCs were distributed evenly in the 14 T225 flasks containing CIK cells (approximately 10^8 DCs per flask). After co-culture for 24–48 h, almost 1 week after cryosurgery, the DC–CIKs were harvested and suspended in 100 ml saline for intravenous injection (cells were collected on four consecutive days; 6 to 10×10^9 cells were collected on each day). The final cell products included $CD3^-CD56^+$ (natural killer) cells (15 ± 4%), $CD3^+$ (T) cells (87 ± 8%) and $CD3^+CD56^+$ (natural killer T) cells (27 ± 5%); these were determined by flow cytometry, assessed for viability by the dye-exclusion test and checked twice for possible contamination by bacteria, fungi and endotoxins.

Thirty-five patients refused to undergo cryo- or immunotherapy owing to its cost or their health or age. These patients received combination chemotherapy with a fluoropyrimidine, platinum/fluoropyrimidine and irinotecan/fluorouracil, or docetaxel/cisplatin/fluorouracil and docetaxel/cisplatin [41].

Evaluation and statistical analysis

Complications and side effects were recorded and classified in accordance with the Common Terminology Criteria of Adverse Events version 4.0. Radiographic local tumor control was assessed using image-guided tumor ablation criteria [16] and the revised Response Evaluation Criteria in Solid Tumors version 1.1 [12]. Thoracic and/or abdominal ultrasonography was performed both 1 day and 1 week after treatment according to the site of ablation. Follow-up dynamic CT was performed 1 month post-cryotherapy and then at 3–4 month intervals. Three diagnostic radiologists reviewed CT scans for every case to determine whether progression or recurrence had occurred. OS was calculated from the time of diagnosis of MEC to death and analyzed using the Kaplan–Meier test with log-rank analysis. Significant differences were indicated by $P < 0.05$, $P < 0.01$ or $P < 0.001$. All analyses were conducted using GraphPad software (San Diego, CA, USA).

Results

Clinical data

The basic clinical data of the 140 patients in this study are shown in Table 1, including sex, nationality, pathologic type, age, metastatic site and symptoms. There were no obvious differences between the two treatment groups.

Perioperative outcomes

Twenty-five patients in the cryotherapy group and nine patients in the chemotherapy group underwent esophageal stent implantation for symptoms of compression caused by neck or thyroid metastases; no stent-associated complications were observed during or after implantation and dysphagia was completely relieved after treatment. All metastases were ablated successfully during the first comprehensive cryotherapy procedure. Seven patients (25%) who also underwent immunotherapy developed mild fever (body temperature <39 °C) but recovered within 3 days. Severe complications that are often associated with ablation in the lung, thyroid, liver or peripheral stomach, such as thyroid shrinkage, damage to neck nerves and blood vessels, respiratory failure, liver rupture and gastrointestinal perforation, did not occur after cryoablation and there were no complication-associated deaths. Many mild side effects associated with cryoablation in the neck, lung, liver or peripheral stomach were observed in the 105 patients after the first cryosurgery procedure, but these were resolved with or without symptomatic treatment.

Cryoablation of the 73 neck and thyroid metastases was performed with meticulous care and no pathologic damage to vital nerves or blood vessels occurred. Cryoablation of the 17 perigastric metastases was associated with the following complications: abdominal distension in six patients (35%, gradually resolved within 2 weeks); anorexia in 15 patients (88%, relieved within 1 week to 1 month); and dyspepsia in 16 patients (94%, relieved within 1 week). Cryoablation of the 75 lung and mediastinal metastases had the following complications: transient hemoptysis in 19 patients (25%) and pneumothorax in 23 (31%) (both resolved in 2 days after symptomatic treatment); bradycardia in 9 (12%) and hypotension in 11 (15%) (recovered spontaneously within a few hours); dull pain in the anterior chest soon after treatment, possibly due to damage to the intercostal nerves, in 16 (21%) (usually resolved naturally within a few months); and cough with blood-streaked sputum in 21 (28%) (improved within 3–5 days without treatment). Cryoablation of the 25 hepatic metastases was associated with the following complications: mild liver hemorrhage in four patients (16%) (healed within 5 days after injection of hemostatic agents); rupture of liver capsule in two (8%) (recovered after blood transfusion); transient thrombocytopenia within 1 week after cryoablation in four (16%) (two received platelet transfusion); and liver abscess at a previous cryoablation site 2 and 4 days after cryoablation in two patients (8%) (recovered after antibiotic treatment and drainage).

Table 1
Basic clinical data of the 140 patients in this study.

	105 Patients with comprehensive cryotherapy	35 Patients with chemotherapy
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Sex		
Male	88	29
Female	17	6
Nationality		
China	93	30
Southeast Asia	12	5
Pathologic type		
AC	13	8
SCC	92	27
Age (yr)		
Range	36–89	40–76
Average	58	58
Single metastasis		
Neck and thyroid	26 Lesions	5 Lesions
Lung and mediastinum	27 Lesions	9 Lesions
Peritoneum	5 Lesions	3 Lesions
Liver	7 Lesions	4 Lesions
Peripheral stomach	7 Lesions	3 Lesions
Double metastases		
Neck and thyroid	47 Lesions	14 Lesions
Lung and mediastinum	48 Lesions	16 Lesions
Peritoneum	14 Lesions	6 Lesions
Liver	18 Lesions	7 Lesions
Peripheral stomach	10 Lesions	4 Lesions
Symptoms		
Dysphagia	25	9
Anorexia and dyspepsia	21	6
Mild dyspnea	29	9
Cough and expectoration	16	5

AC, adenocarcinoma; SCC, squamous cell carcinoma.

Influence of therapeutic method and cancer characteristics on OS

The survival time of patients treated with comprehensive cryotherapy ($n = 105$) or chemotherapy ($n = 35$) from May, 2003 to March, 2011 in our hospital was determined from follow-up data. The OS of all patients with metastases after surgery was 39 ± 23 months, with a median survival time (MST) of 39 months. The OS of the cryotherapy group was 44 ± 20 months (MST, 42 months), which was significantly longer than that of the chemotherapy group (23 ± 24 months; MST, 18 months; $P = 0.0006$) (Fig. 1A).

Among patients with neck and thyroid metastases, 25 in the cryotherapy group and nine in the chemotherapy group had obvious symptoms of esophageal compression and underwent stent implantation. There was no difference in OS between those with and those without esophageal compression after treatment (Fig. 1B) in either the cryotherapy group (with and without stent, 49 ± 19 and 42 ± 20 months, respectively; $P = 0.2667$) or the chemotherapy group (with and without stent, 23 ± 22 and 23 ± 25 months; $P = 0.7386$). The sensitivity of the different pathologic types to treatment method was also analyzed (Fig. 1C). In the cryotherapy group, the OS of patients with squamous cell carcinoma (SCC) was 45 ± 19 months, which was longer than that of patients with adenocarcinoma (AC) (33 ± 18 months; $P = 0.0435$). In

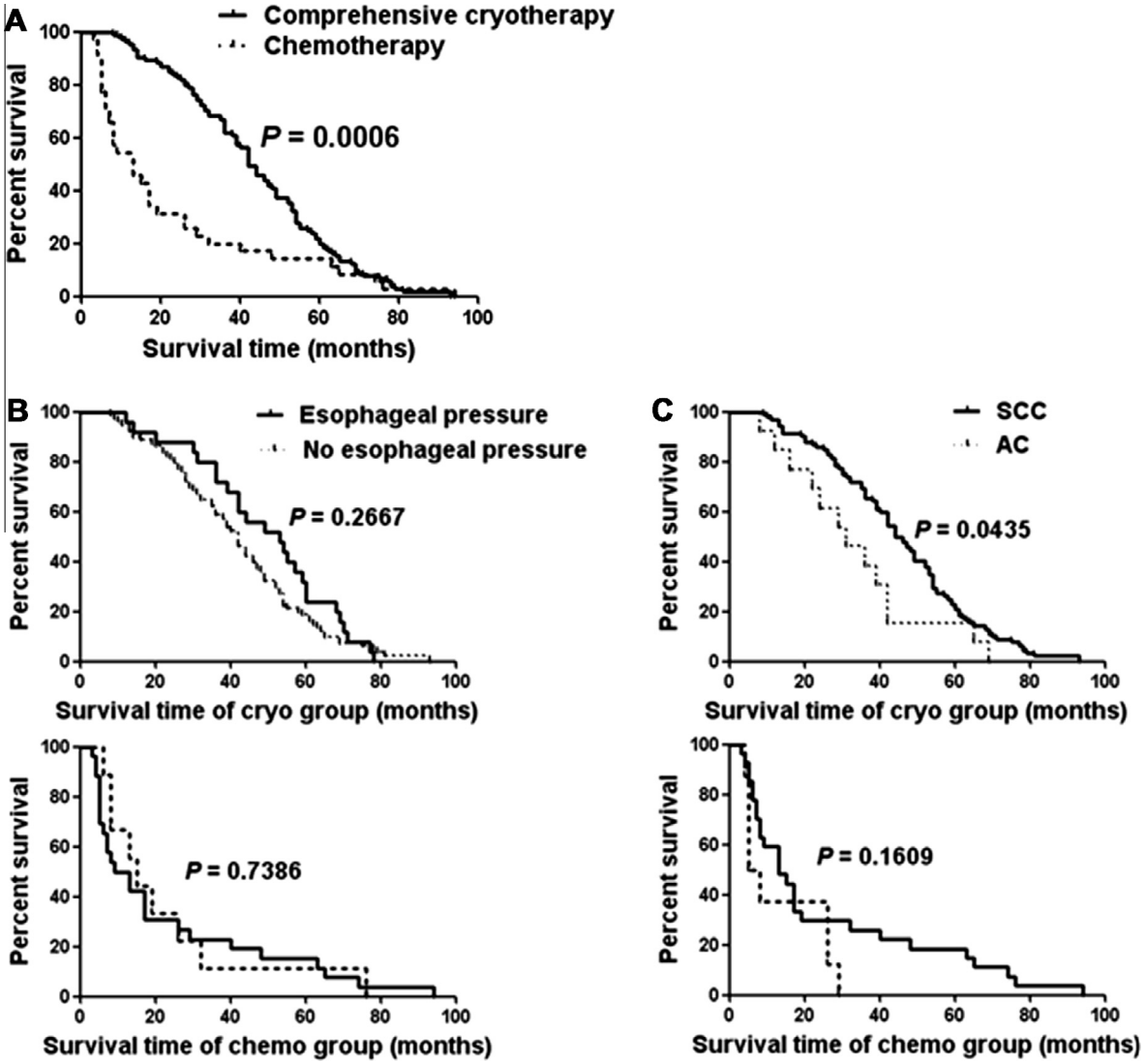

Fig. 1. Overall survival of patients who underwent comprehensive cryotherapy or chemotherapy. The data were analyzed using the Kaplan–Meier test with log-rank analysis. (A) One hundred and forty patients were enrolled; 105 received comprehensive cryotherapy and 35 received chemotherapy; (B) OS between the patients with esophageal pressure and the patients without esophageal pressure (comparison was done in two respective groups of patients (cryotherapy and chemotherapy)); (C) OS between the patients of SCC and the patients of AC (comparison was done in two respective groups of patients (cryotherapy and chemotherapy)). SCC, squamous cell carcinoma; AC, adenocarcinoma.

the chemotherapy group, the OS of SCC patients was 25 ± 27 months, which was not significantly different from that of the AC patients (14 ± 11 months; $P = 0.1609$).

In the cryotherapy group, the timing and number of procedures and the use of immunotherapy varied with patients' geographical distance from our hospital, opinions about the treatment and economic circumstances. After tumor metastasis, 85 patients came to our hospital within 6 months (timely) and 20 patients came in 6–15 months (delayed); the OS of patients who received timely cryotherapy was 46 ± 19 months (MST, 46 months), which was longer than that of those who received delayed cryotherapy (33 ± 20 months; MST, 31 months; $P = 0.0193$) (Fig. 2A). After their first comprehensive cryotherapy procedure, 48 patients did not undergo further physical examination or treatment (single cryoablation), whereas the other 57 continued with examination and treatments (multiple cryoablation); the OS of patients who underwent multiple cryotherapy was 50 ± 17 months (MST, 49 months), which was longer than that of those who underwent single cryotherapy (37 ± 20 months; MST, 31.5 months; $P = 0.0172$) (Fig. 2B). To enhance the antitumor function of the immune system and delay tumor recurrence and metastasis, 28 patients underwent immunotherapy with cryotherapy (cryo-immunotherapy), whereas 77 patients accepted cryotherapy only (cryotherapy alone); the OS of patients who received cryo-immunotherapy was 56 ± 17 months (MST, 56.5 months), which was longer than that of those who received cryotherapy alone (39 ± 19 months; MST, 39 months; $P = 0.0011$) (Fig. 2C).

Discussion

In MEC patients, appropriate choice of treatment is vital for survival. Chemotherapy alone has response rates of only 20–40% and MSTs of 8–10 months [13], with no significant effect of prognostic factors on survival time [1]. Postoperative radiotherapy increased mortality, with a MST of only 8.7 months [14]. Some targeted drugs can increase the survival time of patients with MEC to 13.8 [3], 14.9 [39], 16.2 [36] or even 17 [18] months. Endoscopic treatment

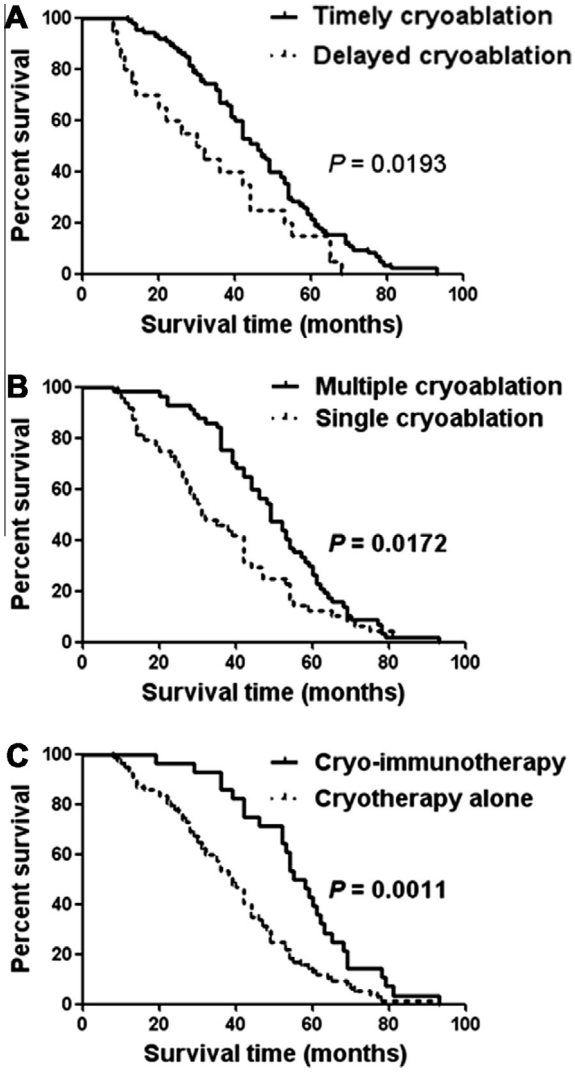

Fig. 2. Comparison of overall survival (OS) by timing and number of cryoablation procedures and use of immunotherapy. The data were analyzed using the Kaplan–Meier test with log-rank analysis. (A) OS according to timing of ablation; (B) OS according to number of ablation procedures; (C) OS according to use of cryotherapy alone or cryo-immunotherapy.

modalities such as stents or laser therapy play an important role in palliation, with a MST of about 5 months [38]. Immunotherapy with DCs has an obvious curative effect [24,44]. In the present study, the OS of the comprehensive cryotherapy group was 44 ± 20 months (MST, 42 months), which was significantly longer than that of the chemotherapy group (23 ± 24 months; MST, 18 months; $P = 0.0006$). Although this primary retrospective study is at an early stage, the results indicate that comprehensive cryotherapy in MEC has a survival advantage over chemotherapy.

Several factors can influence the therapeutic outcome in MEC, including pathologic type [5], stage of the disease, tumor length [4,45], lymphatic invasion [40,42], degree of histopathologic response to treatment [6,10], performance status and, possibly, genetic variations [22,34]. In this study, we found that the OS of SCC patients who underwent cryotherapy was longer than that of AC patients, the OS of SCC patients who underwent chemotherapy had no difference with that of AC patients. Timing of cryoablation [8,9,25,27,46] also influences survival. The OS of patients who received timely cryotherapy was longer than that of those in whom cryotherapy was delayed. Compared with the OS of patients who underwent a single cryotherapy procedure, multiple treatments showed a clear advantage. Four-fifths of our patients underwent comprehensive cryoablation 1–6 months after metastasis was found; delays were due to the patients' geographical distance from our hospital, opinions about the treatment and economic circumstances. Due to the rapid progression of this disease [1,10,15], significant decline in the patient's condition or even death can occur during this time. Thus, the effect of delayed treatment on lifespan is unsurprising, even in patients receiving the same treatment program. This finding indicates that treatment timing is as important as the treatment program for extending survival time. In summary, pathologic type, treatment timing and use of single or multiple cryotherapy procedures can influence the survival of patients with MEC.

The OS of patients who received cryo-immunotherapy was 56 ± 17 months (MST, 56.5 months), which was significantly longer than that of those who received cryotherapy alone (39 ± 19 months; MST, 39 months; $P = 0.0011$). Cryosurgery achieves physical ablation of tumor in situ and cannot effectively eliminate circulating tumor cells or tumor micrometastases [23]. Recurrence and metastasis will thus continue to occur even after complete ablation of the primary lesion and it is necessary to combine cryoablation with another, systemic therapeutic method to prolong life. It is well known that cryosurgery has two basic functions: destruction of the tumor microenvironment and the release of tumor antigens to induce antitumor immunity. The tumor microenvironment is hostile toward immune cells and this can result in local immune tolerance, due to which the tumor cells escape immune surveillance [30]. Destruction of the tumor microenvironment by cryosurgery provides opportunities for immunotherapy. In addition, destruction of the tumor by cryosurgery and in situ delivery of antigen presenting cells promotes antineoplastic immune responses [33]. These tumor antigens can act as in vivo targets for DCs [17]. Activated DCs after cryoablation have been found to be potent stimulators of both $CD4^+$ and $CD8^+$ T cells in experimental [11] and human [2,35] studies. It has been observed that temporary immunotherapy reduced tumor recurrence; the level of tumor-specific lymphocytes decreased before tumor recurrence and increased again with booster immunizations [43]. Thus, it seems plausible that booster immunizations prevent tumor recurrence and cryo-immunotherapy may be a better strategy for treatment of MEC.

The present results for percutaneous comprehensive cryoablation in MEC are only preliminary findings. There are limitations to this research. Our patients had received several types of therapy in different medical centers before their metastases were detected, and our treatment program determined their survival time only after metastasis had occurred. The esophagus is a cavity viscera, and tumor necrosis induced by cryoablation can cause esophageal perforation [31,32]. All of our patients had MEC, and metastases rather than primary tumors were treated by percutaneous comprehensive cryoablation.

In conclusion, we found that comprehensive percutaneous cryoablation prolonged the survival time of patients with MEC by 24 months. Timely therapy, multiple treatments and supplementary immunotherapy may all have contributed to the prolonged survival time observed in this study.

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