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Cryoprotective therapy for huge hepatocellular carcinoma: A study of 14 patients with a single lesion ☆

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ABSTRACT

Percutaneous cryoablation is a potential cure for hepatocellular carcinoma (HCC). This study reviewed retrospectively clinical data from 14 patients who underwent cryoablation of huge HCC (long diameter >7 cm). The side effects of cryosurgeries and liver function reverse were recorded and compared everyday. All the patients survived cryosurgery and none died before leaving hospital 2 weeks later. Despite liver-protective treatment before cryosurgery, alanine transaminase (ALT) and aspartate transaminase (AST) levels were increased significantly, but returned to preoperative levels 2 weeks post-cryosurgery. Before cryosurgery, mean total bilirubin (T.BIL) and direct bilirubin (D.BIL) levels were normal; 8–10 days after cryosurgery, they increased more than two-fold, but returned to the preoperative level 2 weeks post-cryosurgery. Serum transaminase and bilirubin levels were compared between hepatitis B positive and negative patients. The hepatitis B negative group's AST level increased significantly 1 day post-cryosurgery (mean, 186 U/L) and decreased to the preoperative level at day 14. In the hepatitis B positive group, means transaminase and bilirubin reached peak values at different days post-cryosurgery. Overall, ALT and AST are valuable indicators of liver function impairment following cryosurgery. In patients with hepatitis B virus, close attention to the serum bilirubin level should be paid 8–10 days after cryosurgery. Liver-protective treatment may alleviate liver function impairment caused by cryosurgery of huge HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, and its incidence is increasing [5]. HCC is associated with chronic liver disease (i.e. hepatitis or cirrhosis) in about 80% of patients, which has major implications for prognosis and therapeutic options [10]. Many patients are unsuitable for tumor resection because of poor hepatic reserve, cirrhosis or the presence of multicentric tumors or extrahepatic disease [3,9]. Fortunately, non-surgical percutaneous tumor ablation therapies – namely, percutaneous ethanol injection, microwave coagulation, radiofrequency ablation and cryoablation – have been introduced, and now play an important role in the treatment of HCC [16,22]. Cryoablation is an attractive option because the therapy can prolong the patient's life and the complications are acceptable. A long-term study of medium to large lesions (mean diameter 4.6 cm) treated by cryoablation and/or transarterial chemoembolization showed a 5 year survival rate of 23% and a local progression rate of 24% [14,23]. The complication rate following cryoablation may be as high as 40%. Side effects include liver parenchyma fracture, cryoshock, biliary fistula, hemorrhage, cold-induced lesions in nearby structures, thrombocytopenia and coagulopathy; however, these can be reduced by skilled operators, strict patient selection and symptomatic treatment [15,18,19,21]. Ablating the tumor as completely as possible inevitably damages the normal liver tissue, affecting liver function. Preservation of liver function is at least as important as ablation of HCC nodules in patients liver function is already impaired [11]. After liver cryosurgery, rapid elevation of transaminases [1,7,8,17,20,26] and bilirubin [1,2,8,12] is common; however, the elevation is usually transient and usually normalizes within a few days.

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Previous studies have reported on either laparotomy [2,7,8,17,20,26] or percutaneous ablation of small tumors (long diameter <7 cm) [12]; however, there is only one report of percutaneous ablation of huge tumors (long diameter >7 cm) [1]. Thus, the effectiveness and safety of single percutaneous cryosurgery on huge tumors is not clear. In this study, 14 HCC patients were observed for hepatic functional reserve after single percutaneous cryoablation. Liver-protective drugs and symptomatic treatment were provided to these patients after cryosurgery (so called "cryoprotective therapy"). Serum transaminase and bilirubin levels were compared between hepatitis B virus positive and hepatitis B virus negative patients.

Materials and methods

Ethics

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

Patient selection

For all patients, liver pathology imaging (usually magnetic resonance imaging (MRI)) and tumor markers confirmed the diagnosis of unresectable HCC. Ideal patients for this research were those with: only one significant tumor in the liver; Karnofsky performance status score $\geqslant 70$; platelet count $\geqslant 100 \times 10^9$/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 (total bilirubin (T.BIL) <75 μmol/L, direct bilirubin (D.BIL) <39 μmol/L and Child-Pugh score A or B) and renal function (serum creatinine <130 μM, serum urea <10 mM). Between November 2008 and November 2011, 14 patients met our inclusion criteria and received cryoprotective therapy.

Cryotherapy

Each procedure comprised two freeze–thaw cycles, accomplished using an argon gas-based cryosurgical unit (Endocare, Irvine, CA, USA). Six to ten cryoprobes (3 mm in diameter) were inserted into the tumor mass under ultrasonographic guidance, each reaching a temperature of −150 °C at the tip of the probe. The duration of freezing was dependent on the achievement of an ice ball that extended 1 cm beyond the boundary of the tumor and was visible as a hypoechoic region on ultrasonography. Generally, the maximal freezing time was 15 min, followed by thawing for 5 min; this cycle was then repeated. The tracts formed were sealed with fibrin glue immediately after removal of the cryoprobes to ensure hemostasis.

Tests of hepatic functional reserve

An automatic biochemical analyzer (Hitachi-7100; Hitachi, Tokyo, Japan) measured hepatic function. The alanine transaminase (ALT) and aspartate transaminase (AST) activities were detected using the velocity method. A kit (Biosino Biotechnology and Science Inc., Beijing, China) determined the T.BIL and D.BIL using the vanadate method. Blood was drawn in the morning on an empty stomach. The tests were performed every 1–2 days until patient discharge. Normal ranges were defined as 5–35 U/L for ALT, 8–40 U/L for AST, 0–25.5 μmol/L for T.BIL and 0–13 μmol/L for D.BIL. Values higher than the upper limits were considered abnormal.

Liver-protective therapy and symptomatic treatment

Diammonium glycyrrhizinate capsules (150 mg twice per day orally; Chia Tai Tianqing Pharmaceutical Co. Ltd, Jiangsu, China) [25] and Atomolam (reduced glutathione tablets, 180 mg/day intravenous injection; Yaopharma, Chongqing, China) [24] were administered to all patients. In patients with abnormal transaminase activity, the two drugs were given until the liver function returned to normal. In patients with normal transaminase activity, the drugs were usually stopped 3–4 days after cryoablation to help maintain normal liver function. In patients with thrombocytopenia (platelet count $\leqslant 150 \times 10^9$/L), the platelet count was closely supervised and recombinant human interleukin-11 (rhIL-11, 25 μg/kg/day; Northland Biotech, Beijing, China) was given if coagulopathy (platelet count $\leqslant 100 \times 10^9$/L) was detected [21]. Regular hemostatic agents were used to treat liver hemorrhage after cryosurgery. Antibiotics and drainage were used to treat liver abscesses.

Evaluation and statistical analysis

All cases of impaired hepatic function in this study were unambiguous. Abdominal ultrasonography was performed both 1 day and 1 week after treatment. Complications were recorded and classified in accordance with the Common Terminology Criteria of Adverse Events v4.0. MRI was performed 2 weeks after treatment. Image-guided tumor ablation criteria assessed radiographical local tumor control [6]. The revised Response Evaluation Criteria in Solid Tumors v1.1 were used to assess the response of the hepatic tumors [4]. Bonferroni's multiple comparison tests were used to compare data between time points, which were day 0, 1, 2, 4, 6, 8, 10, 12 and 14 after cryosurgery. The test results were expressed as the mean ± standard error and statistical significance was indicated by $P < 0.05$, $P < 0.01$ or $P < 0.001$. Prism 5 (GraphPad, San Diego, CA, USA) performed all the analyses and generated the figures.

Results

Clinical data

Detailed data for the 14 patients before single hepatic cryoablation are shown in Table 1. All of the patients were men. Tumor long diameters were 7–10 cm in 8 patients, 10–14 cm in 6 patients. Twelve (86%) had stage IV disease, 8 (57%) had a history of hepatitis, 6 (43%) had initially been treated surgically and 4 (29%) had been treated with systemic chemotherapy in other centers, and 5 (36%) had thrombocytopenia, and 4 (29%) had small to medium amount of ascites.

Perioperative outcomes

All percutaneous cryoablations of hepatic lesions were performed successfully. No severe complications (such as liver rupture or failure, myoglobinuria or acute renal failure) were discovered post-cryoablation. Mild side effects occurred after cryosurgery, but the affected patients recovered with or without symptomatic treatment. Slight liver hemorrhage occurred in three patients (21%), but all cases resolved within 5 days with injection of hemostatic agents. Liver capsular rupture occurred in one patient (7%), but resolved after a blood transfusion. New thrombocytopenia

Table 1
Detailed data of 14 patients before cryoprotective therapy.

No.	Age	Nationality	Cancer stage	Size of liver tumor (cm × cm)	ALT/AST (U/L)	T/D.BIL (μmol/L)	Hepatitis	Ascites
1	54	China	IV	10.7 × 7.6	29/28.3	15.7/5.9	–	–
2	48	China	IV	11.1 × 7.8	41/126.4	20.6/10.2	B+	Medium amount (~700 mL)
3	63	Indonesia	IV	9.4 × 8.2	24.5/88.8	36.1/25.3	–	–
4	58	China	III	9.2 × 8.8	94/11	23.8/15.9	B+	–
5	40	China	IV	11.9 × 11.3	46.7/206.2	14.9/6.7	B+	Small amount (~400 mL)
6	40	China	IV	8.7 × 7.9	81.6/70	19.5/9.4	B+	–
7	58	Thailand	IV	9.6 × 8.5	38.2/109.4	26/11	B+	–
8	79	China	IV	8.3 × 7	11.8/24	5.8/3.2	–	–
9	55	China	IV	13.8 × 10	45.2/105.9	12.3/8.5	B+	–
10	68	Indonesia	IV	10.1 × 9.5	20.2/26.2	24.6/10	B+	Small amount (~450 mL)
11	47	China	IV	10.4 × 8.5	41.2/74.2	14.5/7.4	–	Small amount (~350 mL)
12	60	China	IV	8.1 × 7	44.2/45.8	10.4/5.4	–	–
13	60	China	III	8 × 7	13.8/25.9	11.7/4.4	–	–
14	48	Thailand	IV	9 × 8	35.7/54.7	5.7/3.7	B+	–

Note: All of the patients were men; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin.

was found in five patients after cryosurgery: all patients recovered within 2 weeks after administration of rhIL-11. Liver abscess was found in one patient (7%) at the site of cryoablation; however, the patients recovered within 2 weeks after antibiotic and drainage treatment. Nearly all patients had pain in the abdominal puncture site, and they all recovered within one week after administration of analgesic drugs. Before leaving our hospital, radiographical assessment confirmed that all the patients had good control of their liver functions.

Changes of hepatic functional reserve after cryoprotective therapy

Before cryosurgery, 10 of the 14 patients had abnormal transaminase levels and three had abnormal bilirubin levels. The test results were as follows: ALT 41 ± 6 U/L; AST 71 ± 14 U/L; T.BIL 17 ± 2 μmol/L; and D.BIL 9 ± 2 μmol/L. Within 2 days after cryosurgery, the serum transaminase values rose rapidly to a peak (ALT 196 ± 57 U/L; AST 313 ± 67 U/L; both $P < 0.01$) and then fell gradually. Two weeks after cryosurgery, the transaminase levels recovered to their preoperative levels (Fig. 1A and B). Within 10 days after cryosurgery, the serum bilirubin values rose gradually to a peak (T.BIL 39 ± 12 μmol/L, $P < 0.05$; D.BIL 24 ± 7 U/L, $P < 0.01$) and then fell gradually. Two weeks after cryosurgery, the bilirubin levels recovered to their preoperative levels (Fig. 1C and D).

For the six hepatitis negative patients (Fig. 2), only the AST values rose rapidly at day 1 after cryosurgery (186 ± 75 U/L, $P < 0.01$; Fig. 2B) and then fell gradually. For the eight hepatitis positive patients, peaks of transaminase and bilirubin appeared at different time points after treatment. The peak of ALT was at day 2 (293 ± 85 U/L, Fig. 3A, $P < 0.001$), the peak of AST was at day 1 (408 ± 93 U/L, Fig. 3B, $P < 0.001$), the peak of T.BIL was in day 10 (57 ± 19 μmol/L, Fig. 3C, $P < 0.01$), and the peak of D.BIL was at day 8 (36 ± 10 μmol/L, Fig. 3D, $P < 0.01$). All 14 patients were given liver-protective therapy before the ablations because of their abnormal liver function. All indexes had returned to their preoperational levels 2 weeks after cryosurgery.

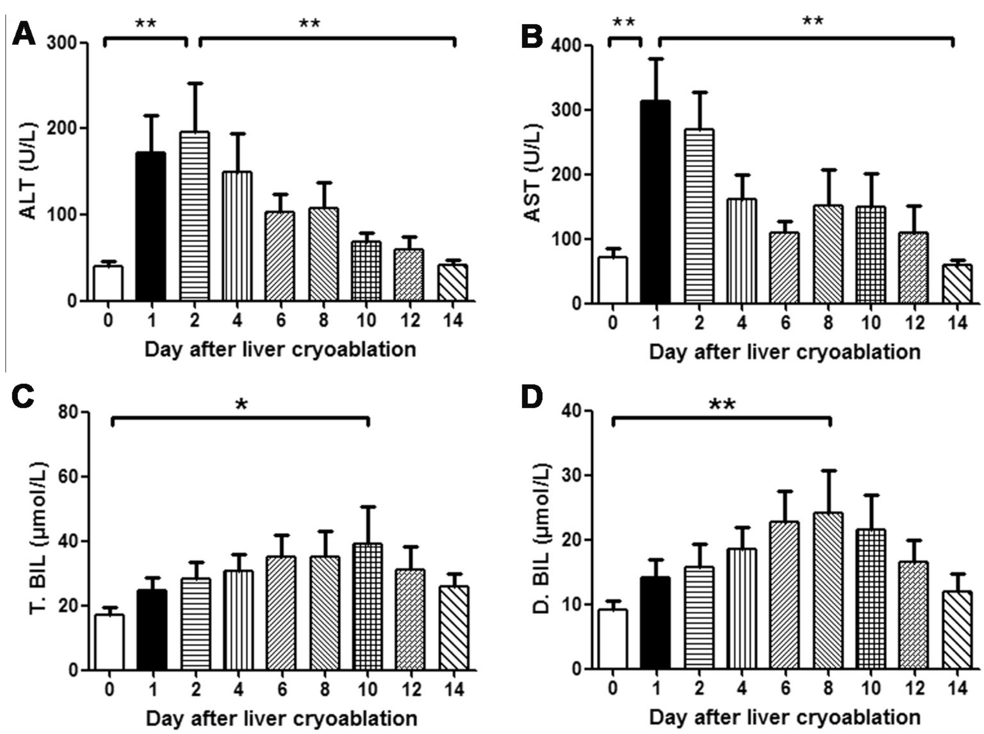

Fig. 1. Changes in hepatic functional reserve after cryosurgery of all 14 patients. Bonferroni's multiple comparison test was used to compare the results from different days. The results were tested at day 0, 1, 2, 4, 6, 8, 10, 12 and 14. Indices of hepatic functional reserve were alanine transaminase (ALT) (A), aspartate transaminase (AST) (B), total bilirubin (T.BIL) (C) and direct bilirubin (D.BIL) (D). $^{*}P < 0.05$, $^{**}P < 0.01$.

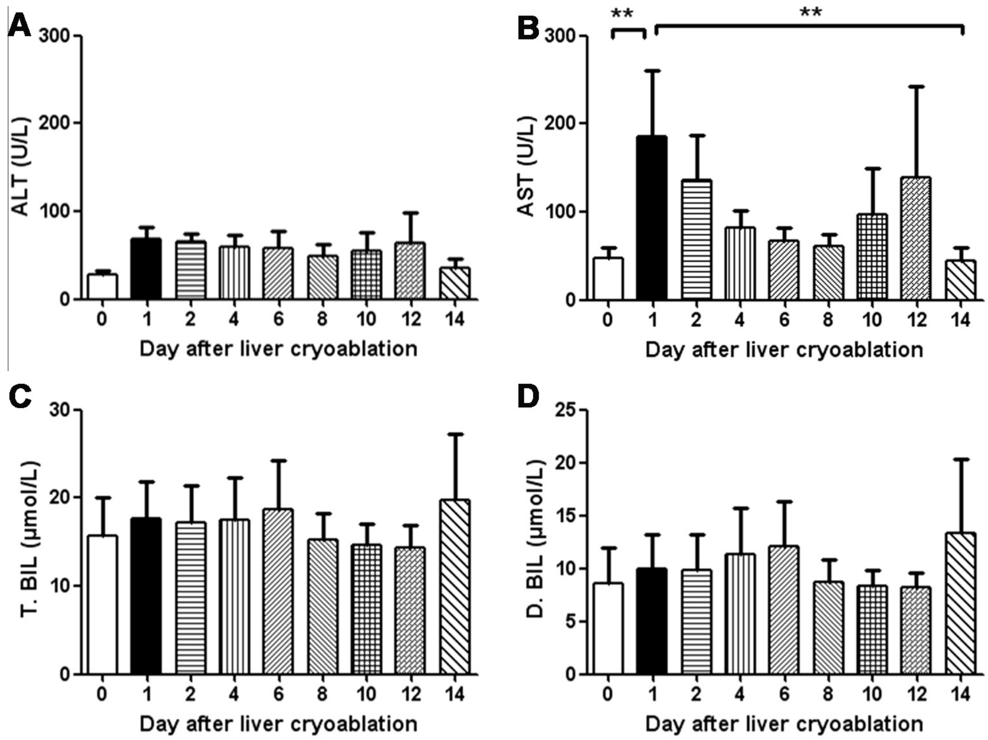

Fig. 2. Changes in the hepatic functional reserve of six non-hepatitis patients. Bonferroni's multiple comparison test was used to compare the results from different days. The results were tested at day 0, 1, 2, 4, 6, 8, 10, 12 and 14. Indices of hepatic functional reserve were alanine transaminase (ALT) (A), aspartate transaminase (AST) (B), total bilirubin (T.BIL) (C) and direct bilirubin (D.BIL) (D). $^{**}P < 0.01$.

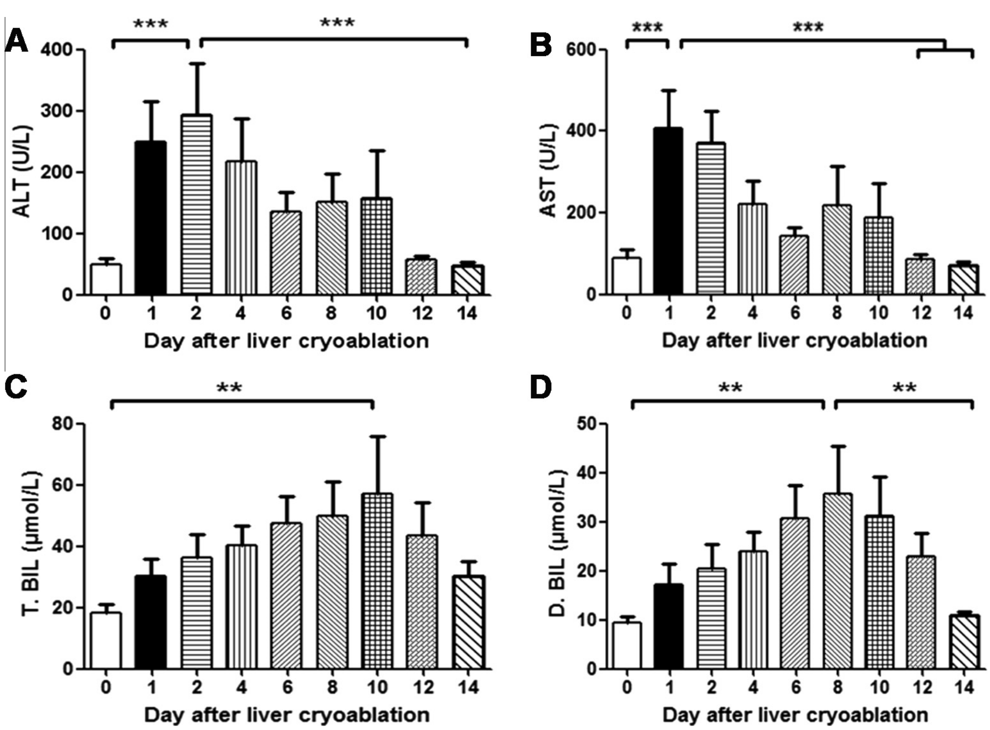

Fig. 3. Changes in the hepatic functional reserve of eight hepatitis patients. Bonferroni's multiple comparison test was used to compare the results from different days. The results were tested at day 0, 1, 2, 4, 6, 8, 10, 12 and 14. Indices of hepatic functional reserve were alanine transaminase (ALT) (A), aspartate transaminase (AST) (B), total bilirubin (T.BIL) (C) and direct bilirubin (D.BIL) (D). $^{**}P < 0.01$, $^{***}P < 0.001$.

According to our former follow-up data, the overall survival (OS) of patients who received cryosurgery was significantly longer than those received no treatment (Median OS: 26 vs. 3.5 months, $P < 0.001$) [13], which showed a huge advantage for tumor-reducing cryosurgery.

Discussion

Cryosurgery is becoming an attractive technique for non-resectable hepatic tumors. The complications of cryoablation for HCC are acceptable and the therapy can prolong the patient's life, especially for those who undergo multiple cryoablations, which can further improve survival [13]. In this study, for tumors with a long diameter (>7 cm), only one session of cryosurgery were performed according to the patients' wish. To maximize the protection of liver function, liver-protective therapy was given before and after the ablations. Based on our clinical observations, 2 weeks' rest was usually sufficient for patients to recover their strength and for most tumor breakage products to be metabolized. All of the patients in this study tolerated the treatment and no severe complications occurred after cryoablation, demonstrating the safety of this therapy. Meanwhile, radiographical assessment indicated that all the patients obtained good control of their liver functions, which demonstrated the efficacy of this therapy.

Cryosurgery for liver cancer inevitably induces hepatocyte destruction and the subsequent release of transaminases and bilirubin, which are important indices of hepatic functional reserve. From 1988 to 2005, much research was undertaken in the field of cryotherapy, which found obvious elevations of transaminases [1,7,8,17,20,26] and bilirubins [1,2,8,12]; these elevations are usually transient – especially in patients with a healthy liver – and usually normalize within a few days. Other studies did not provide any liver protection before or after ablation, whereas in the present study, with the aid of liver-protective therapy, the rapid release of transaminases was controlled and recovered quickly. It seems that the use of liver-protective therapy is beneficial for cryoablation.

Elevation of serum bilirubin was observed in the present study, which was similar to the findings of Goodie et al. [7] and Hamad and neifeld [8], but occurred on different days post-cryosurgery. Goodie et al. demonstrated an increase in serum T.BIL from 11 μmol/L preoperatively to 28 μmol/L on postoperative day, and Hamad showed that serum T.BIL almost tripled on postoperative day 1. These differences may be explained by the fact that the patients in the present study were all treated with liver-protective drugs. Perhaps the continued use of these drugs delayed the damage to normal hepatocytes, causing a gradual accumulation of bilirubins.

The different reactions after cryosurgery indicated that the degree of liver damage is more serious for hepatitis patients (all indexes were elevated significantly after cryosurgery). The causes of this phenomenon may be the overlap of double damage by the virus and the low temperature. Fortunately, liver-protective drugs could play a strong protective role, and helped the serum values of transaminase and bilirubin to return to normal after 2 weeks.

Overall, ALT and AST are valuable as indicators of liver function impairment following cryosurgery. In patients with hepatitis B virus, serum bilirubin should also be close monitored 8–10 days after cryosurgery. Liver-protective treatment may alleviate liver function impairment caused by cryosurgery of huge HCC.

References

[1] A.M. Ali, N. Lizhi, L. Jialiang, Y. Fei, W. Yuan, Z. Jianying, Y. Jin, C. Jibing, M. Feng, X. Kecheng, Cryoprotective therapy for hepatocellular carcinoma: study of 51 patients with a single lesion, Cryobiology 69 (2014) 61–67.

[2] P. Bayjoo, G. Jacob, Hepatic cryosurgery: biological and clinical considerations, J. R. Coll. Surg. Edinburgh 37 (1992) 369–372.

[3] G.M. Dusheiko, K.E. Hobbs, R. Dick, A.K. Burroughs, Treatment of small hepatocellular carcinomas, Lancet 340 (1992) 285–288.

[4] E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij, New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), Eur. J. Cancer 45 (2009) 228–247.

[5] H.B. El-Serag, A.C. Mason, Rising incidence of hepatocellular carcinoma in the United States, N. Engl. J. Med. 340 (1999) 745–750.

[6] S.N. Goldberg, C.J. Grassi, J.F. Cardella, J.W. Charboneau, G.D. Dodd 3rd, D.E. Dupuy, D. Gervais, A.R. Gillams, R.A. Kane, F.T. Lee Jr., T. Livraghi, J. McGahan, D.A. Phillips, H. Rhim, S.G. Silverman, Image-guided tumor ablation: standardization of terminology and reporting criteria, J. Vasc. Interv. Radiol. 16 (2005) 765–778.

[7] D.B. Goodie, M.D. Horton, R.W. Morris, L.S. Nagy, D.L. Morris, Anaesthetic experience with cryotherapy for treatment of hepatic malignancy, Anaesth. Intensive Care 20 (1992) 491–496.

[8] G.G. Hamad, J.P. Neifeld, Biochemical, hematologic, and immunologic alterations following hepatic cryotherapy, Semin. Surg. Oncol. 14 (1998) 122–128.

[9] H.J. Hodgson, Primary hepatocellular carcinoma, Br. J. Hosp. Med. 29 (1983) (240, 246, 250 passim).

[10] P.J. Johnson, Hepatocellular carcinoma: is current therapy really altering outcome?, Gut 51 (2002) 459–462

[11] Y. Koike, H. Yoshida, S. Shiina, T. Teratani, S. Obi, S. Sato, M. Akamatsu, R. Tateishi, T. Fujishima, Y. Hoshida, M. Kanda, T. Ishikawa, Y. Shiratori, M. Omata, Changes in hepatic functional reserve after percutaneous tumor ablation for hepatocellular carcinoma: long-term follow up for 227 consecutive patients with a single lesion, Hepatol. Int. 1 (2007) 295–301.

[12] S.M. Lee, J.Y. Won, D.Y. Lee, K.H. Lee, K.S. Lee, Y.H. Paik, J.K. Kim, Percutaneous cryoablation of small hepatocellular carcinomas using a 17-gauge ultrathin probe, Clin. Radiol. 66 (2011) 752–759.

[13] F. Mu, L. Niu, H. Li, M. Liao, L. Li, C. Liu, J. Chen, J. Li, J. Zuo, K. Xu, Percutaneous comprehensive cryoablation for metastatic hepatocellular cancer, Cryobiology 66 (2013) 76–80.

[14] A. Orlacchio, G. Bazzocchi, D. Pastorelli, F. Bolacchi, M. Angelico, C. Almerighi, S. Masala, G. Simonetti, Percutaneous cryoablation of small hepatocellular carcinoma with US guidance and CT monitoring: initial experience, Cardiovasc. Interv. Radiol. 31 (2008) 587–594.

[15] A.S. Pearson, F. Izzo, R.Y. Fleming, L.M. Ellis, P. Delrio, M.S. Roh, J. Granchi, S.A. Curley, Intraoperative radiofrequency ablation or cryoablation for hepatic malignancies, Am. J. Surg. 178 (1999) 592–599.

[16] R.T. Poon, S.T. Fan, F.H. Tsang, J. Wong, Locoregional therapies for hepatocellular carcinoma: a critical review from the surgeon's perspective, Ann. Surg. 235 (2002) 466–486.

[17] T.S. Ravikumar, R. Kane, B. Cady, R. Jenkins, M. Clouse, G. Steele Jr., A 5 year study of cryosurgery in the treatment of liver tumors, Arch. Surg. 126 (1991) 1520–1523 (discussion 1523–1524).

[18] T. Sarantou, A. Bilchik, K.P. Ramming, Complications of hepatic cryosurgery, Semin. Surg. Oncol. 14 (1998) 156–162.

[19] J.K. Seifert, D.L. Morris, World survey on the complications of hepatic and prostate cryotherapy, World J. Surg. 23 (1999) 109–113 (discussion 113–114).

[20] M. Shafir, R. Shapiro, M. Sung, R. Warner, A. Sicular, A. Klipfel, Cryoablation of unresectable malignant liver tumors, Am. J. Surg. 171 (1996) 27–31.

[21] J. Shi, L. Niu, Z. Huang, F. Mu, J. Chen, J. Li, K. Xu, Diagnosis and treatment of coagulopathy following percutaneous cryoablation of liver tumors: experience in 372 patients, Cryobiology 67 (2013) 146–150.

[22] S. Shiina, T. Teratani, S. Obi, K. Hamamura, Y. Koike, M. Omata, Nonsurgical treatment of hepatocellular carcinoma: from percutaneous ethanol injection therapy and percutaneous microwave coagulation therapy to radiofrequency ablation, Oncology 62 (Suppl. 1) (2002) 64–68.

[23] T. Shimizu, Y. Sakuhara, D. Abo, Y. Hasegawa, Y. Kodama, H. Endo, H. Shirato, K. Miyasaka, Outcome of MR-guided percutaneous cryoablation for hepatocellular carcinoma, J. Hepatobiliary Pancreat. Surg. 16 (2009) 816–823.

[24] D.M. Townsend, K.D. Tew, H. Tapiero, The importance of glutathione in human disease, Biomed. Pharmacother. 57 (2003) 145–155.

[25] H.M. Xu, Y. Chen, J. Xu, Q. Zhou, Drug-induced liver injury in hospitalized patients with notably elevated alanine aminotransferase, World J. Gastroenterol. 18 (2012) 5972–5978.

[26] X.D. Zhou, Z.Y. Tang, Y.Q. Yu, Z.C. Ma, Clinical evaluation of cryosurgery in the treatment of primary liver cancer. Report of 60 cases, Cancer 61 (1988) 1889–1892.