JBUON 2018; 23(4): 985-991
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

CT combined with tumor markers in the diagnosis and prognosis of hepatocellular carcinoma

Xingwen Huang[1]*, Jianlin Li[2]*, Fuzheng Wang[3], Mingda Hao[4]

[1]Imaging Department, Yantaishan Hospital, Yantai 264000, China; [2]Department of Radiology, the Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai 264000, China; [3]Department of Radiology, the Second People's Hospital of Laiyang, Laiyang 265200, China; [4]Imaging Department, Yantai Hospital of Traditional Chinese Medicine, Yantai 264013, China

*These authors contributed equally to this work

Summary

Purpose: *To investigate the application values of CT combined with tumor markers (TM) in the diagnosis and prognosis of hepatocellular carcinoma (HCC).*

Methods: *62 HCC patients were selected as the study group and all of them had undergone hepatectomy. In addition, 40 healthy subjects composed the control group. Blood samples were collected preoperatively from the subjects of the study group, and serum alpha fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) levels were determined by radioimmunoassay. The correlation between serum TM levels and clinicopathological features was investigated by pathological examination. The imaging features of HCC were explored by spiral CT plain scanning and enhanced scanning. The diagnostic efficiency of single detection of TM, combined detection of TMs and combined detection of TMs and CT was analyzed by receiver operating characteristic (ROC).*

Results: *The levels of serum AFP, CA19-9 and CEA in HCC patients were significantly higher than those in the control group ($p<0.05$). Patients with low differentiation and distant metastasis had higher levels of AFP, CA19-9 and CEA ($p<0.05$) compared with patients with high differentiation and no distant metastasis. There were no significant differences in TM levels in HCC patients with different age, gender and clinical stages ($p>0.05$). The ROC analysis showed that the single detection of serum AFP, CEA or CA19-9 had lower specificity and sensitivity compared with the combined detection of the three TMs ($p<0.05$). The combined CT examination could achieve a specificity of 95.71% and a sensitivity of 87.47%, superior to the combined detection of the TMs ($p<0.05$). Eight HCC patients relapsed within 6 months after operation. Compared with those before operation, the levels of serum AFP, CA19-9 and CEA in the relapsed patients were increased significantly, and the TM levels were higher than those in patients without relapse ($p<0.05$).*

Conclusions: *The detection of serum AFP, CA19-9 and CEA combined with CT can overcome the deficiency of single detection, avoid misdiagnosis and missed diagnosis, and increase significantly the positive detection rate of HCC.*

Key words: *CT, diagnosis, HCC, tumor markers*

Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor with a high mortality rate. There are 500,000-1,000,000 new cases of HCC across the world every year [1]. The occurrence of this disease has an obvious regional difference, especially in the Asian-Pacific region [2]. According to statistical data, the incidence rate of liver cancer in the Asian-Pacific region exceeds 20/100000. About 80% of HCC is caused by infection with hepatitis (HBV or HCV). Patients infected with chronic hepatitis are prone to develop cirrhosis in 10-30 years, and die of liver cancer [3,4]. The effective treatment options for early-stage HCC patients are surgical resection and liver transplantation, but about 85%

Correspondence to: Mingda Hao, BM. Imaging Department, Yantai Hospital of Traditional Chinese Medicine, 39 Xingfu Rd, Zhifu Disctrict, Yantai 246013, Shandong, China.
Tel: +86 0535 6597128, E-mail: 1972485736@qq.com
Received: 02/01/2018; Accepted: 27/01/2018

of patients with advanced liver cancer die since they cannot undergo surgical treatment [5]. Therefore, this situation calls for an urgent development of a reasonable early HCC screening scheme.

At present, the sole clinically confirmed tumor marker (TM) for HCC is AFP, which has been widely used as a part of HCC screening, however, its sensitivity is very limited (39-65%) and its specificity is not high (76-94%), especially for small and early HCCs [6,7]. Currently, the TM identification is still in its infancy, and researchers are trying to evaluate the performance of TM alone or in combination. CEA and CA19-9 can be used to predict a variety of tumors as classic TMs, with important clinical significance [8]. The biomarkers of HCC at the level of genomics, transcriptomics and proteomics, supplemented with the traditional CT imaging, can help form a systematic understanding of disease. In this study, we identified the early biomarkers of HCC through the proteomic expression analysis method and evaluated the clinical feasibility of new biomarkers in plasma.

Methods

Clinical data

A total of 62 HCC patients of the Oncology Department of our hospital were selected from May 2013 to May 2016 and composed the study group. All patients had undergone hepatectomy and were pathologically identified as HCC [9]. There were 36 males and 26 females, aged from 36 to 62 years with an average age of 47.5±13.3 years. In addition, 40 healthy subjects were recruited and composed the control group, including 18 males and 22 females, aged from 34 to 56 years with an average age of 45.3±12.6 years. There were no statistically significant differences in the gender and age between the two groups (p<0.05), and they were comparable.

Detection of serum TMs

For the two groups of patients, 15 mL whole blood was drawn and centrifuged at 1000 rpm for 10 min, and then the upper layer of serum was drawn. The levels of AFP, CA19-9 and CEA were tested quantitatively by radioimmunoassay. The specific operation procedures were as follows: Different concentrations of standard antigens and test samples were added to different test tubes, and equal amount of radio-labeled antigen and a certain amount of antibody were added to each test tube, which were kept at 4°C or 37°C. After equilibrium of reaction, the antigen-antibody complex (B) and the free labeled antigen (F) were separated and the radioactive intensity was evaluated. Then, according to the radioactive intensity (B/T) ratio, a standard curve of the amount of silver (Ag) was plotted. The amount of unknown samples could be checked from the standard curve. In this study, AFP>8.54 ng/L, CA19-9>15.4 U/L and CEA>2.8 ng/L were defined as positive results.

CT examination

Double-spiral CT (Elscint, Israel) scanning was performed with 512×512 reconstruction matrix and conventional layer thickness and spacing of 10 mm. In the lesion area, 5 mm thin-layer scanning was performed. For some cases, enhanced scan was performed in the lesion area. In this study, the CT signs were summarized by conventional CT. Spiral CT data were evaluated by two imaging physicians in a double-blind manner. The imaging findings were compared with the histopathological findings for all cases.

Statistics

Statistical analyses of the experimental results was performed using GraphPad Prism software (Version 5.01, GraphPad Software, Santiago, Chile). The correlations of AFP, CA19-9 and CEA levels with pathological features of HCC patients were tested by chi-square test. The differences in samples indexes between the two groups were compared by independent-samples t-test. The diagnostic efficiency of single detection of TM, combined detection of TMs and combined detection of TMs and CT was analyzed by the ROC test. p<0.05 indicated statistically significant difference.

Results

Comparisons of serum TM levels in HCC patients and healthy subjects

As shown from Table 1 and Figure 1, the mean levels of serum AFP, CA19-9 and CEA in HCC patients were 133.27±88.21 ng/mL, 43.48±20.67 U/L and 11.18±2.61 ng/mL, respectively, which were significantly higher than those in the control group (p<0.05).

Table 1. Comparisons of serum AFP, CA19-9 and CEA levels between the study group and the control group (mean±SD)

Group	*n*	*AFP*		*CA19-9*		*CEA*	
		Detection level (ng/mL)	*Positive rate n (%)*	*Detection level (U/L)*	*Positive rate n (%)*	*Detection level (ng/mL)*	*Positive rate n (%)*
Study group	62	133.27±88.21	55 (88.71)*	43.48±20.67*	52 (83.87)	11.18±2.61*	42 (67.74)
Control group	40	2.16±1.14	2 (5)	8.42±2.82	1 (2.5)	1.06±0.13	1 (2.5)

Compared with the control group, *p<0.05

Comparisons of serum TM levels in HCC patients with different clinicopathological features

As shown from Table 2, the HCC patients with low differentiation and distant metastasis had higher levels of AFP, CA19-9 and CEA ($p<0.05$), while the TM levels of HCC patients with different age, gender and clinical stage showed no significant differences ($p>0.05$).

CT imaging signs of HCC

Of the 62 pathologically confirmed HCC patients, the CT scan showed a clear boundary, an irregular low-density lesion with a pseudocapsule, while some calcifications could be seen inside the lesions. Rare cases had a single massive liver mass, with small satellite lesions, double lesions, and diffuse multiple lesions around the mass. The enhanced CT showed continuous enhancement of tumor parenchyma from the arterial phase to the venous phase, while there was no enhancement of the central scar tissues, showing low-density areas, the latter of which was histologically confirmed as ischemic fiber scar structure. Sometimes, enhancement signals of the hilar region and enlarged lymph nodes in the abdomen could be observed (Figure 2).

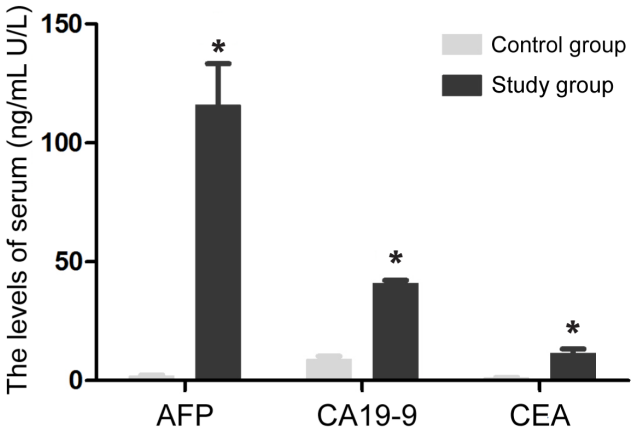

Figure 1. Comparisons of serum tumor marker levels in HCC patients and healthy subjects. Compared with the control group, *$p<0.05$.

Comparison of diagnostic efficiency of serum TM and CT scan in HCC

The single detection of TM, combined detection of TMs and combined detection of TMs and CT were analyzed by the ROC test, hoping to enhance the diagnostic level. If one of the three indexes (AFP, CEA and CA19-9) was positive, then the diagnosis result was positive. The results (Table 3 and Figure 3) showed that the single detection of TM had a low specificity and sensitivity, while the combined detection of TMs significantly increased both the specificity and sensitivity. The combined detection of TMs and CT could achieve a specificity of 95.71% and a sensitivity of 87.47%, superior to the combined detection of TMs ($p<0.05$).

Table 2. Comparisons of serum tumor marker levels in HCC patients with different clinicopathological features (mean±SD)

Pathological staging	*n*	*AFP (ng/mL)*	*p value*	*CA19-9 (U/L)*	*p value*	*CEA (ng/mL)*	*p value*
Gender			>0.05		>0.05		>0.05
Male	36	123.17±44.73		38.83±10.34		10.43±3.47	
Female	26	118.29±37.42		37.94±13.26		11.75±4.20	
Age, years			>0.05		>0.05		>0.05
≥50		124.74±52.41		38.32±14.42		9.24±2.66	
≤50		116.34±44.08		40.21±13.95		10.73±4.62	
Differentiation grade		46.23±13.82	<0.05	34.43±9.92	<0.05	4.23±1.83	<0.05
Highly differentiated		63.74±28.58		37.23±16.63		8.34±4.40	
Moderately differentiated		109.43±57.72		46.23±18.85		13.23±3.81	
Poorly differentiated	13	119.51±17.93	>0.05	37.52±16.87	>0.05	6.21±2.34	>0.05
Clinical stage			>0.05		>0.05		>0.05
T1	-	-		-		-	
T2	14	116.52±23.52		35.83±24.53		8.55±5.23	
T3	28	122.28±66.23		35.04±14.55		9.63±5.04	
T4	7	136.05±73.41		46.87±24.62		12.03±6.82	
Distant metastasis			<0.05		<0.05		<0.05
Yes		88.24±24.57		45.54±9.83		13.72±2.88	
No		47.34±37.24		33.87±11.94		9.72±1.62	

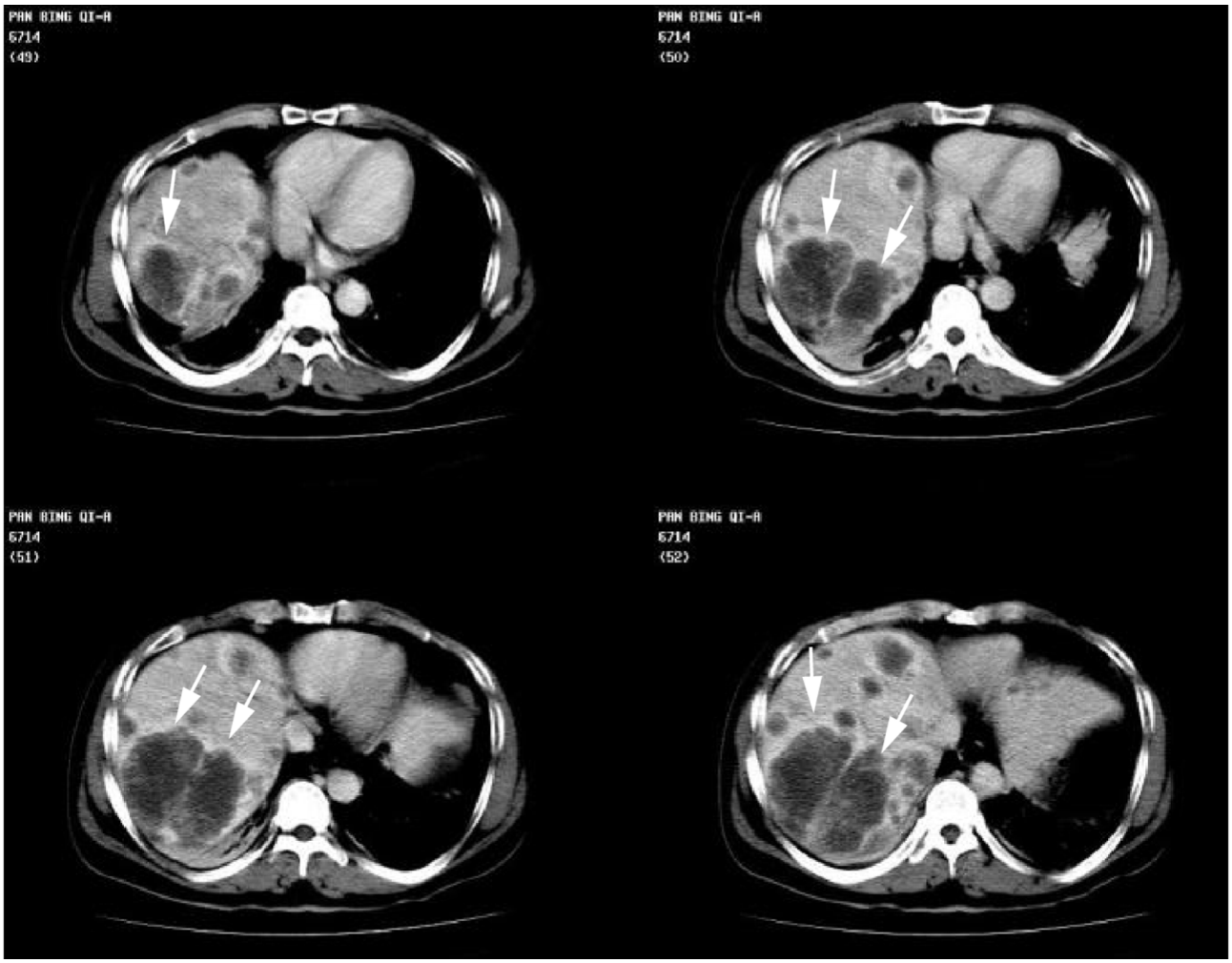

Figure 2. Imaging findings of plain and enhanced CT in HCC patients. This patient had persistent pain in the right upper quadrant, with progressive aggravation and emaciation for more than one month. The CT scan showed multiple intrahepatic low-density lesions with varying size and irregular shape, which were considered liver cancer with multiple intrahepatic metastases. After enhancement, the lesions showed significant annular enhancement, while the middle low-density lesions showed no apparent enhancement, which was considered as necrotic tissue. Arrows show the liver cancer.

Table 3. Comparison of diagnostic efficiency of serumtumor markers and CT scan in HCC

Diagnostic method	*n*	*Specificity (%)*	*Sensitivity (%)*
Serum AFP	62	61.54	61.23
Serum CA19-9	62	68.36	68.43
Serum CEA	62	65.23	66. 09
Combined detection of TMs	62	90.66	75.27
CT scan	62	85.54	72.31
Combined detection of TMs and CT	62	95.71	87.47
x^2		6.36	5.82
p		0.025	0.018

TM: tumor markers, CT: computed tomography

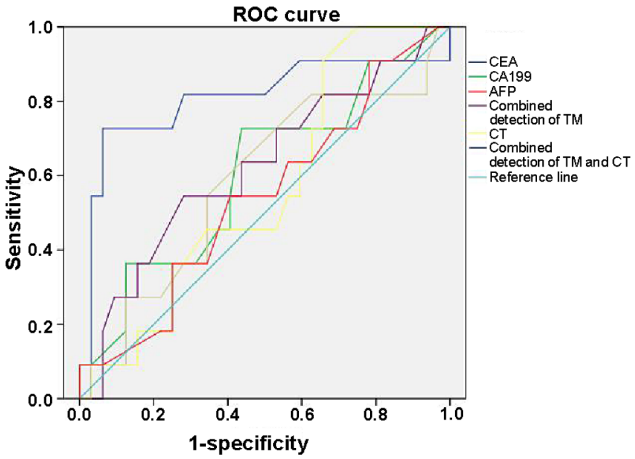

Figure 3. Detection of diagnostic efficiency of serum TM and CT scan in HCC by ROC curves. Single detection of TM showed low specificity and sensitivity. Combined detection of TMs and CT achieved high specificity (95.71%) and high sensitivity (87.47%).

Table 4. Comparisons of tumor recurrence and tumor marker levels of HCC patients 6 months after the operation (mean±SD)

Tumor markers	*Group*	*n*	*Before operation*	*6 months after operation*
AFP	Recurrent patients	8	92.64±32.37	134.46±63.65*
	Non-recurrent patients	54	88.27±29.44	53.77±27.31**
CA19-9	Recurrent patients	8	35.15±828	45.39±0.17*
	Non-recurrent patients	54	38.20±9.39	34.88±0.44**
CEA	Recurrent patients	8	8.56±3.74	12.62±4.75*
	Non-recurrent patients	54	8.34±3.61	5.22±2.38**

Compared with the recurrent patients before operation, *p<0.05. Compared with the recurrent patients after operation **p<0.05

Comparisons of tumor recurrence and TM levels in HCC patients

Table 4 shows that there were 8 recurrent cases 6 months after the operation. There were no statistically significant differences in the levels of serum AFP, CA19-9 and CEA before operation between recurrent and non-recurrent patients (p>0.05). The levels of serum AFP, CA19-9 and CEA of recurrent patients 6 months after operation were significantly increased compared with those before operation, and significantly higher than those of non-recurrent patients (p<0.05).

Discussion

In recent years, the incidence and mortality rates of liver cancer remain high, showing increasing trends year by year [10]. With the development of oncology molecular biology, immunology, human genome project and the clinical application of modern new testing equipments, TM has become a hot topic in basic and clinical tumor research [11]. The monitoring of TM levels can provide the following clinical information: 1) The cancer screening and screening of high-risk populations. 2) Differential diagnosis and auxiliary pathological diagnosis of tumor. 3) Identify if operation, radiotherapy and/or drug treatment are effective. 4) Detection of the tumor changes. The detection of TM levels at different time points in the treatment can help the detection of the tumor residual, recurrence and metastasis. At present, serum TM, as a routine test technique, has been widely used in clinical applications and scientific research in numerous grade A class 3 hospitals. The application of TM combined with imaging techniques facilitates the development of combined detection, which has been widely accepted in early diagnosis, staging and classification, and prognosis judgment [12].

In China, about 90% of HCC cases are complicated with cirrhosis. The most clinically effective detection mean is still AFP. However, AFP-negative HCC cases account for 20-30%. Even in AFP-positive cases, the diagnosis and localization of lesions must rely on imaging examination [13]. CA19-9 is a pancreatic cancer TM. The serum CA19-9 in healthy people is <20U/mL, while it is significantly increased in patients with pancreatic cancer [14]. Studies have shown that when CA19-9 combined with CEA is used to detect pancreatic cancer, the positive rate can be as high as 90%. In other gastrointestinal tumors, such as liver cancer, gastric cancer, biliary cancer and colon cancer, the positive rate of CA19-9 is found to be about 30-60% [15]. Therefore, CA19-9 can also be used to monitor tumor recurrence and prognosis. CEA was initially found in colon cancer and fetal intestinal tissue, so named carcinoembryonic antigen. Serum CEA concentration is below 2.5 ng/mL in 97% of healthy adults. Increased CEA level is often found in colorectal cancer, pancreatic cancer, gastric cancer, small cell lung cancer, breast cancer, medullary thyroid cancer, etc. CEA higher than 20 ng/mL may imply the existence of gastrointestinal cancer [16]. However, serum CEA levels are also elevated in 15-53% of individuals with smoking, gestational and cardiovascular diseases, diabetes and nonspecific colitis, therefore, CEA is only used as an auxiliary diagnostic marker [17].

In this study, we detected the levels of serum AFP, CA19-9 and CEA in HCC patients. The results showed that the positive rates of these TMs in HCC patients were 88.71, 83.87 and 67.74%, respectively, which were significantly higher than those in healthy controls. The diagnostic specificity of detecting AFP, CA19-9 and CEA for HCC was 61.54, 68.36 and 65.23%, respectively, while it was 88.71% in the combined serum TM detection, significantly superior to the single detection of TM. Although the detection accuracy of TM has been greatly improved, there are still many deficiencies. Different TMs are used for different pathological

types and stages. The single and combined detection of TMs may cause missed diagnosis and misdiagnosis due to the great variations in sensitivity and specificity.

As one of the important means of diagnosing HCC, imaging examination is especially important for patients with chronic liver diseases, such as hepatitis B and cirrhosis, who are at risk of developing HCC. The focal liver examination should be performed regularly. In addition, distinguishing HCC from other solid lesions such as regenerative nodules, atypical nodules and fusion hepatic fibrosis is important and difficult [18]. CT plays an important role in the diagnosis of HCC. The features of CT plain scan include low-density foci with clear boundary, pseudocapsule, and calcifications of some lesions. Most of the patients have round or oval low-density lesions, infiltrative growth of tumor without capsules, and vague boundary. In a few patients, a single massive liver mass, with small satellite lesions, double lesions, diffuse multiple lesions around the mass can be seen, and ischemic necrosis, hemorrhage and calcification may occur in the center of the lesions [19]. The enhanced CT sometimes shows enhancement of the hilar region and enlarged lymph nodes in the abdomen, and continuous enhancement of tumor parenchyma from the arterial phase to the venous phase and also low-density cancer embolus. Hepatic vein and inferior vena cava tumor embolus show filing defects and more importantly, lymph node metastasis could be seen [20]. However, the early-stage liver cancer less than 1 cm in diameter cannot be well distinguished by CT. Since the blood supply of liver cancer is different, and the pathological types and cell differentiation are varying, it is necessary to perform diagnosis by pathological examination combined with serum TMs. In this study, the specificity of the combined detection of serum TMs (AFP, CA19-9 and CEA) was 90.66%, while that of combined detection of serum TMs and CT was significantly increased (95.71%). In addition, the detection of serum TMs combined with CT reduces the misdiagnosis rate of HCC, with great significance for the clinical diagnosis of HCC.

Conclusions

With the research development, more and more new TMs will be found in the future. A rational combination of TMs can improve the positive detection rate of HCC and achieve more accurate and specific pathological typing, so as to achieve early diagnosis, early treatment and prolong the survival of patients.

Conflict of interests

The authors declare no conflict of interests.

References

1. Bruix J, Sherman M. Management of hepatocellular carcinoma: An update. Hepatology 2011;53:1020-2.
2. Deng Y, Zhou X, Xiang X, Ou Y, He J. NOR1 expression and its relationship with prognosis in patients with hepatocellular carcinoma. JBUON 2017;22:1186-90.
3. Llovet JM, Ricci S, Mazzaferro V et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-90.
4. Zhai YP, Wang Y. Effect of the combination treatment of high-intensity focused ultrasound and cryocare knife in advanced liver cancer. JBUON 2017;22:495-9.
5. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52-60.
6. Durazo FA, Blatt LM, Corey WG et al. Des-gamma-carboxyprothrombin, alpha-fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol 2008;23:1541-8.
7. Volk ML, Hernandez JC, Su GL, Lok AS, Marrero JA. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers: A comparison of AFP, DCP, and AFP-L3. Cancer Biomark 2007;3:79-87.
8. Lai IR, Lee WJ, Huang MT, Lin HH. Comparison of serum CA72-4, CEA, TPA, CA19-9 and CA125 levels in gastric cancer patients and correlation with recurrence. Hepatogastroenterology 2002;49:1157-60.
9. Qin S. Guidelines on the diagnosis and treatment of primary liver cancer (2011 edition). Chin Clin Oncol 2012;1:10.
10. Singal A, Volk ML, Waljee A et al. Meta-analysis: Surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. Aliment Pharmacol Ther 2009;30:37-47.
11. Kirchner H, Chused TM, Herberman RB, Holden HT, Lavrin DH. Evidence of suppressor cell activity in spleens of mice bearing primary tumors induced by Moloney sarcoma virus. J Exp Med 1974;139:1473-87.
12. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 2005;93:387-91.

13. Pawlik TM, Delman KA, Vauthey JN et al. Tumor size predicts vascular invasion and histologic grade: Implications for selection of surgical treatment for hepatocellular carcinoma. Liver Transpl 2005;11:1086-92.
14. Steinberg WM, Gelfand R, Anderson KK et al. Comparison of the sensitivity and specificity of the CA19-9 and carcinoembryonic antigen assays in detecting cancer of the pancreas. Gastroenterology 1986;90:343-9.
15. Pleskow DK, Berger HJ, Gyves J, Allen E, McLean A, Podolsky DK. Evaluation of a serologic marker, CA19-9, in the diagnosis of pancreatic cancer. Ann Intern Med 1989;110:704-9.
16. Cedres S, Nunez I, Longo M et al. Serum tumor markers CEA, CYFRA21-1, and CA-125 are associated with worse prognosis in advanced non-small-cell lung cancer (NSCLC). Clin Lung Cancer 2011;12:172-9.
17. Aggarwal C, Meropol NJ, Punt CJ et al. Relationship among circulating tumor cells, CEA and overall survival in patients with metastatic colorectal cancer. Ann Oncol 2013;24:420-8.
18. Colli A, Fraquelli M, Casazza G et al. Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: A systematic review. Am J Gastroenterol 2006;101:513-23.
19. Sahani DV, Holalkere NS, Mueller PR, Zhu AX. Advanced hepatocellular carcinoma: CT perfusion of liver and tumor tissue-initial experience. Radiology 2007;243:736-43.
20. Ma WL, Hsu CL, Wu MH et al. Androgen receptor is a new potential therapeutic target for the treatment of hepatocellular carcinoma. Gastroenterology 2008;135:947-55.