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ORIGINAL ARTICLE

A Comparative Study on Dual-phase Enhanced CT, CT Perfusion and Histopathology in Edges of Hepatocellular Carcinoma

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A R T I C L E I N F O

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A B S T R A C T

Background: The morphological characteristics of HCC edge not only predict the biological behavior of the tumor, but also determine the treatment.

Method: 47 cases of HCC borders were divided into three morphological types based on enhanced CT findings. Clear edges (Type I): The tumor boundary was clearly displayed at dual-phase CT. Blurry edges (Type II): The boundary between tumor and normal liver tissues was blurry at dual-phase CT. Mixed type (Type III): Part of tumor edge was clear or had an incomplete capsule formation, another part of tumor edge had a blurry boundary with normal liver tissues, and the above two types of performance were seen at dual-phase CT. All patients underwent CT perfusion (CTP) imaging. The correlations between microvessel density (MVD) and the CTP parameters were analyzed.

Results: In 47 cases of HCC, the MVD in edge areas of type II was higher than that in type I, and the difference was statistically significant ($P < 0.05$).The MVD in clear edge areas of type III was lower than that in Blurry edges ($P < 0.01$). Positive linear correlations existed between the values of HAP, HAF, and HBF versus MVD ($P < 0.05$). HPP showed a negative correlation with MVD measurements ($P < 0.05$).

Conclusion: The CT classification of the tumor edges can roughly evaluate the characteristics of the tumors at the macro level, and the CTP imaging in tumor edges can acquire more information at the micro level and reflect the tumor characteristics more specifically and comprehensively.

INTRODUTION

The morphological characteristics of HCC edge not only predict the biological behavior of the tumor, but also determine the treatment (1). With the widespread application of the enhanced multi-slice spiral CT scanning, the diagnostic value for HCC

patients of hepatic arterial and portal venous phase imaging (referred to as dual-phase enhanced CT) has been recognized(2, 3). Further, at the micro level, CT CTP imaging can quantitatively analyze physiological and pathological hemodynamic changes that occured in the HCC, and indicate the extent of angiogenesis in **HCC(4, 5)**.Nevertheless, there has been no systematic evaluation on different types of HCC edges using CTP imaging.Thus, this paper aims to explore the relationships between CTP parameters and their histopathological basis in different types of HCC edges.

MATERIAL AND METHODS

Patients :

This study was approved by IRB and ethics committee of Harbin Medical University, written informed consent was gained from all persons before the study was conducted. From June 2011 to July 2013, 37 patients with 47 lesions identified as HCC were made on the basis of the European Association for the Study of the Liver (EASL) criteria, including 23 males and 14 females, aged 30 to 79 years (mean age 56 years).The 37 patients with HCC underwent dual-phase enhanced CT and CTP imaging. After CT scanning, the patients underwent surgical resection in the hospital, pathological data were obtained and the tumors were **proven to be** HCC. Immunohistochemical results of Microvessel Density (MVD) measurement and hematoxylin and eosin (HE) were assessed.

CT protocol

All patients were examined with a GE Lightspeed 64-slice spiral CT scanner; a **U.S. MEDRAD binocular** high-**pressure syringe was also employed for imaging**. At first, all subjects underwent a plain CT scan to localize the central slices for perfusion, and the maximum slice of the HCC focus was selected as the central slice for perfusion scanning, with an attempt made to make the selected slice close to the hepatic portal. After preparation, the contrast agent was intravenously injected according to the dosage of 1.0 ml/kg body weight, with 4-5 ml/s flow rate; subsequently, 20 ml physiological saline was injected at the same rate, and the delay time was shortened or postponed according to the patient's heart function. The CT perfusion scanning parameters were as follows: **layer** thickness, 5 mm; **layer spacing**, 0 mm; 8-layer successive scanning; scan range, 40 mm; 120 kV; 60 mAs; matrix, 512 × 512; exposure time, 50 s; and rotating 360°/1 s. Enhanced dual-phase CT was performed 10 minutes after the CT perfusion scanning completed. The mean scanning time delay of the arterial phase (AP) was 15–20 s after injection. The portal venous phase (PVP) was acquired at 45-60 s.

Morphological classification of tumor edges based on ehanced dual-phase CT

According to enhanced CT characteristics, morphological classification of the tumor edges had following types:

Clear edges (Type I): The tumor boundary was clearly displayed at dual-phase CT. Type I a: the complete tumor capsule was visible at dual-phase CT. Type I b: the tumor capsule was not formed yet at dual-phase CT.

Blurry edges (Type II): The boundary between tumor and normal liver tissues was blurry at dual-phase CT.

Mixed type (Type III): Part of tumor edge was clear or had an incomplete capsule formation, another part of tumor edge had a blurry boundary with normal liver tissues, and the above two types of performance were seen at dual-phase CT.

Quantitative analysis of CT perfusion parameters

The perfusion images were transmitted to a workstation (Advantage Windows 4.3, GE Medical Systems Ltd., Milwaukee, WI, USA). A time-density curve (TDC) was automatically generated after the obtained data were processed using the Perfusion 3 software package. The red zones on CTP pseudocolor images indicates high perfusion areas with rich blood flow, while the blue zones indicates low perfusion areas with decreased blood flow. The perfusion parameters related to hepatic blood flow were obtained using the deconvolution algorithm for each tissue region of interest (ROI): Hepatic blood flow (HBF) was expressed as mL/min/100 mg of body weight, Hepatic arterial fraction (HAF) as the percentage of total blood flow of arterial origin. Hepatic arterial perfusion (HAP) and Hepatic portal perfusion (HPP) were expressed as mL/min/100 mg. HBF was calculated using the following equation: HBF=HAP+HPP, where HAP=HBF×HAF and HPP= HBF×(1-HAF).

Immunohistochemistry analysis

141 slices of the specimen was cut up layer by layer as far as possible according to the corresponding slices and the layer thickness in preoperative CT scan, three pieces of tissues were drawn respectively in the edges of maximum tumor slices for type I and type II, the tissues were drawn in different edge positions according to different edge morphologies for type III. Tumor tissues were fixed in 10% neutral buffered formalin and embedded routinely in paraffin for immunohistochemical study, with HE and CD34 staining. MVD was measured by anti-CD34 staining.

The MVD assessment was performed using a two-step protocol (Beijing Zhongshan Golden Bridge Biotechnology Co., China) according to the method described by Weidner(6). The CD34-stained sections were screened at low power (×40), and 3 areas with the most intense neovascularization (hot spots) were selected. Microvessel counts of these areas were performed at high power (×200). Any brown-stained endothelial cells or cell clusters clearly separated from adjacent microvessels, tumor cells, and other connective-tissue elements were counted as a single microvessel, irrespective of the presence of a vessel lumen. The mean microvessel count/mm² of the 5 richest vascular areas was taken as the MVD. If MVD>30, this indicated that neovascularization was **abundant** in the tumor edges (7, 8).

Statistical Analysis

All measurement data were expressed as mean ± standard deviation (mean ± s). The SPSS19.0 statistical package was used for analysis. Changes in the different perfusion parameters. Differences with a *P* value <0.05 were considered statistically **significant**.

RESULTS

Features of tumor edges on dual-phase enhanced CT and perfusion CT

Type I a: A total of 9 cases, of which, tumor edges in 5 cases showed low densities around the mass at hepatic arterial phases and enhanced rings at portal venous phases (Figure I a,b). Type I b: A total of 7 cases, of which, the tumor edges in 6 cases showed irregular spots or stripy shadows of feeding arteries at hepatic arterial phases, the liver parenchyma surrounding tumors were obviously enhanced at portal venous phases, and the boundaries were clearer than those at hepatic arterial phases (Figure I e,f). In the perfusion map of HAF, the tumor edges showed hyper perfusion, there was no obvious enhancement in the center, a relatively low perfusion was seen in the perfusion parameters analysis map, but the central perfusion was still higher than that in the surrounding liver parenchyma. The tumors showed high perfusion in the HAF and HBF perfusion parameter map. This type of tumors had obvious boundaries with surrounding liver tissues; the tumor contours were also displayed clearly in the corresponding perfusion maps (Figure I c,d,g,h).

Type II: A total of 20 cases, of which, the tumor edges in 13 cases showed irregular enhanced vascular shadows at hepatic arterial phases, were unclear at portal venous phases, and crisscrossed with non-cancerous liver tissues, the enhancement degree was higher than that at hepatic arterial phases (Figure I i,j).

Type III: A total of 11 cases, partial tumor edges were clear or coated, partial tumor edges were blurry and had no obvious boundary with normal liver tissues, this type had performances of the above two types. 1 patient had portal vein tumor thrombus (Figure I m,n).

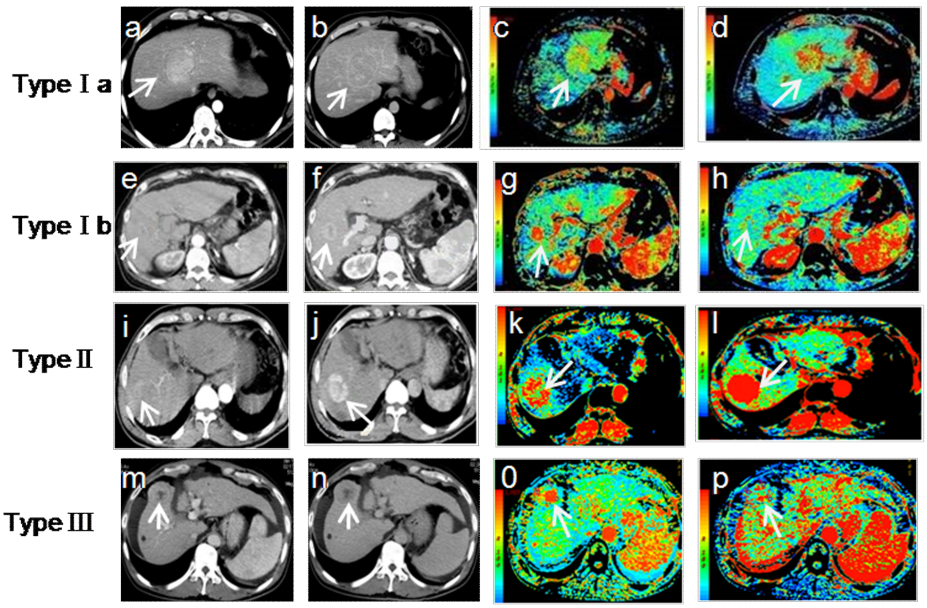

Figure I: Images of enhanced CT and perfusion CT of tumor edges of different types.

White arrows indicate the edges of the tumors. a**,e,i,m**: Enhanced CT images in the arterial phase; **b,f,j,n**: Enhanced CT images in the portal venous phase; **c,g,k,o**: Perfusion CT images of HAF; **d,h,l,p**: Perfusion CT images of BF

For tumor edges of type II and type III, in the corresponding HAF perfusion parameter map, the marked enhancement areas showed disordered hyperperfusion, there was no enhanced necrosis area in the center, the hypoperfusion was seen in the perfusion map. In the HBF perfusion parameter map, the tumor edges with rich blood flow showed relatively high perfusion. The tumor edges were blurry in the perfusion map, and had a staggered distribution with the surrounding liver parenchyma (Figure I k,l,o,p)

Pathologic findings of tumor edges on dual-phase enhanced CT and CT Perfusion

Table I showed that, Type Ia: A total of 27 slices, the tumor tissues showed that fibrous connective tissue capsules were seen significantly in the tumor edges. Type I b: A total of 21 slices, of which, the formation of incomplete capsules was seen in 3 slices, the edges were blurred in 2 slices, part of interstitial fibrosis was seen in 16 slices. Type II: A total of 60 slices, the tumor tissues showed that no capsule formation, of which, there were no obvious boundaries between cancer tissues and surrounding liver tissues in 52 slices, and the edges were clear with no capsule in 8 slices. Type III: A total of 33 slices, of which, the interstitial fibrosis in thin layers was seen in 6 slices, the tumor cells showed invasive growth in 27 slices.

Table I: CT morphological classification and pathologic findings of tumor edges

CT typing (Slice no.)	Capsules are clear and complete (%)	Capsules are clear and incomplete (%)	The edges are blurry with no capsule (%)	The edges are clear with no capsule (%)
Type I(48)				
Ia (27)	23 (85.19)	4 (14.81)	0	0
Ib (21)	0	3 (14.29)	2 (9.52)	16 (76.19)
Type II (60)	0	0	52 (86.67)	8 (13.33)
Type III (33)	0	0	27 (81.82)	6 (18.18)

Table II: CT morphological classification and MVD of the tumor edges

CT Typing	Slice No.	MVD Count
Clear edges (I)		
With capsules (Ia)	27	14.3±10.4
Without capsules (Ib)	21	19.3±12.5
Blurry edges (II)	60	46.1±17.3*
Mixed type (III)		
Clear area	10	20.4±11.2
Blurry area	23	41.8±13.6**

Note: * represents that by t-test analysis, there is a significant difference between two sets of data from clear edges (I) and Blurry edges (II) ($P < 0.05$).** represents that there is a significant difference between data from Clear edge and Blurry edge in the Mixed type (III)($P < 0.01$).

Table II showed that, all cases of Type I had a MVD≤30. All cases of Type II had a MVD>30. In Type III, part cases had a MVD≤30 and part cases had a MVD>30. In 47 cases of HCC, the MVD in edge areas of type II was higher than that in type I, and the difference was statistically significant ($P < 0.05$).The MVD in clear edge areas of type III was lower than that in Blurry edges ($P < 0.01$). There was no significant difference between type Ia and type Ib ($P > 0.05$). The mean HAP, HAF, HBF and MVD was significantly higher in Type II than Type I and Type III($P<0.05$). The mean HPP was significantly lower in Type II than Type I and Type III ($P<0.05$) (Figure II). Positive linear correlations existed between the values of HAP, HAF, and HBF versus MVD ($P < 0.05$). HPP showed a negative correlation with MVD measurements ($P < 0.05$) (FigureIII).

DISCUSSION

Tumors need nutrients to survive, for HCC, the tumor angiogenesis is important for tumor growth, it is the premise and foundation of tumor formation, and also provides the conditions for tumor metastasis (9). MVD is currently considered the gold standard for histological assessment of the degree of angiogenesis within a tumor. Studies involving HCC have shown that angiogenesis is the most active at HCC borders (10), so it is significant to study the angiogenesis of tumors with different morphological edges. The application of dual-phase enhanced CT and CT perfusion imaging has opened up a new field for imaging studies of tumor angiogenesis (11,12).

In the present study, MVD was lower in Clear edges based on ehanced dual-phase CT (Ia : 14.3±10.4 and Ib : 19.3±12.5), indicating the malignant degrees of tumor were lower, also their invasive abilities were reduced. As the tumor edges changed, beginning with Clear edges (Type I), then to Blurry edges (Type II), there was a significant increase in HAP of CTP parameters, and a decrease in HPP in Blurry edges (Type II) than the Clear edges (Type I; $P < 0.05$), this indicates that the tumor edges were supplied gradually by hepatic arterial blood as the tumor grow. The gradual increase of tumor edges being supplied by the hepatic arterial blood resulted in the formation of tumors characteristics. Therefore, the variations in CTP parameters quantitatively reflected the blood supply changes of the hepatic artery and portal vein accurately.

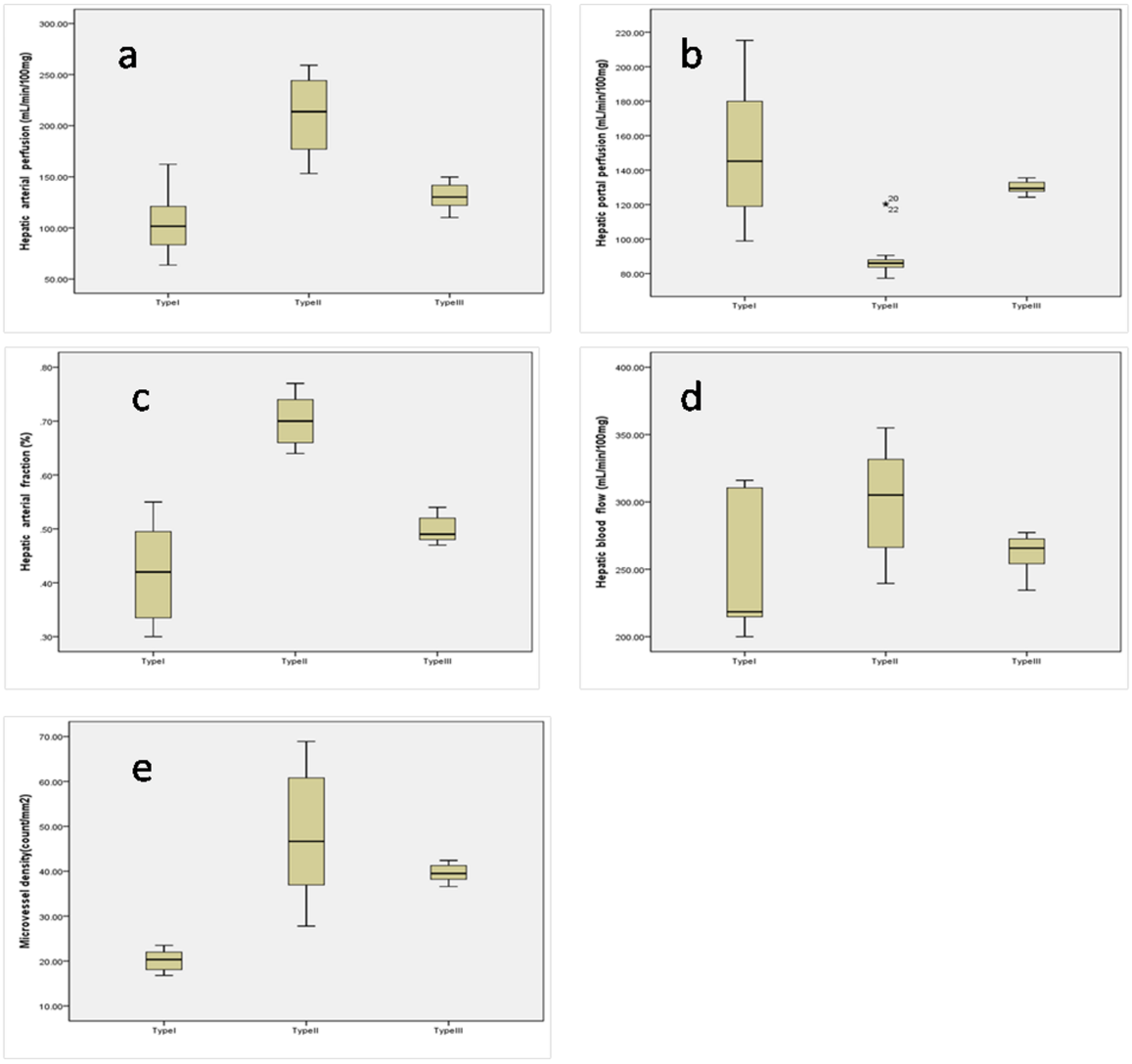

Figure II: CT perfusion parameters and MVD of Type I (Clear edges), Type II (Blurry edges) and Type III (Mixed edges) of HCC. The mean HAP, HAF, HBF and MVD was significantly higher in Type II than Type I and Type III(P<0.05). The mean HPP was significantly lower in Type II than Type I and Type III(P<0.05).

Figure III indicates that positive linear correlations existed between the values of HAP, HAF, and HBF versus MVD ($P < 0.05$). HPP showed a negative correlation with MVD measurements ($P < 0.05$). The higher HAP, HAF and HBF, MVD value also increased. But HPP showed a negative correlation with MVD measurements ($P < 0.05$).The results were slightly different from previous studies. Yang et al (13)considered that HBF and HAP were correlated with MVD (P <0.05), they had a high diagnostic value for tumor angiogenesis; while HPP and HAF were not correlated with the MVD expression (P> 0.05). It is expected for researchers to further study whether HAF is correlated with MVD in the future. The results showed that HAP, HAF, HBF and HPP could reflect the degree of tumor differentiation and neovascularization to a certain extent. It could provide an in vivo real-time dynamic evaluation of the liver cancer neovascularization and its biological behaviours.

The CT classification of the tumor edges can roughly evaluate the characteristics of the tumors at the macro level, and the CTP imaging in tumor edges can acquire more information at the micro level. It can reflect the tumor characteristics more specifically and comprehensively. CTP parameters could reflect the blood flow changes in local areas caused by tumor foci which provide quantitative information on local blood flow changes, and open up a new way for preoperative evaluation of tumor in vivo effectively.

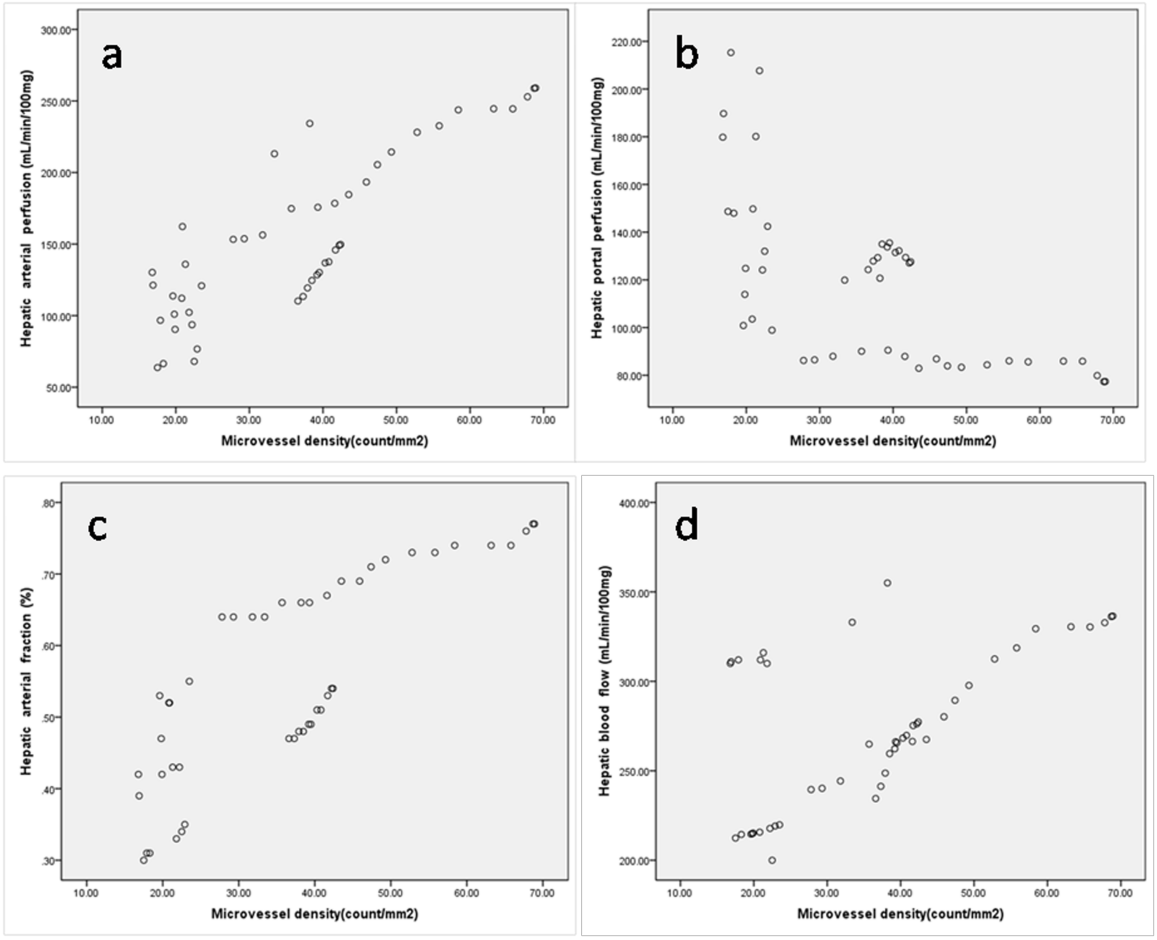

Figure III: Correlation dots of MVD and CT perfusion parameters. A-C:Positive linear correlations existed between the values of HAP, HAF, and HBF versus MVD ($P < 0.05$). D:HPP showed a negative correlation with MVD measurements ($P < 0.05$).

Acknowledgments

The experiments were performed with the approval of the human subject's ethics committee of the second affiliated hospital of Harbin medical university. The liver tissue specimens were obtained in this study by operation. This study was supported by grants from the Ethics Committee for the resection of all liver tumor masses and known not to have changed in appearance over many years to be respected. All patients in this study participated voluntarily and agreed to this experiment checks. The feasibility of this experiment was permitted by the Ethics Committee of Harbin medical university. The permission was granted by the publisher and author to reproduce any previously published figures, including permission to reproduce in both print and electronic formats. The patients gave informed consent for the study.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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