The value of MRI enhancement in the differential diagnosis of hepatocellular carcinoma and combined hepatocellular-cholangiocarcinoma

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Research Article

Keywords: combined hepatocellular-cholangiocarcinoma, hepatocellular carcinoma, preoperative clinical information, magnetic resonance imaging, differential diagnosis

Posted Date: April 21st, 2022
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Abstract

Background Combined hepatocellular-cholangiocarcinoma (cHCC-CC) is a rare primary liver malignant tumor, the distinction between cHCC-CC and HCC before operation has important clinical significance for optimizing the treatment plan and predicting the prognosis of patients. This study intends to study the value of preoperative clinical date and enhanced MRI in the differential diagnosis of HCC and cHCC-CC and to obtain independent risk factors for predicting cHCC-CC.

Methods The clinical and imaging data of 157 HCC and 59 cHCC-CC patients confirmed by pathology were collected, and the differences between the two groups of patients were compared by t test, chi-square test, and logistic regression analysis.
Results CHCC-CC was more likely to show multiple lesions than HCC (28.81% Vs 10.83%, $P=0.001$) and more prone to microvascular invasion (MVI) (36.31% Vs 61.02%, $P<0.001$). However, HCC had a higher incidence of liver cirrhosis than cHCC-CC (50.85% Vs 72.61%, $P=0.003$). The incidence of non-smooth margin was higher in cHCC-CC group (84.75% Vs 52.23%, $P<0.001$). The incidence of peritumor enhancement in arterial phase was higher in cHCC-CC group (11.46% Vs 62.71%, $P<0.001$) Multivariate analysis showed that liver cirrhosis and arterial peritumor enhancement were independent risk factors for predicting cHCC-CC. In addition, the imaging sign of arterial phase peritumor enhancement had high sensitivity (62.71%) and specificity (88.54%) in the diagnosis of cHCC-CC.

Conclusions Liver cirrhosis and the imaging findings of GD-DTPA-enhanced MRI are helpful for the differential diagnosis of HCC and cHCC-CC. In addition, the imaging sign of peritumoral enhancement in the arterial phase has high sensitivity and specificity for the diagnosis of cHCC-CC.

Keywords combined hepatocellular- cholangicarcinoma; hepatocellular carcinoma; preoperative clinical information ;magnetic resonance imaging; differential diagnosis

Abbreviations
HCC: hepatocellular carcinoma
CHCC-CC: combined hepatocellular- cholangicarcinoma
ICC: intrahepatic cholangiocarcinom
PLCs: primary liver carcinoma
HH: hepatic hemangioma
LI-RADS: liver imaging reporting and data system
MRI: magnetic resonance imaging
GD-DTPA: gadolinium diethylenetriamine pentaacetic acid
TACE: transarterial chemoembolization
MVI: microvascular invasion
HBsAg: hepatitis B surface antigen
HBeAg: hepatitis Be antigen
Background

Combined hepatocellular-cholangiocarcinoma (cHCC-CC) is a rare primary liver malignant tumor, which was first reported by Allen and Lisa [1], accounting for about 0.4-14.2% of primary liver carcinoma (PLCs) [2][3]. The pathological diagnosis of cHCC-CC requires the presence of both Hepatocellular carcinoma (HCC) cells and intrahepatic cholangiocarcinoma (ICC) cells, accompanied by intermediate transition areas. Some studies have shown that cHCC-CC has the biological behavior of both HCC and ICC, and the prognosis is poor [4]. Liver transplantation as an effective treatment for HCC [5], there is no consistent conclusion on the therapeutic effect of in cHCC-CC, though radical resection and liver transplantation are the main surgical treatments for HCC and cHCC-CC. Moreover, most studies believe that [6][7] liver transplantation is performed in patients with cHCC-CC. It can not improve the survival rate of patients. If cHCC-CC patients are misdiagnosed as HCC and liver transplantation is performed, it will bring unnecessary treatment risk and economic burden to patients. As a consequence, the distinction between cHCC-CC and HCC before operation has important clinical significance for optimizing the treatment plan and predicting the prognosis of patients.

Magnetic resonance imaging (MRI) has been widely used in the preoperative diagnosis and evaluation of primary liver malignant tumors [8][9][10]. However, almost all existing MRI studies only simple describe the imaging or clinical features of cHCC-CC compared with HCC and ICC. They are not analyzed together [11][12]. As a consequence, this study intends to evaluate the preoperative clinical and GD-DTPA-enhanced MRI features (based on the morphological and enhanced features defined by LI-RADS), and to study its value in the differential diagnosis of HCC and cHCC-CC, and to find independent risk factors for predicting cHCC-CC, providing more useful information for the differential diagnosis of the two.

Methods

Study population

Clinical date: A retrospective review of 366 cases of liver tumors from June 2010
to October 2021 in Shanghai Oriental Hepatobiliary surgery Hospital who underwent
Gadolinium meglumine (GD-DTPA) enhanced MRI scans was done (HCC cases from
January 2014 to October 2021 were selected for inclusion in the study), and 279
cases of HCC were obtained, 87 cases of cHCC-CC. After screening, 157 cases of
HCC and 59 cases of cHCC-CC were finally included in the study.

The selection criteria were as follows: 1. The patients who aged 18-80 years old.
2. MRI suggested that liver tumor ≥ 1 cm was found. 3. The patients who conducted
MRI enhancement check and complete record; 4. Surgical resection of liver tumor
was completed; 5. Postoperative pathology confirmed HCC or cHCC-CC.

Exclusion criteria: 1. The patients age < 18 years old or > 80 years old; 2. The
image quality of tumor MRI scan was poor or the phase was not complete.3.Those
who did not undergo liver surgery; 4. Postoperative pathology revealed intrahepatic
cholangiocarcinoma or other liver tumors. 5. Patients underwent contrast - enhanced
MRI examination after transarterial chemoembolization (TACE) or radiotherapy (Fig. 1).

Our study complied with the Declaration of Helsinki. This study was a
retrospective study, which was approved by the Ethics Committee of Shanghai
Oriental Hepatobiliary surgery Hospital, exemption of Informed Consent. The
enhanced MRI test had been widely used in clinic to exempt patients from informed
consent.

MRI scan

MRI scanning parameters: GE optima MRI360 was adopted with field strength
1.5T, and 8-channel abdominal surface coil. The patients fasted for 4 hours before
scanning, and the patients were trained to breathe before scanning. The patients lied
back on the inspection bed with the feet entering firstly. The 0.1 mmoL/Kg of
GD-DTPA was injected into the median cubital vein through a high-pressure injector
with a flow rate of 2.0 ml/s. The enhanced scanning time was 22-28s in arterial phase,
50-70s in portal phase and 90s-120s in delayed phase after injection of contrast agent.
Precontrast scan: T1WI: gradient double echo sequence was adopted, holding breath
at the end of breath, with TR / TE = 190 / (4.3,2.1) ms, slice thickness= 6mm, layer
interval= 2 mm, matrix = 256x160, and FOV=44x40cm. DWI: b=600sec/mm², T2WI: fast spin echo sequence, fat suppression, and respiratory gating was conducted, with TR / TE = 6667/85 ms, layer thickness =6mm, layer interval= 2mm, matrix = 320x224, and FOV=44x40cm. Breath-holding scan was applied with TR / TE = 3000/74 ms, layer thickness 8mm, layer interval 2mm, matrix = 128x160, and FOV = 44x40cm. Enhancement: cross section adopted 3D LAVA, with TR/TE= 3.6/1.7ms, layer thickness / layer spacing = 5/-2.5 mm, matrix: 256x192, and FOV = 44x40cm.

**Analysis of MRI appearances**

Two radiologists engaged in abdominal imaging diagnosis for more than 10 years analyzed the MRI appearance of all cases on PACS system, including lesion size, boundary, capsule, smooth edge, arterial phase peritumoral enhancement and so on. They would reach an agreement through discussion when in doubt.

The tumor size was measured by selecting the length and diameter of the largest plane according to the liver imaging reporting and data system 2018 standard[13], including the mass capsule, when the mass was shown most clearly in the MRI enhanced portal phase images. Besides, the tumor edge was divided into smooth edges (nodular tumors with smooth edges) and non-smooth edges (budding processes on cross-sectional and coronal images[14]. The capsule was defined as the enhancement degree of the smooth edge of the tumor in the portal vein or delayed phase was higher than that of the mass, and pathologically it was mainly fibrous components[15][16]. The peritumoral enhancement in the arterial phase was defined as a crescent or polygonal enhancement area outside the edge of the tumor in the arterial phase. The degree of overall or partial enhancement was higher than that of the hepatic parenchyma. In addition, there was extensive contact with the edge of the tumor, and the signal in the delayed phase was similar to that of the normal hepatic parenchyma[17]. Arterial rim enhancement is defined as ring-shaped enhancement at the edge of the arterial mass.

**Pathological analysis**

The postoperative tumor tissues were standardized and stained with hematoxylin-eosin staining by an experienced pathologist (engaged in pathological
diagnosis of liver cancer for 20 years). If necessary, immunohistochemical staining was performed. All pathological sections were reviewed according to the 2019 WHO classification, and the classification and diagnosis of HCC and cHCC-CC were performed[18]. That is, cHCC-CC is a mixed area of HCC and CC in tumor cells, and there is a transitional area of the two types at the same time. The growth pattern of cancer focus included peri-cancerous infiltration, capsule formation, microvascular invasion (MVI) and satellite nodules, etc; Liver cirrhosis showed extensive fibrosis of liver tissue with pseudolobule formation.

**Statistical analysis**

This study was divided into two groups with the following statistical methods:

Basic table: continuous variables (measurement data): the variables were consistent with normal distribution, using t-test, and were presented as "mean+sd"; non-normal distribution adopted non-parametric test (kruskal-Wallis), and were presented as "median (1/4-3/4IQR)"; classified variables (count / grade data) adopted chi-square or Fisher test, and were presented "count (percentage)".

Univariate and multivariate analysis: Logistic regression: SPSS19.0 statistical software package, and chi-square test and independent sample t-test were used for statistical analysis. At the same time, combined with clinical significance, the parameters were analyzed by single multi-factor Logistic regression analysis, and the odds ratio (OR) and 95% confidence interval (CI) of each parameter were calculated. The results of multi-factor analysis and statistically significant indicators were analyzed comprehensively. In addition, the sensitivity and specificity under different combinations were calculated. (α = 0.05, bilateral test).

**Results**

**Clinical group**

157 cases of HCC met the inclusion criteria, with an average age of 55.80 ± 10.44 and male to female ratio was 134:23; 59 cases of cHCC-CC met the inclusion criteria with an mean age of 52.80 ± 10.23 and male to female ratio was 48:11. There was no significant difference in onset age and gender ratio between the two group (P
There was no significant difference in tumor diameter (defined by 5 cm), AFP, HBsAg and HBeAg between HCC and cHCC-CC cases ($P = 0.116, 0.407, 0.589, 0.159$, respectively).

The proportion of multiple lesions in HCC is 17:157 (10.83%). However, that of cHCC-CC was 17:59 (28.81%). The probability of HCC complicated with liver cirrhosis was (114/157, 72.61%), and the probability of cHCC-CC was (30/59, 50.85%). The differences between the two group were statistically significant ($P = 0.001, 0.003$ Table 1). At the same time, cHCC-CC is more prone to MVI than HCC manifestations. ($36.31\%$ Vs $61.02\%$, $P < 0.001$ Table 1)

**Image sign**

There were significant differences in arterial peritumor enhancement and tumor margin between HCC and cHCC-CC groups ($P < 0.001$). Besides, the incidence of non-smooth margin in cHCC-CC group was higher than that in HCC group ($84.75\%$ vs $52.23\%, P < 0.001$). Moreover, the HCC group had a lower incidence of peritumor enhancement in arterial phase ($11.46\%$ Vs $62.71\%$, $P < 0.001$) The capsular appearance rates of the two groups on MRI were 40/157 (25.48%) and 8/59 (13.56%), respectively. There was no significant difference between the two group ($P = 0.061$, Table 1) (Figs.2, 3 and 4).

**Risk factor analysis**

According to multivariate analysis, liver cirrhosis (OR=0.373,95%CI:0.175,0.793, $P=0.010$) and arterial peritumor enhancement (OR=8.833,95%CI:4.033,19.346, $P<0.001$) were independent risk factors for cHCC-CC ($P < 0.001$) (Table 2). Besides, arterial peritumor enhancement imaging signs had high sensitivity (62.71%) and specificity (88.54%) in the diagnosis of cHCC-CC (Table 3).

**Discussion**

It was found that there was no statistical significance in other aspects except the ratio of single focus to multiple lesions and the probability of mass complicated with liver cirrhosis through the study of the clinical date of the two groups of patients. Lin[19] found that the incidence of cHCC-CC in men is higher. However, in this study,
these two types of liver cancer are not statistically significant in terms of gender and age, the author believes that it is related to the small number of cases collected. And clinical manifestations of HCC and cHCC-CC are not typical, may only show skin itching, weight loss and other symptoms[20], different patients have different sensitivity and attention to the changes of the body. As a consequence, patients come to see a doctor in different stages of the disease, so the difference between the two sizes is not statistically significant. Studies have shown that both HBsAg and HBeAg are closely related to the occurrence of hepatocellular carcinoma[21][22], AFP is mainly synthesized in the fetal liver, decreases gradually after birth, and approaches the adult level in about a week, which is of great significance for the diagnosis of HCC[23]. However, it is usually related to the size of the tumor, especially when the tumor is smaller, the proportion of normal value can reach up to 35%-40%[24]. At the same time, some other lesions in the liver, such as hepatoblastoma, will also increase[25]. Because most of the cases collected in this study were smaller than 5cm in diameter, and cHCC-CC contained both hepatocellular carcinoma and cholangiocarcinoma, there was no significant difference in AFP, HBsAg and HBeAg between them. Most scholars believe that it originated from hepatic progenitor cells though there are different opinions on the origin of cHCC-CC[26][27]. Hepatic progenitor cells are a group of undifferentiated cells with hepatocyte function in normal human liver, which are mainly distributed in the Hering duct of portal vein and bile canaliculi[28]. On the other hand, the Hering duct constructs the relationship between the biliary system and the intralobular anatomy and microscopic system of the liver. Once cHCC-CC occurs, it is easy to have multiple lesions. At the same time, although the blood supply of cHCC-CC is less than that of HCC, its ability to invade large hepatic vessels, such as portal vein and hepatic vein, is similar to that of HCC. Besides, it is more likely to have metastasis of peripheral lymph nodes[29]. As a consequence, the probability of multiple lesions in cHCC-CC is significantly higher than that in HCC, and the difference is statistically significant. Liver cirrhosis is a very important link in the occurrence of hepatocellular carcinoma. Studies have shown that up to 85%-90% of hepatocellular carcinoma patients have liver
Although cHCC-CC contains liver cancer cells, liver cancer cells are only one of its components. As a consequence, the probability of cHCC-CC patients with liver cirrhosis is low, and the difference between the two is statistically significant.

MRI-enhanced scanning is widely used in the diagnosis and differential diagnosis of benign and malignant liver lesions and the evaluation of liver function with the advantages of non-radiation and multi-mode imaging[31][32]. It was found that there was significant difference in tumor margin and peritumoral enhancement between HCC and cHCC-CC groups. The analysis showed that the focus of HCC was mainly composed of hepatocellular carcinoma cells with few fibrous components. However, the fibrous tissue contained in cHCC-CC cholangiocarcinoma components would pull the mass in the process of growth and lead to lobular changes, so sometimes it could be shown as budding protuberance on imaging[33]. Moreover, because cHCC-CC contains both HCC and ICC, HCC is more prone to hematogenous metastasis, and ICC is more likely to have lymph node metastasis. The invasiveness is stronger[29][34], and the probability of rough edge on the image is greater. The difference between the two is statistically significant. Studies have shown that[35][36]. Peritumoral enhancement is an important index for predicting microvascular infiltration. It is generally believed that the main reason for this phenomenon is that the normal liver tissue is mainly supplied by the portal vein, and the tumor thrombus caused by microvascular infiltration will lead to obstruction of portal vein branches around the mass. As a result, the surrounding arteries are compensated[37]. As a consequence, when the enhanced scan is performed, the area can be obviously enhanced in the arterial phase. The analysis of the pathological results of the two groups showed that the microvascular infiltration ability of the cHCC-CC group was higher than that of the HCC group. Therefore, the cHCC-CC group was more likely to have peritumoral enhancement in the arterial phase than the HCC group. The difference is statistically significant.

"Capsule sign" is one of the main signs of LI-RADS. It is mainly an imaging manifestation of collagen fibers produced by liver cancer cells, hepatocytes and various factors activating stellate cells [15]. This sign has
a good heterogeneity in the diagnosis of HCC[38], and can better distinguish HCC from benign liver lesions or other non-HCC liver malignant lesions. The hepatoma cell components contained in cHCC-CC can also activate some stellate cells to produce collagen fibers, showing a "capsule sign" in the image. As a consequence, there is no significant difference between the two.

It was found that liver cirrhosis and arterial peritumor enhancement were independent risk factors for predicting cHCC-CC, and the imaging feature of arterial peritumor enhancement had high sensitivity (62.71%) and specificity (88.54%) for the diagnosis of cHCC-CCA through the multivariate analysis of the collected data. It has a certain hint for the diagnosis of cHCC-CC.

The main shortcomings of this study are as follows: 1. The number of cases was relatively small, which might cause certain errors. 2. Because the imaging data provided by MRI plain scan was limited, the display of some lesions was poor, and the blood supply of lesions can not be well observe. As a consequence, this study did not choose MRI plain scan data for analysis. 3. This study did not make a more detailed pathological classification of cHCC-CC. In the future, the author will conduct a more detailed study on these deficiencies.

**Conclusion**

Generally speaking, it can be concluded that combining the two data, we can make a better differential diagnosis between HCC and cHCC-CC before operation according to the analysis of the preoperative clinical features and the imaging findings of contrast-enhanced MRI. Compared with cHCC-CC, HCC is more likely to be complicated with liver cirrhosis However, the incidence of multiple lesions, peritumoral enhancement in arterial phase, and rough margin is lower. Liver cirrhosis and arterial peritumoral enhancement are independent risk factors for predicting cHCC-CC. Arterial peritumoral enhancement has high sensitivity and specificity in the diagnosis of cHCC-CC.
References


**Figure Legends:**

Fig.1 Flowchart detailing the patient selection process and exclusion criteria. In total, 157 patients with HCC and 59 patients with cHCC-CCA were enrolled in the final analysis.

Fig.2 cHCC-CC presenting in a 42-year-old man. (A-B): multiple lesions (red arrow) and non-smooth margin (white arrow) on T2WI; (C): The arterial phase shows obvious peritumoral enhancement (yellow arrow); (D): without capsule shows in enhanced portal phase images.

Fig.3 cHCC-CC presenting in a 51-year-old man. (A-B): Non-smooth tumor margin on T1WI, T2WI (white arrow) (C): Without obvious peritumoral enhancement in the arterial phase (yellow arrow) (D): Capsule obvious shows in enhanced portal phase images (red arrow).
Fig.4 HCC presenting in a 58-year-old man. (A-B) smooth margin on T1WI、T2WI（white arrow）（C): The arterial phase shows obvious peritumoral enhancement(yellow arrow)（D) capsule obvious shows in enhanced portal phase images (red arrow).

**Declarations**

**Ethics approval and consent to participate:** This study was a retrospective study, which was approved by the Ethics Committee of our hospital, exemption of Informed Consent. The enhanced MRI test had been widely used in clinic to exempt patients from informed consent.

**Consent for publication:** Not applicable

**Availability of data and materials:** All data generated or analysed during this study are included in this published article.

**Competing interests:** Not applicable

**Funding:** This study has received funding by the Shanghai clinical project [SHDC2020CR1029B], Natural science foundation of guangdong province [2018A030313951], Sponsored by Natural Science Foundation of Chongqing [cstc2018jxjl130081].

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Ethical Approval for Research: N.A.
obtained funding: Xian Yu, Ningyang Jia, Ling Zhang
study supervision: Lun Lu, SiSi Zhang
Acknowledgements: Not applicable
From June 2015 to October 2017, 366 cases of clinically suspected liver cancer underwent enhanced MRI scans, of which HCC: n=279, cHCC-CCA: n=87.

Less than 18 years old or more than 80 years old (n=32)
HCC: n=27
cHCC-CCA: n=5

Patients undergoing enhanced MR examination after TACE or radiotherapy (n=13)
HCC: n=9
cHCC-CCA: n=4

Tumor MRI scan image quality is poor or incomplete (n=5)
HCC: n=3
cHCC-CCA: n=2

No liver surgery (n=58)
HCC: n=51
cHCC-CCA: n=7

Postoperative pathology suggests ICC or other liver tumors (n=42)
HCC: n=32
cHCC-CCA: n=10

HCC: n=157
cHCC-CCA: n=59

**Figure 1**

Flowchart detailing the patient selection process and exclusion criteria. In total, 157 patients with HCC and 59 patients with cHCC-CCA were enrolled in the final analysis.
Figure 2

cHCC-CC presenting in a 42-year-old man. (A-B): multiple lesions (red arrow) and non-smooth margin (white arrow) on T2WI; (C): The arterial phase shows obvious peritumoral enhancement (yellow arrow); (D) without capsule shows in enhanced portal phase images.

Figure 3

cHCC-CC presenting in a 51-year-old man. (A-B): Non-smooth tumor margin
HCC presenting in a 58-year-old man. (A-B) smooth margin on T1WI\(\rightarrow\)T2WI. White arrow (C): Without obvious peritumoral enhancement in the arterial phase (yellow arrow) (D) Capsule obvious shows in enhanced portal phase images (red arrow).

Figure 4