PD1-6　Gd-EOB-DTPA 造影 MRI の肝細胞相にて非定形的信号を呈する肝腫瘤症例の検討

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Gd-EOB-DTPA 造影 MRI は、血流画像を描出するとともに肝細胞相にて胆汁酸の取り込み・排泄能を疑似評価できる新しい modality である。肝細胞相では、EOB 取り込み能をもたない腫瘤を低信号域として陰性描出するが、肝細胞癌脱分化シークエンスにおける早期の脱分化をも検出することができ、その感度には疑う余地がない。一方で検出不能な肝細胞癌症例や高信号に描出される結節を経験し、その解釈に戸惑うことがある。すなわち、血流診断からは明らかな多血性肝細胞癌が、EOB-MRI 肝細胞相にて高信号や等信号を呈することがあり、EOB-MRI による診断のみで十分といえない要素を残している。 この例外的な事象をも、一つの画像パターンとして認識することにより「例外」から「積極的診断」に変え、EOB-MRI による診断をより精度の高いものにすることができると考え、今回われわれは、EOB-MRI の肝細胞相にて高～等信号を呈した結節、およびリング状の高信号染影を呈した結節について検討したので報告する。

【対象と方法】

2007 年から 2010 年 4 月の間に EOB-MRI の肝細胞相画像を評価できた 530 症例を review し、腫瘤が高信号を呈した症例および結節と判定しうる等信号を呈した症例を拾い上げた。治療後による変化は除外した。合致する計 60 症例につき、画像的特徴や他の modality における描出を検討した。可能な症例については組織学的検討を追加し、一部の症例では OATP8 や MRP3 など、EOB の取り込み、排泄に関わるトランスポーターの発現を検討した。

【結果 1】

検討症例のうち 11 例では、腫瘤辺縁がドーナツ状の高信号を呈した。うち 3 例は FNH と考えられた。4 例は CTAP 濃染結節で、組織学的には 7 例が過形成結節または再生結節であった。1 例は結節内結節型を呈した HCC で、内包腫瘍の周囲にドーナツ状染影を認めた。早期濃染域の直径とドーナツ状染影の内径外径の比較検討では、結節内結節型を呈した HCC を除いて高信号域は腫瘤側であり、HCC 症例では高信号域はコロナ様に外側に見られた。

【結果 2】

ドーナツ状を呈さない 49 症例のうち、2 例は FNH と診断され、22 例は臨床的に HCC と診断された。病理組織を検討できた 14 例の HCC のうち 3 例は偽腺管構造を呈した。EOB-MRI の肝細胞相画像において、HCC と診断されたうちの 4 例では腫瘤の外側に低信号帯を認め、4 例では内部に低信号結節を内包していた。すなわち EOB-MRI 肝細胞相で高信号を呈する結節は、淡い低信号の内部に生じ、内部に強い低信号を生じることがある。EOB-MRI 肝細胞相における

信号強度はOATP8やMRP3等のトランスポーターの発現と相関し、結節内結節型では層状に発現域が描出された。EOB-MRI肝細胞相で高信号を呈する肝細胞癌は、動脈性多血と門脈血流低下を呈する。動脈性多血は軽度のことが多いが、強い多血を呈することもある。

【考察】

EOB-MRI肝細胞相で高信号を呈する結節には、1）高分化～中分化多血性肝細胞癌、2）乏血性の再生結節、過形成結節、3）FNH、良性のCTAP濃染結節、がみられ、1）は軽度多血～多血を呈し、3）はドーナツ型 の高信号を呈する。1）には結節内結節型症例（EOB高信号領域が内包される症例やEOB高信号結節の内部に低信号域をもつ症例）が見られ、EOB取り込みトランスポーターであるOATP8の発現と相関することが示され、脱分化過程の一段階としてEOB取り込みや排泄が制御されている可能性が示唆された。高信号を呈する理由として、1'）EOB取り込みトランスポーター（OATP 8）の発現増加、2'）造影前のT1Wで、すでに高信号を呈する、3'）肝細胞機能を保ち、かつ、血流増加がみられる、ことが挙げられる。

【結語】

EOB-MRI肝細胞相で非定型的画像（高信号）を呈する結節も、画像パターンを十分に解析し認識しすれば、積極的な診断が可能である。EOB-MRIの肝細胞相で高信号を呈する結節の画像診断におけるdecision treeを作成し、呈示した。

PD1-8 Gd-EOB-DTPA 造影 MRI の肝細胞相にて低信号を呈する乏血性肝腫瘤症例の検討

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【緒言】

Gd-EOB-DTPA MRI(以下 EOB-MRI) が出現し、その肝細胞相は前癌病変および高分化肝細胞癌の検出能が高いことが明らかにされたが、その臨床上の取り扱いにはしばしば苦慮する。今回我々は、EOB-MRI の肝細胞相にて低信号を呈し、血管造影下 CT にて非多血性を確認された肝腫瘍について CTHA/CTAP の造影パターン、病理組織、およびその後の経過を検討し、臨床上の取り扱いについて考察した。

【対象と方法】

2008 年 7 月から 2010 年 5 月に施行された EOB-MRI の肝細胞相で、周囲肝より低信号を呈し、その前後 2 カ月以内の血管造影下 CT にて非多血性を確認した 55 症例 83 結節を対象とした。対象症例は男性 32 例、女性 23 例、平均年齢 72.2±6.9 歳、背景肝は慢性肝炎 4 例、肝硬変 51 例 (Child-Pugh 分類 grade A：43 例、Child-pugh 分類 grade B：8 例) で、慢性肝疾患の成因は HCV50 例、HBV4 例、非 B 非 C1 例、平均腫瘍径は 1.5±0.5cm であった。
47 結節は組織学的検査を施行した（生検 45 結節、手術切除 2 結節)。治療を行なわず 3 か月以上経過観察できた 55 結節において、腫瘍径の増大および多血化の有無を検討した。

【結果】

83 結節の血流パターンは CTHA/CTAP：iso/iso が 12 結節、low/iso が 13 結節、low/high が 11 結節、low/low が 26 結節、iso/low が 21 結節であった。病理組織が得られた 47 結節の内訳は、高分化型 HCC が 39 結節 (83.0%)、中分化型 HCC が 3 結節 (6.4%)、dysplastic nodule が 3 結節 (6.4%)、特異所見なしが 2 結節 (4.3%) であった。CTAP 低吸収群では 89.3%、CTAP 非低吸収群では 89.5% が HCC で、両群間に差はなかった。腫瘍径別では 1.5cm 以上の群で 92.7%、1.5cm 未満の群で 85.7% が HCC で、両群間に有意差は認めなかった (p=0.64：Fisher's exact test)。
3 か月以上経過観察し得た 55 結節中、多血化が 13 結節 (23.6%) に、非多血性の腫瘍径増大が 16 結節 (29.1%) にみられたが、26 結節 (47.3%) は血流パターン、腫瘍径とも変化を認めなかった (平均観察期間 9.3±4.6 か月)。多血化結節の多血化までの期間は 11.9±4.6 か月であった。平均腫瘍径で多血化結節 (1.4±0.5cm) は不変結節 (1.2±0.4cm) に比し大きい傾向にあり (p=0.060：T test)、非多血性増大結節 (1.6±0.4cm) は不変結節に比し有意に大きかった (p=0.027：T test)。Kaplan-Meier 法では 1 年間での累積多血化率は 31.4% であった。多血化

結節における、非多血時の血管造影下 CT の血流パターンは iso/low(53.8％：7/13 結節) からの多血化が最も多く、CTAP 低吸収の結節 (76.9％：10/13 結節) からの多血化が多かった。腫瘍径別の検討では 1.0cm 以上の結節 (n=47 結節) は 1.0cm 未満の結節 (n=8 結節) に比し累積多血化率が高い傾向にあったが、有意差は認めなかった (p=0.25:Log-Rank test)。多血化結節に、非多血性のまま増大する結節を加え、その累積出現率を検討したところ、1.5cm 以上 (n=20 結節)、1.5cm 未満 (n=35 結節) の 2 群間では、1.5cm 以上の結節において有意にその累積出現率が高い結果であった (p=0.017：Log-Rank test)(図 1)。

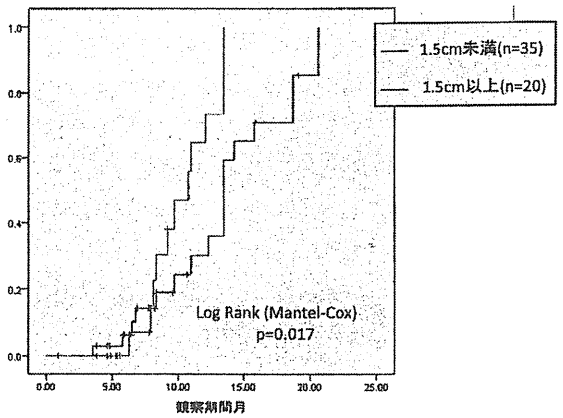

図 1　多血化 + 非多血性増大結節の累積出現率

【考察】

EOB-MRI の肝細胞相において低信号結節として検出された乏血性結節は、血流診断、腫瘍径によらず組織学的に高頻度に肝細胞癌であった。しかし乏血性腫瘍の増大は緩徐であり [1]、組織学的に癌であることと臨床的な治療対象とするかは別次元の問題として捉える必要がある。一方、腫瘍径 1.5cm 前後より増大、多血化傾向がみられた。この結果は、早期肝癌において腫瘍径が 1.5~2.0cm に至ると、活発に増殖し、脱分化する傾向にあるという [2] 文献的報告に合致する結果であった。肝癌診療マニュアル第 2 版においても、EOB-MRI 肝細胞相で低信号を呈する 1.5cm 以上の非多血性肝癌は、腫瘍生検のうえ高分化肝細胞癌と診断されれば治療対象とすると提案されている [3]。今回の我々の検討結果は、その提案を支持する結果であった。しかし、乏血性結節を治療することが、患者予後の改善につながるかは不明であり、今後検証していく必要がある。EOB-MRI 時代の到来により、新たな肝癌診療の展開が望まれ、今回のような検証の蓄積が必要と思われる。

(文献)

(1) Kudo M, Tochio H. Oncology. 2008;75 Suppl 1:55-64. Epub 2008 Dec 17.

(2) The International Consensus Group for Hepatocellular Neoplasia. Hepatology, 2009 Feb;49(2):658-64.

(3) 肝癌診療マニュアル第 2 版 . 編集：日本肝臓学会

W1-9　当院における Sorafenib の使用経験

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【背景】

本邦において肝細胞癌に対し Sorafenib が保険適応となり約 1 年が経過した。しかし経カテーテル治療（TACE）が普及している本邦の患者に対する臨床効果や治療アルゴリズムにおける位置付けは未だ不確定であり、明らかにすることが急務である。今回我々は当院における Sorafenib 投与例の治療効果および副作用を検討したので報告する。

【対象および方法】

1．対象

当院における Sorafenib 投与の適応は、遠隔転移のある Stage IV B 症例、RFA または手術の適応がなくかつ TACE も不応と判定された症例を主な対象としている。

今回の対象は 2009 年 6 月から 2010 年 7 月の間に当院にて Sorafenib を導入した肝細胞癌患者 46 例（1 例は 2008 年に導入された治験症例）。男女比 38:8，平均年齢 69.0（46-87）歳，肝炎ウイルスマーカーは B/C/B+/non-B non-C がそれぞれ 26/8/3/9 例，肝予備能（Child-Pugh）は 5/6/7/8 点が 16/14/12/4 例，肉眼的進行度は III / IV A/ IV B が 23/5/18 例であった。肝癌診断後平均 6.8 年（1 カ月 -9.7 年）が経過し、Sorafenib 導入前治療は肝切除，RFA，IVR，全身化学療法がそれぞれ 8，24，41，3 例に施行されていた（重複あり）。

2．方法

1）当院における投与方法

投与量については当初 800mg/da にて投与を行っていたが後述の副作用多発により 400mg/day にて開始し、その後症例によって増量する方針としている。

2）検討項目

Kaplan-Meier 法を用いた累積生存率，Modified RECIST を基調とした抗腫瘍効果，副作用の 3 項目について検討した。

【成績】

1．累積生存率（46 例）

投与開始後 1，3，6 ヶ月の累積生存率はそれぞれ 95.3，82.6，52.8%，生存期間の中央値は 289 日であった。

2．画像評価

Sorafenib 導入前 1 カ月以内に TACE を施行されておらず、導入の前後に Dynamic CT が撮像され評価可能であった 18 症例における 3 カ月後時点での CR/PR/SD/PD はそれぞれ 0/2/8/8

例であった。PR及びSD計10症例に投与継続したところ6カ月後時点でのCR/PR/SD/PDは0/0/3/5例であった（2例は全身状態増悪のため投与中止）。

3．副作用

副作用による投与中止例は21例（45.6%）で、投与中止までの平均期間は1.3ヶ月であった。投与前肝予備能別の投与中止率はChild-Pugh 5点31%（5/16例），6点43%（6/14例），7点62%（8/13例），8点66%（2/3例）と点数と中止率は相関傾向にあった。副作用の内訳は肝障害10例，手足症候群3例，消化管症状（下痢）4例，消化管出血3例，精神症状（鬱）1例であった。投与量別を見てみるとSorafenib 800mg群で51%(16/31例)と，400mg群(33%(5/15例)より頻度が高い傾向を認めた。

【考案】

累積生存率については、Child-Pugh Bの肝機能低下例（16/46）を多く含むにも関わらずSHARP試験に遜色ない数字であったが、画像評価では腫瘍縮小効果が見られた症例はごく一部であった。やはり、本薬剤の主目的は腫瘍増大のコントロールであり、長期投与により価値を発揮するが、それを可能とするには副作用コントロールの重要性が再確認された。沼田らによりSorafenib 400mg減量投与による副作用の減少が報告されているが当院でも同様の傾向であった。

【結語】

Sorafenibの当院での使用経験を報告した。今後症例を重ね、引き続き有用性，安全性について検討していきたい。

(文献)

(1) Llovet JM, Ricci S, MazzaferroV, et al. N Engl J Med 2008; 359: 378—390.

(2) 沼田和司，ら．肝臓2010; 51:400-402.

(3)Livraghi T, et al. Hepatology 2008;

第46回　日本肝癌研究会記録集

発行日：2011年5月
監　修：林　紀夫
編　集：竹原徹郎
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　　TEL：06-6879-3621
発行所：三鳩堂

J Gastroenterol
DOI 10.1007/s00535-011-0505-8

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Decrease in alpha-fetoprotein levels predicts reduced incidence of hepatocellular carcinoma in patients with hepatitis C virus infection receiving interferon therapy: a single center study

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Received: 28 July 2011 / Accepted: 24 October 2011

Abstract
Background Increasing evidence suggests the efficacy of interferon therapy for hepatitis C in reducing the risk of hepatocellular carcinoma (HCC). The aim of this study was to identify predictive markers for the risk of HCC incidence in chronic hepatitis C patients receiving interferon therapy.
Methods A total of 382 patients were treated with standard interferon or pegylated interferon in combination with ribavirin for chronic hepatitis C in a single center and evaluated for variables predictive of HCC incidence.
Results Incidence rates of HCC after interferon therapy were 6.6% at 5 years and 13.4% at 8 years. Non-sustained virological response (non-SVR) to antiviral therapy was an independent predictor for incidence of HCC in the total study population. Among 197 non-SVR patients, independent predictive factors were an average alpha-fetoprotein (AFP) integration value $\geq$10 ng/mL and male gender. Even in patients whose AFP levels before interferon therapy were $\geq$10 ng/mL, reduction of average AFP integration value to <10 ng/mL by treatment was strongly associated with a reduced incidence of HCC. This was significant compared to patients with average AFP integration values of $\geq$10 ng/mL ($P = 0.009$).
Conclusions Achieving sustained virological response (SVR) by interferon therapy reduces the incidence of HCC in hepatitis C patients treated with interferon. Among non-SVR patients, a decrease in the AFP integration value by interferon therapy closely correlates with reduced risk of HCC incidence after treatment.

Keywords Alpha-fetoprotein · Hepatocellular carcinoma · Hepatitis C · Interferon

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Published online: 23 November 2011

Introduction

Hepatitis C virus (HCV) infection is a predominant cause of liver cirrhosis and hepatocellular carcinoma (HCC) in many countries, including Japan, the United States, and countries of Western Europe [1–5]. The annual incidence of HCC in patients with HCV-related cirrhosis ranged from 1 to 8% [6–9]. Even in the absence of liver cirrhosis, patients with chronic hepatitis caused by HCV infection are at a high risk of developing HCC. Indeed, a large-scale Japanese cohort study showed that the annual incidence of HCC is 0.5% among patients with stage F0 or F1 fibrosis and 2.0, 5.3, and 7.9% among those with F2, F3, and F4 fibrosis, respectively [9]. Periodic surveillance is recommended to detect HCC as early as possible in patients with HCV-related chronic liver disease; however, this may not be cost-effective. For patients with chronic hepatitis C, more effective detection and prevention of HCC is being sought by two important routes: (1) the attempt to discover noninvasive predictive markers and (2) development of treatment strategies to reduce the risk of HCC. There have been several attempts to discover non-invasive markers capable of predicting the risk of HCC incidence in patients with chronic hepatitis C [6, 10]. For example, a cohort

derived from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) Trial identified older age, African American race, lower platelet count, higher alkaline phosphatase, and esophageal varices as risk factors for HCC [11].

There have also been a number of studies to evaluate the effect of anti-viral treatment of chronic hepatitis C on the incidence of HCC [12–19]. The results were summarized in a meta-analysis, which concluded that the effect of interferon on risk of HCC is mainly apparent in patients achieving a sustained virological response (SVR) to interferon therapy [13]. In addition, a number of studies have suggested the incidence of HCC is reduced in treated patients compared to historical controls [12, 15, 16, 19]. However, the recent HALT-C randomized control trial revealed that long-term pegylated interferon therapy does not reduce the incidence of HCC among patients with advanced hepatitis C who do not achieve SVRs. Reduction in the risk of HCC by maintenance therapy was shown only in patients with cirrhosis [14, 17]. These controversial results suggest that interferon therapy reduces the risk of HCC only in a group of patients with HCV-related chronic liver disease. Thus, it is important to evaluate the risk of HCC development in hepatitis C patients receiving interferon therapy and it will be clinically useful to discover markers distinguishing high- and low-risk groups.

Serum alpha-fetoprotein (AFP) has been widely used as a diagnostic marker of HCC [20–22]. However, elevation of serum AFP levels is often found in non-neoplastic liver diseases without evidence of HCC, including acute liver injury and chronic viral hepatitis [23–27], especially among patients with advanced chronic hepatitis C [28]. An increase of AFP after liver damage is interpreted as a sign of dedifferentiated hepatic regeneration [27]. There have been some reports that AFP is a significant predictor of HCC in patients with chronic hepatitis C [4, 5, 29]. In addition, it has recently been shown that AFP levels decrease in response to interferon administration in patients with chronic hepatitis C [30, 31], and that long-term interferon therapy for aged patients with chronic HCV infection is effective in decreasing serum AFP levels and preventing hepatocarcinogenesis [32, 33]. However, little is known about the relationship between changes in serum AFP level over time during interferon therapy and the development of HCC.

The aim of this large single center study was to identify predictive markers for the risk of HCC development in patients receiving interferon therapy for chronic hepatitis C. For this purpose, patients treated with standard or pegylated interferon, in combination with ribavirin, for chronic hepatitis C were enrolled and subjected to scheduled periodic surveillance for HCC and a number of potential predictive markers, including AFP and alanine aminotransferase (ALT) integration values, at a single center.

Materials and methods

Patients

Between January 2002 and April 2010, 528 patients with chronic hepatitis C received combination therapy with standard interferon and ribavirin ($n = 84$) or pegylated interferon and ribavirin ($n = 444$) at Osaka Red Cross Hospital. Eligibility criteria for treatment were positivity for serum HCV RNA and histological evidence of chronic hepatitis C ($n = 427/444$; 80.9%), or positivity for serum HCV RNA, liver enzyme levels greater than the normal upper limit, and an ultrasound image demonstrating chronic liver damage ($n = 101/444$; 19.1%). Exclusion criteria for treatment were as follows: neutrophil count <750 cells/μL, platelet count <50,000 cells/μL, hemoglobin level ≤9.0 g/dL, and renal insufficiency (serum creatinine levels >2 mg/dL).

Of 528 patients who received interferon therapy for chronic hepatitis C, 146 were excluded from this study for the following reasons: follow-up <24 weeks after the termination of the interferon therapy ($n = 122$), previously treated for HCC ($n = 22$), or occurrence of HCC during or within 24 weeks after treatment ($n = 2$). Therefore, 382 patients were enrolled for the study and were retrospectively analyzed.

To detect early-stage HCC, ultrasonography, dynamic contrast enhanced computed tomography (CT), dynamic contrast enhanced magnetic resonance imaging (MRI), and/or measurement of tumor markers (including AFP) were performed for all patients at least every 6 months. HCC was diagnosed radiologically as liver tumors displaying arterial hypervascularity and venous or delayed phase washout by dynamic contrast enhanced CT or MRI.

The study protocol was approved by the Ethics Committee at Osaka Red Cross Hospital and performed in compliance with the Helsinki Declaration.

Treatment protocol and definition of responses to treatment

The basic treatment protocol for patients with chronic hepatitis C consisted of 6 mega units of interferon-α-2b 3 times a week or 1.5 μg/kg of pegylated interferon α-2b once a week, combined with ribavirin at an oral dosage of 600–1000 mg/day. Duration of the treatment was 48–72 weeks for those with HCV genotype 1 and serum HCV RNA titer of >5 log IU/mL, and 24 weeks for all other patients.

Patients who were negative for serum HCV RNA for >6 months after completion of interferon therapy were defined as showing an SVR. Patients whose serum ALT levels decreased to the normal range and remained normal for >6 months after the termination of interferon therapy were defined as showing a sustained biochemical response (SBR).

Patients who did not achieve SVR received ursodeoxycholic acid and/or glycyrrhizin containing preparation (Stronger Neo-Minophagen C), when serum ALT levels were higher than the upper limit of normal.

Virological assays

HCV genotype was determined by polymerase chain reaction (PCR) amplification of the core region of the HCV genome using genotype-specific PCR primers [34]. Serum HCV RNA load was evaluated once a month during and 24 weeks after treatment using a PCR assay (Cobas Amplicor HCV Monitor, Roche Molecular Systems, Pleasanton, CA, USA).

Measurement of AFP and calculation of average integration value

AFP was measured in serum samples obtained from each patient at intervals of 1–3 months. The median number of examinations was 15 (range 1–70) in each patient. Serum AFP levels were determined by enzyme-linked immunosorbent assay, which was performed using a commercially available kit (ELISA-AFP, International Reagents, Kobe, Japan). Integration values of AFP and ALT were calculated as described in previous reports [35]. For example, the integration value of AFP was calculated as follows, $(y_0 + y_1) \times x_1/2 + (y_1 + y_2) \times x_2/2 + (y_2 + y_3) \times x_3/2 + (y_3 + y_4) \times x_4/2 + (y_4 + y_5) \times x_5/2 + (y_5 + y_6) \times x_6/2$, i.e., the area of each trapezoid representing an AFP value was measured the sum of the resulting values used to calculate the integration value (Fig. 1). The average integration value was obtained by dividing the integration value by the observation period from initiation of the treatment.

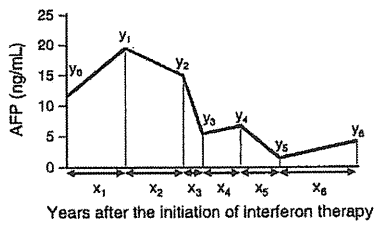

Fig. 1 Example plot of data used for calculation of average integration value of alpha-fetoprotein (AFP)

Statistical analysis

The Kaplan–Meier method was used to estimate the rates of development of HCC in patients after interferon therapy. Log-rank tests were used to evaluate the effects of predictive factors on incidence of HCC. Significance was defined as $P < 0.05$. Multivariate Cox regression analysis using the stepwise method was used to evaluate the association between HCC incidence and patient characteristics, and to estimate hazard ratio (HR) with a 95% confidence interval (CI). A P value of 0.1 was used for variable selection and was regarded as statistically significant. SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Characteristics of patients and incidence of HCC

This study included 382 patients treated for chronic hepatitis C with standard interferon or pegylated interferon in combination with ribavirin. Baseline clinical and virological characteristics of patients included in the study are summarized in Table 1. The median age of the patients at the outset of therapy was 59.0 years (range 18–81 years) and the median follow-up period was 4.1 years (range 0.1–8.4 years). The majority of patients were infected with HCV genotype 1b ($n = 229$; 60%), and median serum HCV RNA load was 6.1 log IU/mL (range 2.3–7.3 log IU/mL). Baseline (before interferon therapy) median serum AFP level was 6.9 ng/mL (range 1.6–478.3 ng/mL).

During follow-up, 23 patients (4.9%) developed HCC. The cumulative incidences of HCC, which was estimated using the Kaplan–Meier method, were 3.1, 6.6, and 13.4% at 3, 5, and 8 years, respectively (Fig. 2).

Predictive factors for incidence of HCC in all patients

Predictive factors for incidence of HCC in all 382 patients were analyzed using log-rank tests (Table 2). Univariate analysis showed that age ≥70 years ($P = 0.040$), non-SVR ($P < 0.0001$), non-SBR ($P = 0.027$), average ALT integration value ≥40 IU/L ($P = 0.001$), baseline AFP ≥10 ng/mL ($P = 0.005$), average AFP integration value ≥10 ng/mL ($P < 0.0001$), and baseline platelet count <150,000 platelets/μL ($P = 0.001$) were all significantly associated with the incidence of HCC. After multivariate analysis, the only variable remaining in the model was non-SVR (HR 8.413, 95% CI 1.068–66.300, $P = 0.043$).

Table 1 Characteristics of 382 patients with hepatitis C treated with interferon therapy in this study

Age (years)	59.0 (18–81)
[a]Males/females	192/190
Observation period (years)	4.1 (0.1–8.4)
[a]IFN + RBV/PEG-IFN + RBV	69/313
HCV genotype 1/2/unclassified	229/57/96
HCV RNA (log IU/mL)	6.1 (2.3–7.3)
White blood cell count (/μL)	4950 (2050–9970)
Hemoglobin (g/dL)	14.0 (10.3–18.8)
Platelet (10^4/μL)	15.0 (5.3–36.4)
AST (IU/L)	56 (17–244)
ALT (IU/L)	67 (16–416)
Bilirubin (mg/dL)	0.8 (0.3–2.4)
AFP (ng/mL)	6.9 (1.6–478.3)

Qualitative variables ([a]) are shown in number, and quantitative variables expressed as median (range)

IFN interferon, *RBV* ribavirin, *PEG-IFN* pegylated interferon, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein

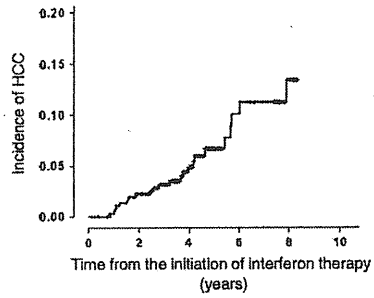

Fig. 2 Incidence of hepatocellular carcinoma (HCC) in 382 patients with hepatitis C who received interferon therapy, estimated using the Kaplan–Meier method

Further, although patients with average AFP integration values ≥10 ng/mL also appeared to have an increased risk of HCC, the difference did not reach statistical significance in the multivariate analysis ($P = 0.050$) (Table 3).

Predictive factors for incidence of HCC in non-SVR patients

Because non-SVR was the only predictive factor across the entire study cohort, to clarify predictive factors for incidence of HCC within this group, the same variables were further analyzed in non-SVR patients alone. By univariate analysis, average AFP integration value ≥10 ng/mL ($P = 0.019$) and baseline platelet count <150,000 ($P = 0.0022$) (Table 2) were again identified as significant predictive factors for incidence of HCC. In addition, male gender was significantly associated with incidence of HCC in non-SVR patients ($P = 0.022$). Multivariate analysis, however, indicated that only two variables were independently associated with incidence of HCC in non-SVR patients: average AFP integration value ≥10 ng/mL (HR 4.039, 95% CI 1.570–10.392, $P = 0.004$), and male gender

Table 2 Univariate analysis of predictive factors for incidence of hepatocellular carcinoma in all 382 and 197 non-SVR patients

Factors	All ($n = 382$)			Non-SVR ($n = 197$)		
	No.	Incidence of HCC ($n = 23$)		No.	Incidence of HCC ($n = 22$)	
		No. (%)	*P* value[a]		No. (%)	*P* value[a]
Age (years)						
<70	359	19 (5)	0.040	182	18 (10)	0.089
≥70	23	4 (17)		15	4 (27)	
Sex						
Female	190	8 (4)	0.125	111	8 (7)	0.022
Male	192	15 (8)		86	14 (16)	
HCV genotype						
1	229	12 (5)	0.452	137	12 (9)	0.796
Non-1	57	1 (2)		10	1 (10)	
Virological response						
SVR	185	1 (1)	<0.0001			
Non-SVR	197	22 (11)				
Biochemical response						
SBR	282	12 (4)	0.027	102	11 (11)	0.857
Non-SBR	86	11 (13)		81	11 (14)	
ALT before IFN therapy						
<40	79	2 (3)	0.274	39	2 (5)	0.319
≥40	301	21 (7)		158	20 (13)	
ALT integration value						
<40	238	6 (3)	0.001	79	5 (6)	0.153
≥40	142	17 (12)		118	17 (14)	
AFP before IFN therapy						
<10	230	7 (3)	0.005	102	7 (7)	0.124
≥10	116	14 (12)		75	13 (17)	
AFP integration value						
<10	258	8 (3)	<0.0001	115	8 (6)	0.019
≥10	63	12 (19)		53	11 (21)	
Platelet before IFN therapy						
<150,000	187	20 (11)	0.001	121	19 (16)	0.022
≥150,000	194	3 (2)		76	3 (4)	

[a] Log-rank test

SVR sustained virological response, *SBR* sustained biochemical response, *ALT* alanine aminotransferase, *IFN* interferon, *AFP* alpha-fetoprotein

Table 3 Multivariate analysis of the predictive factors for incidence of hepatocellular carcinoma in all 382 patients

Factors	Hazard ratio	95% CI	*P* value
Virological response			
SVR	1		
Non-SVR	8.413	1.068–66.300	0.043
AFP integration value			
<10	1		
≥10	2.580	0.999–6.659	0.050

SVR sustained virological response, *IFN* interferon, *AFP* alpha-fetoprotein

Table 4 Multivariate analysis of predictive factors for incidence of hepatocellular carcinoma in 197 non-SVR patients

Factors	Hazard ratio	95% CI	*P* value
AFP integration value			
<10	1		
≥10	4.039	1.570–10.392	0.004
Sex			
Female	1		
Male	3.636	1.383–9.563	0.009

AFP alpha-fetoprotein

(HR 3.636, 95% CI 1.383–9.563, $P = 0.009$) (Table 4). There was no significant difference in other variables including those identified as predictive factors in the entire study population (i.e., age, non-SBR, ALT integration value, AFP before interferon therapy) (Table 2).

AFP integration value as a predictive factor for HCC

Further analysis focused on the AFP integration value as this was the strongest predictive factor for incidence of HCC in non-SVR patients. Of the 382 patients, both baseline and AFP integration values were available for 321. These were divided into four groups: (1) AFP "low–low," (2) AFP "low–high," (3) AFP "high–low," and (4) AFP "high–high," for baseline AFP-average AFP integration values, respectively, where "high" is ≥10 ng/mL and "low" is <10 ng/mL. As shown in Fig. 3a, of the 321 patients, 211 (65.7%) showed baseline AFP levels <10 ng/mL. Of these 211, 207 (98%), were in the AFP low–low group, and only four in the AFP low–high groups. Baseline characteristics, including age, gender, serum HCV-RNA, aspartate aminotransferase (AST), ALT, bilirubin, white blood cell, hemoglobin, platelet, observation periods, and number of times of AFP measurement, were not different between AFP high–low group and high–high group. However, AFP-low group, which is a combination of the low–high and low–low groups, showed significantly lower AST level ($P < 0.00001$), lower ALT level ($P < 0.00001$), higher platelet count ($P < 0.00001$), shorter observation period ($P = 0.01448$), and fewer number of times of AFP examination ($P = 0.00035$), compared to both AFP high–high and AFP high–low group. Six patients (2.8%) with baseline AFP levels <10 ng/mL developed HCC in the follow-up period and none of these patients were among the four low–high group patients. Even in patients with high baseline AFP levels, incidence of HCC was only 3.9% among the AFP high–low group (2 of 51 patients). In contrast, 20.3% of patients in the AFP high–high group developed HCC during the follow-up period.

The incidence rate of HCC in three patient groups, "AFP-low" (a combination of the "low–high" and "low–low" groups),"high–low," and "high–high", was estimated using the Kaplan–Meier method and compared using log-rank tests (Fig. 3b). The rate of HCC incidence was significantly higher in the AFP high–high group compared to both the AFP high–low group and patients with low baseline AFP levels ($P = 0.009$ and 0.001, respectively). There was no significant difference between patients with low baseline AFP levels and the AFP high–low group. The 7-year incidence rate of HCC was 32.3% in the AFP high–high group, compared to only 6.6% in the AFP high–low group, and 8.1% in all patients with low pre-treatment levels.

Discussion

It is well recognized that the most effective strategy for the prevention of HCC development in patients with chronic hepatitis C is likely to be the complete elimination of the HCV infection accompanied by the resultant normalization of liver function [7, 12, 13, 15, 16, 19]. Indeed, we confirmed here that non-SVR is the most significant predictive factor for incidence of HCC in patients receiving interferon therapy for chronic hepatitis C. However, it should be noted that the risk of HCC, even in non-SVR patients, differs between individuals. In the current study, we identified AFP integration value and male gender as independent risk factors for incidence of HCC in non-SVR patients. The incidence of HCC was significantly reduced in individuals with average AFP integration values < 10 ng/mL after interferon therapy, which suggests that the decrease of AFP by interferon therapy lowers the risk of developing HCC. Indeed, even where patients had high baseline AFP levels, incidence of HCC was reduced when the AFP integration value decreased after interferon therapy. Thus, our current findings identify AFP integration value as a useful predictive marker of HCC development in non-SVR patients.

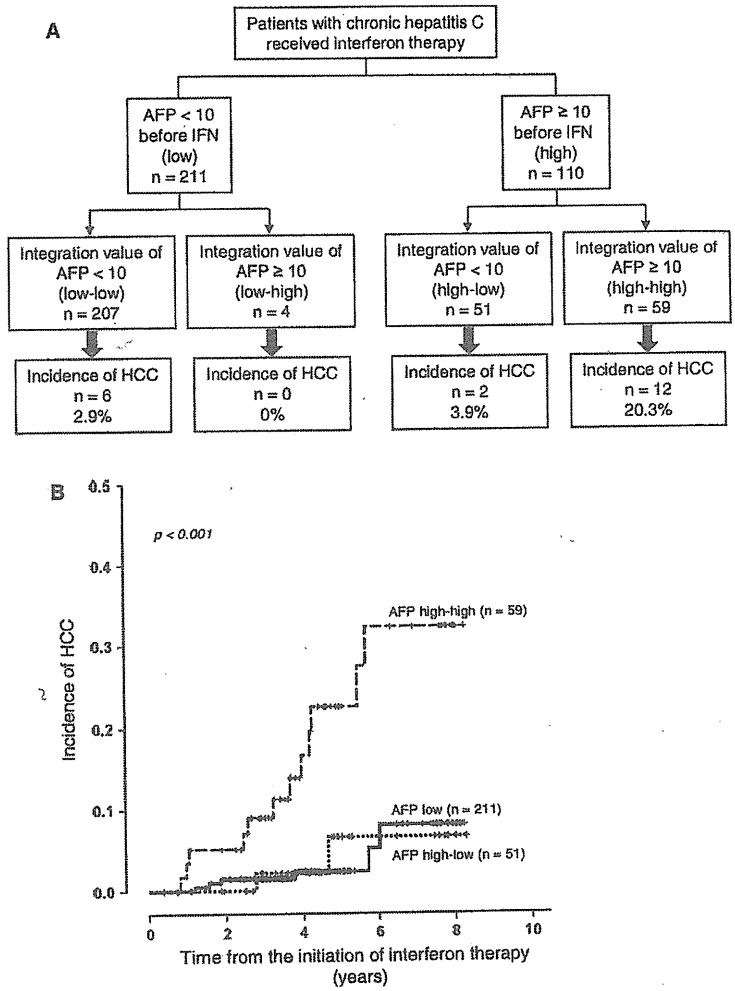

Fig. 3 AFP integration value as a predictive factor for HCC. **a** *Flow diagram* showing the number of patients (*n*) classified by baseline alpha-fetoprotein (AFP) levels before interferon (IFN) therapy and average AFP integration value, and the incidence of hepatocellular carcinoma (HCC) of each group. **b** Kaplan–Meier estimates of the incidence of HCC. *Solid line* AFP-low group (AFP levels before interferon therapy <10 ng/mL); *dotted line* AFP high–low group (baseline AFP levels ≥10 ng/mL, average AFP integration value <10 ng/mL); *dashed line* AFP high–high group (both baseline and average AFP integration values ≥10 ng/mL)

Data from several previous studies suggest that the continuous normalization of alanine aminotransferase (ALT) levels by interferon therapy can reduce the risk of HCC development [36–39]. In addition, one recent study suggested that the ALT integration value is a predictive factor for HCC [35]. In contrast to published data (22), our multivariate analysis did not identify the ALT integration value as a significant predictive factor for HCC incidence, although it was identified as significant by univariate analysis in all 382 patients. Since the previous study did not evaluate AFP levels as a factor for prediction of HCC [35], our results indicate that the AFP integration value is superior to that of ALT as a predictive factor for incidence of HCC. We do not know the reason for this result, but it is speculated that significance of AFP as a marker of hepatic regeneration resulted in the more accurate prediction of hepatocarcinogenesis by integration value of AFP than that of ALT.

As AFP is a diagnostic marker for the existence of HCC, high integration value of AFP in the present study might be a result of HCC development. However, we concluded that the high AFP integration values in patients who developed HCC were not caused by a result of existence of HCC, because of the following two reasons. First, the last AFP values before detection of HCC were not the highest level in the follow-up periods in 19 of 23 patients who developed

HCC, suggesting that the AFP was not produced by the developing HCC in these patients. Second, to exclude the influence of the remaining four patients whose last AFP levels were the highest in the follow-up periods, we analyzed the same statistical analysis by using average AFP integration values excluded the last two examinations of AFP before the detection of HCC. The results of the analysis also showed average integration value of AFP as a significant predictive factor for incidence of HCC.

Male gender was also identified as an independent risk factor for HCC in non-SVR patients in this study. Several reports have shown that men are at a higher risk of developing HCC than women [6, 10, 33, 40, 41]. The male gender also appears to be a risk factor for more severe disease and a greater risk of developing cirrhosis in chronic hepatitis C [42]. Although the association of male gender with the risk of HCC is as yet unexplained, hormonal or genetic factors may lead to increased risk for HCC and cirrhosis in men as previously discussed [10].

In conclusion, a decrease in the AFP integration value predicts reduced incidence of HCC in patients with hepatitis C receiving interferon therapy. Further prospective studies with a larger number of patients are required to validate the significance of these findings.

Acknowledgments This work was supported by Grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labor and Welfare of Japan.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Bruix J, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, Ventura M, Vall M, Bruguera M, Bru C, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet. 1989;2:1004–6.
2. Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, Dioguardi N, Houghton M. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet. 1989;2:1006–8.
3. Hasan F, Jeffers LJ, De Medina M, Reddy KR, Parker T, Schiff ER, Houghton M, Choo QL, Kuo G. Hepatitis C-associated hepatocellular carcinoma. Hepatology. 1990;12:589–91.
4. Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology. 1993;18:47–53.
5. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med. 1993;328:1797–801.
6. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127:S35–50.
7. Ikeda K, Marusawa H, Osaki Y, Nakamura T, Kitajima N, Yamashita Y, Kudo M, Sato T, Chiba T. Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. Ann Intern Med. 2007;146:649–56.
8. Liang TJ, Heller T. Pathogenesis of hepatitis C-associated hepatocellular carcinoma. Gastroenterology. 2004;127:S62–71.
9. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. Ann Intern Med. 1999;131:174–81.
10. Heathcote EJ. Prevention of hepatitis C virus-related hepatocellular carcinoma. Gastroenterology. 2004;127:S294–302.
11. Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology. 2009; 136:138–48.
12. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. International Interferon-alpha Hepatocellular Carcinoma Study Group. Lancet. 1998;351:1535–9.
13. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol. 2001;34:593–602.
14. Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghany MG, Morishima C, Snow KK, Dienstag JL. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med. 2008;359:2429–41.
15. Fattovich G, Giustina G, Degos F, Diodati G, Tremolada F, Nevens F, Almasio P, Solinas A, Brouwer JT, Thomas H, Realdi G, Corrocher R, Schalm SW. Effectiveness of interferon alfa on incidence of hepatocellular carcinoma, decompensation in cirrhosis type C. European Concerted Action on Viral Hepatitis (EUROHEP). J Hepatol. 1997;27:201–5.
16. Hayashi K, Kumada T, Nakano S, Takeda I, Kiriyama S, Sone Y, Toyoda H, Shimizu H, Honda T. Incidence of hepatocellular carcinoma in chronic hepatitis C after interferon therapy. Hepatogastroenterology. 2002;49:508–12.
17. Lok AS, Everhart JE, Wright EC, Di Bisceglie AM, Kim HY, Sterling RK, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Morgan TR. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. Gastroenterology. 2011;140:840–9.
18. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet. 1995;346:1051–5.
19. Okanoue T, Itoh Y, Minami M, Sakamoto S, Yasui K, Sakamoto M, Nishioji K, Murakami Y, Kashima K. Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. J Hepatol. 1999;30:653–9.
20. Izuno K, Fujiyama S, Yamasaki K, Sato M, Sato T. Early detection of hepatocellular carcinoma associated with cirrhosis by combined assay of des-gamma-carboxy prothrombin and alpha-fetoprotein: a prospective study. Hepatogastroenterology. 1995;42:387–93.

21. Trevisani F, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J Hepatol. 2001;34:570–5.
22. Zoli M, Magalotti D, Bianchi G, Gueli C, Marchesini G, Pisi E. Efficacy of a surveillance program for early detection of hepatocellular carcinoma. Cancer. 1996;78:977–85.
23. Alpert E, Feller ER. Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. Gastroenterology. 1978;74:856–8.
24. Bloomer JR, Waldmann TA, McIntire KR, Klatskin G. Alpha-fetoprotein in noneoplastic hepatic disorders. JAMA. 1975;233: 38–41.
25. Ruoslahti E, Seppala M. Normal and increased alpha-fetoprotein in neoplastic and non-neoplastic liver disease. Lancet. 1972;2: 278–9.
26. Sakurai T, Marusawa H, Satomura S, Nabeshima M, Uemoto S, Tanaka K, Chiba T. *Lens culinaris* agglutinin-A-reactive alpha-fetoprotein as a marker for liver atrophy in fulminant hepatic failure. Hepatol Res. 2003;26:98–105.
27. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. Hepatology. 1990;12:1420–32.
28. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, Wright EC, Everson GT, Lindsay KL, Lok AS, Lee WM, Morgan TR, Ghany MG, Gretch DR. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. J Hepatol. 2005;43:434–41.
29. Tateyama M, Yatsuhashi H, Taura N, Motoyoshi Y, Nagaoka S, Yanagi K, Abiru S, Yano K, Komori A, Migita K, Nakamura M, Nagahama H, Sasaki Y, Miyakawa Y, Ishibashi H. Alpha-fetoprotein above normal levels as a risk factor for the development of hepatocellular carcinoma in patients infected with hepatitis C virus. J Gastroenterol. 2011;46:92–100.
30. Murashima S, Tanaka M, Haramaki M, Yutani S, Nakashima Y, Harada K, Ide T, Kumashiro R, Sata M. A decrease in AFP level related to administration of interferon in patients with chronic hepatitis C and a high level of AFP. Dig Dis Sci. 2006;51: 808–12.
31. Tamura Y, Yamagiwa S, Aoki Y, Kurita S, Suda T, Ohkoshi S, Nomoto M, Aoyagi Y. Serum alpha-fetoprotein levels during and after interferon therapy and the development of hepatocellular carcinoma in patients with chronic hepatitis C. Dig Dis Sci. 2009;54:2530–7.
32. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Kawamura Y, Kumada H. Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. J Med Virol. 2007;79: 1095–102.
33. Asahina Y, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, Yasui Y, Hosokawa T, Ueda K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Enomoto N, Izumi N. Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. Hepatology. 2010;52:518–27.
34. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, Mukaide M, Williams R, Lau JY. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol. 1997;35:201–7.
35. Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Kanamori A, Atsumi H, Takagi M, Nakano S, Arakawa T, Fujimori M. Incidence of hepatocellular carcinoma in hepatitis C carriers with normal alanine aminotransferase levels. J Hepatol. 2009;50:729–35.
36. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Kawamura Y, Kumada H. Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. J Med Virol. 2007;79:1485–90.
37. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K. Risk factors for hepatocellular carcinoma, its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology. 1998;27:1394–402.
38. Kurokawa M, Hiramatsu N, Oze T, Mochizuki K, Yakushijin T, Kurashige N, Inoue Y, Igura T, Imanaka K, Yamada A, Oshita M, Hagiwara H, Mita E, Ito T, Inui Y, Hijioka T, Yoshihara H, Inoue A, Imai Y, Kato M, Kiso S, Kanto T, Takehara T, Kasahara A, Hayashi N. Effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with chronic hepatitis. Hepatol Res. 2009;39:432–8.
39. Suzuki K, Ohkoshi S, Yano M, Ichida T, Takimoto M, Naitoh A, Mori S, Hata K, Igarashi K, Hara H, Ohta H, Soga K, Watanabe T, Kamimura T, Aoyagi Y. Sustained biochemical remission after interferon treatment may closely be related to the end of treatment biochemical response and associated with a lower incidence of hepatocarcinogenesis. Liver Int. 2003;23:143–7.
40. Kurosaki M, Hosokawa T, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, Tamaki N, Ueda K, Tsuchiya K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Asahina Y, Enomoto N, Izumi N. Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy. Hepatol Res. 2010;40:870–7.
41. Takahashi H, Mizuta T, Eguchi Y, Kawaguchi Y, Kuwashiro T, Oeda S, Isoda H, Oza N, Iwane S, Izumi K, Anzai K, Ozaki I, Fujimoto K. Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C. J Gastroenterol. 2011;46:790–8.
42. Forns X, Ampurdanes S, Sanchez-Tapias JM, Guilera M, Sans M, Sanchez-Fueyo A, Quinto L, Joya P, Bruguera M, Rodes J. Long-term follow-up of chronic hepatitis C in patients diagnosed at a tertiary-care center. J Hepatol. 2001;35:265–71.

J Gastroenterol (2011) 46:1418–1426
DOI 10.1007/s00535-011-0452-4

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Percutaneous radiofrequency ablation therapy for hepatocellular carcinoma: a proposed new grading system for the ablative margin and prediction of local tumor progression and its validation

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Received: 16 May 2011 / Accepted: 18 July 2011 / Published online: 16 August 2011

Abstract
Background In the present study we classified the radicality of percutaneous radiofrequency thermal ablation (RFA) therapy according to the extent of the ablated margin. We measured the local recurrence rate for each radicality grade to evaluate the significance of the grading system in assessing the therapeutic effectiveness of RFA and predicting local tumor progression.
Methods This retrospective study involved 269 patients with solitary hypervascular hepatocellular carcinoma who had undergone RFA. The mean ± SD observation period after RFA, number of treatment sessions, and tumor diameter were 25.7 ± 19.9 months, 1.2 ± 0.5, and 2.1 ± 0.7 cm, respectively. Patients were evaluated using dynamic computed tomography. We classified the radicality of RFA treatment into four grades (R grades: A, B, C, and D) according to the extent of the ablated tumor margin, calculated the post-RFA cumulative local recurrence rate for each R grade, and analyzed the factors (patient characteristics, biochemical data, contiguous vessels, and tumor marker) contributing to local recurrence.
Results The cumulative local recurrence rates at 3 years were 6.7, 17.6, 55.8, and 82.2% for Grades A, B, C, and D, respectively. Using univariate analysis, R grade, tumor size (>2 cm), and des-γ-carboxy prothrombin (DCP) (>200 mAU/mL) were shown to be significant factors contributing to local recurrence. However, using multivariate analysis, only the R grade was found to be a significant independent factor.
Conclusions The proposed R grading method is a valid and useful method for assessing treatment efficacy, and for predicting local tumor progression after RFA.

Keywords Hepatocellular carcinoma · Radiofrequency thermal ablation · Treatment outcome · Grading · Local tumor progression

Introduction

Hepatocellular carcinoma (HCC) is a problem worldwide [1–5]. Unlike most solid cancers, the future incidence and mortality rates for HCC are projected to increase substantially in many countries over the next 20 years, mostly as a result of infection with the hepatitis C virus [6].

HCC frequently recurs after treatment, leading to high mortality rates. Current options for the treatment of HCC include surgical resection, transcatheter arterial embolization (TAE), percutaneous ethanol injection therapy (PEIT), and percutaneous radiofrequency thermal ablation (RFA) therapy. Recently, systemic treatments with molecular-targeted drugs, such as sorafenib, have been recommended for advanced-stage HCC [6]. Surgical resection plays only a limited role in the treatment of HCC, because the inclusion criteria are usually very limited. TAE is often performed in patients with multiple hypervascular nodules. However, complete necrosis of the tumor tissue is rarely achieved [7]. PEIT continues to play an important role in the treatment of small HCCs. However, its efficacy depends on tumor size, and a higher rate of local tumor progression has been reported compared with other procedures [8, 9].

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RFA therapy, an alternative technique to PEIT that was introduced in Japan in 1999, has been widely used as a curative treatment for HCC [1–5]. An area of <3 cm in diameter can be ablated with a single application of RFA. This technique has proven to be a safe and effective modality for the treatment of small HCCs in patients who are considered unsuitable for surgical intervention because of insufficient hepatic reserve [1–5]. Moreover, for local tumor control, RFA therapy has proven to be superior to PEIT and percutaneous microwave thermocoagulation therapy (PMCT), both of which have been used previously [8–11].

Many studies have indicated that the incidence of local tumor progression after treatment with RFA has ranged from 2 to 53% [11–17]. Complete response to the initial RFA treatment has been reported to contribute to the long-term survival of HCC patients [18, 19]. The majority of recurrent lesions emerge from the ablated area within 5 mm of the tumor border, which is the area most likely to contain viable tumor cells [16]. Consequently, an adequate ablative margin and an accurate method to assess the treatment efficacy of RFA for HCC are required.

We have routinely classified patients treated with RFA into four groups based on the extent of the ablative margin; this is a novel classification system, which we have referred to as Radicality Grading (R grades: A, B, C, and D). To our knowledge, no other investigators have used this or a similar classification system to grade the extent of the ablative margin. The objective of the present study was to examine the usefulness of the R grading as a predictor of local tumor progression.

Patients and methods

Patients and HCC diagnosis

We performed RFA therapy in 315 treatment-naive patients diagnosed with solitary HCC at the Gastroenterology Department, Osaka Red Cross Hospital, between January 2004 and October 2010. Prior to the performance of RFA, written informed consent was obtained from all patients. The ethics committee of our department approved the protocol for RFA therapy. The present study comprised a retrospective analysis of patient records, and all treatments were conducted in an open-label manner.

HCC was diagnosed using abdominal ultrasound and dynamic computed tomography (CT) scans (hyperattenuation during the arterial phase in all or some part of the tumor, and hypoattenuation in the portal-venous phase). Arterial and portal phase dynamic CT images were obtained at approximately 30 and 120 s, respectively, after injecting contrast material. Of the 315 patients, 21 who were diagnosed with hypovascular HCC (hypoattenuation in the arterial phase) and 25 who were lost to follow-up were excluded. Thus, 269 patients diagnosed with solitary hypervascular HCC were included in this analysis. For all patients, abdominal angiography was performed before RFA. We confirmed solitary and hypervascular HCC with no vascular invasion and no satellite nodules using CT during hepatic arteriography (CTHA) and arterial-portography (CTAP).

Assessment of treatment efficacy

To assess treatment efficacy, we performed dynamic 16-column multi-detector CT (MDCT) using 3-mm slice scans within 1 week after RFA. In assessing the ablated margin, for each patient, using the image in the portal-venous phase, we analyzed not only the maximum frame of the CT scan but also all of the frames that showed tumor. Within 1 week after RFA, the increase of inflammatory arterial blood flow brought about by performing RFA could not be ignored, and this is the reason that we used the image in the portal-venous phase. When using the image in the portal-venous phase, it was considered that the timing of evaluation did not affect the results. The patients were then classified into four groups as follows: Grade A (absolutely curative), a ≥5 mm ablative margin around the entire tumor; Grade B (relatively curative), an ablative margin was present around the tumor, but the diameter was <5 mm in some places; Grade C (relatively non-curative), only an incomplete ablative margin was formed although no residual tumor was apparent; Grade D (absolutely non-curative), the tumor was not completely ablated (Fig. 1). We used a >5 mm ablative margin because Nakazawa et al. [16] reported that an ablative margin of 5 mm or greater was the most important factor for the local control of HCC. Using this method to assess treatment efficacy, we calculated the local tumor progression rate for all 269 patients. In other words, we used the R grading method and examined its usefulness (R Judgment). We defined local tumor progression as the presence of a hypervascular nodule adjacent to the ablated area after RFA using dynamic CT scan. A recurrence that was distant from the ablated area in the same segment was not included in the assessment of local tumor progression. And local tumor progression was determined by three radiologists experienced in liver imaging. This approach was essentially based on the standardized terminology and reporting criteria published by the Society of International Radiology Technology Assessment Committee; International Working Group on Image-Guided Tumor Ablation [20]. Regarding tumors of <5 mm located at the periphery of the liver, if the tumor was ablated just below the liver capsule we considered it an ablative margin of >5 mm. This

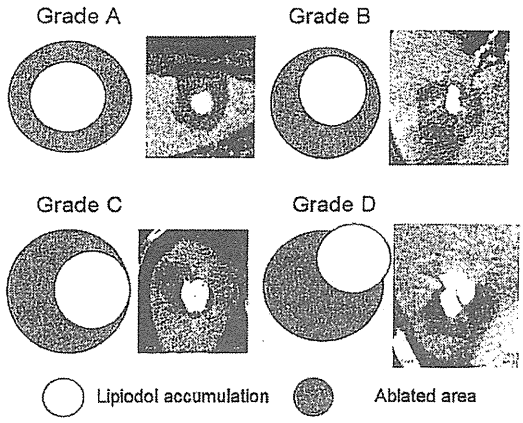

Fig. 1 Diagrammatic representation of the proposed radicality (R) grading method. Grade A (absolutely curative): an ablative margin ≥5 mm is achieved around the entire tumor; Grade B (relatively curative): an ablative margin extends around the entire tumor but the diameter is <5 mm in some places; Grade C (relatively non-curative): a complete ablative margin is not formed although no residual tumor is apparent; Grade D (absolutely non-curative): the tumor has not been entirely ablated

decision, the R Judgment, was made by the three radiologists experienced in liver imaging mentioned above. If the decisions of the three radiologists differed (for example, if two radiologists judged Grade A and the other radiologist judged Grade B), a decision was made by majority rule.

In our department, during abdominal angiography, we routinely perform arterial infusion of iodized oil (Lipiodol Ultra-Fluid; Schering Japan, Osaka, Japan) alone (1–2 mL). Lipiodol was injected to intensify the radiologic visibility of the target tumor. For patients in whom the tumor location was determined because of the dense accumulation of Lipiodol, we assessed treatment efficacy using dynamic CT scans. However, in patients for whom it was difficult to determine the exact location of the tumor because of insufficient Lipiodol accumulation, and for those with Lipiodol accumulation in only part of the tumor, such as nodule-in-nodule HCC, we measured the ablative margin using CTHA with a CTAP image as the reference image. Follow-up consisted of monthly blood tests and monitoring of tumor markers, including des-γ-carboxy prothrombin (DCP), which was measured by a chemiluminescent enzyme immunoassay (Lumipulse PIVKAII Eisai; Eisai, Tokyo, Japan). Dynamic CT scans were obtained every 3–4 months after RFA.

In addition to the R grade, a total of 20 factors, including patient characteristics, clinical biochemical data, contiguous vessels, and tumor markers, were retrospectively examined, using univariate and multivariate analyses, for their contribution to local tumor progression.

RFA procedure

We routinely used a cool-tip needle (Radionics, Burlington, MA, USA) while performing RFA. Using the intercostal or subcostal approach, a 17-gauge, 2- or 3-cm cooled-tip electrode was inserted under real-time ultrasound guidance. The initial treatment was planned with one ablation for tumors of <2 cm in diameter and two or more ablations with the overlapping technique for tumors of ≥2 cm in diameter. We defined a session as a single intervention episode that consisted of one or more ablations performed on a single tumor, and a treatment as the completed effort to ablate the tumor. This approach was in accordance with the working party report on image-guided tumor ablation [21].

After insertion of the electrode into the tumor, we started ablation at 60 W for the 3-cm exposed tip and 40 W for the 2-cm exposed tip. The power was increased to 120 W at a rate of 10 W/min. The duration of a single ablation was 12 min for the 3-cm electrode and 6 min for the 2-cm electrode. After RFA exposure, the pump was stopped and the temperature of the needle tip was measured. When the temperature reached >60°C, additional ablation was not performed. When tumor ablation was complete, thermal ablation was performed along the needle track. All patients were carefully observed for treatment-related complications. All procedures were performed under ultrasound guidance by one of five operators who had at least 3 years of experience of performing RFA. We used the artificial ascites technique to prevent collateral thermal injury when the anticipated RFA zone was in contact with a critical organ, such as the hepatic flexure of the colon. We also used this technique to improve visibility when the index tumor was located in the hepatic dome area.

Complete ablation of HCC was defined as hypoattenuation of the lesion including the surrounding liver parenchyma. Therefore, we routinely performed additional RFA treatment until we had confirmed that the ablative margin surrounded the entire circumference of the tumor (R grade: Grade A or B), provided that patient consent had been given. If we were not able to acquire consent from the patient, or if the patient was judged to be at high risk if given additional treatment, no further RFA treatment was given.

Statistical analysis

Data were analyzed using univariate and multivariate analyses. The cumulative local tumor progression rate was

calculated using the Kaplan–Meier method, and tested using the log-rank test. The Cox proportional hazard model was used for multivariate analyses of factors that were considered significant in univariate analysis. These statistical methods were used to estimate the interval from RFA treatment to local tumor progression. Data were analyzed using SPSS software, version 9.0 (SPSS, Chicago, IL, USA) for Microsoft Windows. Data are expressed as means ± standard deviation (SD). Values of $P < 0.05$ were considered to be statistically significant.

Results

Clinical characteristics

The clinical characteristics of the patients with HCC are shown in Table 1. No patient had major complications after RFA. The mean ± SD tumor diameter, observation period, and number of treatment sessions were 2.1 ± 0.7 cm, 25.7 ± 19.9 months, and 1.2 ± 0.5, respectively. We confirmed 84 cases of local tumor progression (31.2%) and the mean observation period until local tumor progression occurred after RFA was 20.0 months. Using the proposed R grading system, 49 patients were classified as Grade A (18.2%), 113 as Grade B (42.0%), 74 as Grade C (27.5%), and 33 as Grade D (12.3%).

Reasons for not attempting to perform additional RFA in patients with Grade C and D

It was considered that patients with Grade C and D (total 107 patients) should receive additional RFA because a sufficient ablative margin had not been obtained. Despite this decision, we did not attempt to perform additional RFA in some of these patients; details of the reasons why and the number of patients are as follows: (1) patients in whom additional RFA was decided against by the doctors because of sites at which it was extremely difficult to perform additional RFA, such as sites directly under the hepatic dome or the heart (39 patients), (2) patients in whom additional RFA was considered to be difficult to perform because of poor visibility under ultrasonography owing to extreme obesity and impossibility of breath-hold when performing RFA (15 patients), (3) patients in whom high rates of complications were expected, such as when HCC at the site of a hepatic hilar lesion was treated by RFA (23 patients), (4) patients in whom additional RFA was difficult to perform because ascites appeared after the first session of RFA owing to poor hepatic function before RFA (20 patients), (5) and patients whose informed consent could not be obtained for additional RFA for reasons such as a physical burden (10 patients).

Table 1 Clinical characteristics of patients with hepatocellular carcinoma

Characteristics	Number of patients or mean ± SD
Gender	
Male/female	165/104
Age (years)	69.4 ± 8.8
Tumor size (cm)	2.1 ± 0.7
Observation period (months)	25.7 ± 19.9
Number of RFA sessions	1.2 ± 0.5
Cause of liver disease	
Hepatitis C/hepatitis B/nonB, nonC	213/20/36
Child–Pugh classification	
Chronic hepatitis/Child–Pugh A/B/C	61/167/37/4
The R factor	
Grade A/B/C/D	49/113/74/33
Contiguous vessels	
Yes/no	117/152
Local tumor progression	
Yes/no	84/185
BMI (kg/m^2)	
>25/≤25	78/191
DM	
Yes/no	90/179
Post-RFA antiviral therapy	
Yes/no	26/243
BCAA medication	
Yes/no	45/224
Biochemical analysis	
AST (IU/L)	57.2 ± 30.9
ALT (IU/L)	50.3 ± 43.2
ALP (IU/L)	362.1 ± 216.3
γGTP (IU/L)	74.7 ± 73.4
Alb (g/dL)	3.80 ± 0.51
T-Bil (mg/dL)	0.95 ± 0.51
PT (%)	86.9 ± 15.9
Platelets (10^4/mm^3)	11.0 ± 4.73
AFP (ng/mL)	184.8 ± 966.1
DCP (mAU/mL)	352.7 ± 2787

SD standard deviation, *BMI* body mass index, *DM* diabetes mellitus, *RFA* radiofrequency thermal ablation, *R* radicality, *BCAA* branched chain amino acid, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *γGTP* γ-glutamyl transpeptidase, *Alb* albumin, *T-Bil* total bilirubin, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

Quantitative analysis of the ablative margin and local tumor progression

The cumulative local tumor progression rate in all patients at 1, 2, and 3 years was 12.8, 23.6, and 36.6%, respectively (Fig. 2). In terms of extent of the ablation margin, among

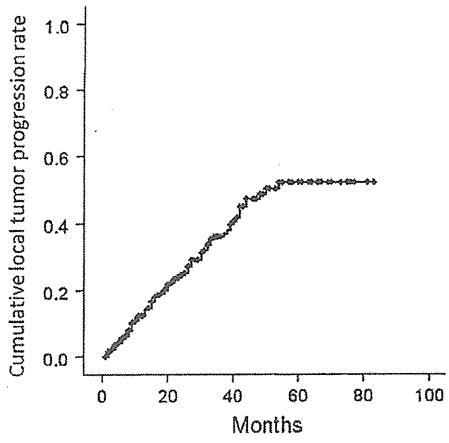

Fig. 2 Cumulative local tumor progression rate in all patients

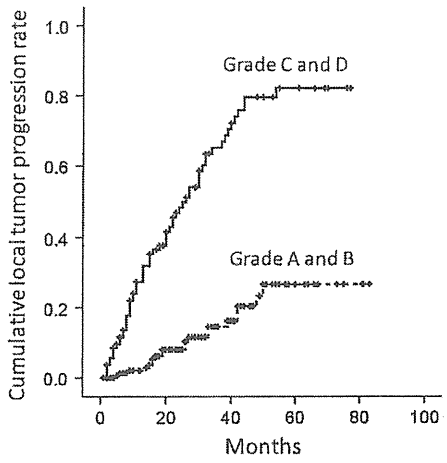

Fig. 3 Cumulative local tumor progression rate in patients with a sufficient or insufficient ablative margin. The local tumor progression rate was significantly lower in patients with a sufficient ablative margin (corresponding to R grade A and B) than in those with an insufficient ablative margin (corresponding to R grade C and D) ($P < 0.001$)

the 162 patients with complete circumferential ablation (corresponding to R grades A and B), the cumulative rates of local tumor progression at 1, 2, and 3 years, were 1.4, 8.2, and 13.8%, respectively. In the 107 patients with incomplete circumferential ablation (corresponding to R grades C and D), the cumulative rates of local tumor progression at 1, 2, and 3 years were 32.2, 49.1, and 63.4%, respectively. Accordingly, the local tumor progression rate was significantly lower in patients with complete circumferential ablation versus those with incomplete circumferential ablation ($P < 0.001$) (Fig. 3).

Analysis by the R grading

We also determined the rates of tumor progression for each of the four grades in the R grading system. The cumulative rates of local tumor progression at 1, 2, and 3 years were as follows: 2.6, 2.6, and 6.7%, respectively, for Grade A; 2.4, 10.2, and 17.6%, respectively, for Grade B; 19.5, 44.0, and 55.8% for Grade C; and 46.2, 53.4, and 82.2% for Grade D. These differences between individual grades reached statistical significance, indicating that a more complete and a larger ablation margin was associated with a lower rate of recurrence (overall significance, $P < 0.001$) (Fig. 4).

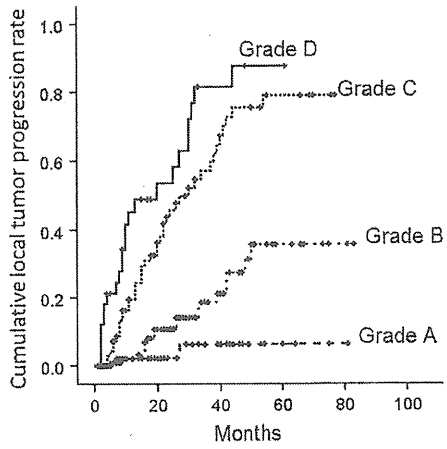

Fig. 4 Cumulative rates of local tumor progression according to R grading. The rate of local progression differed significantly among the four R grades (overall significance, $P < 0.001$)

Analysis including all factors other than the R grading

Using univariate analysis, the R grade ($P < 0.001$), tumor size >2 cm ($P = 0.014$), and des-γ-carboxy prothrombin (DCP >200 mAU/mL) ($P = 0.007$) were found to be significant factors for predicting local tumor progression (Table 2). In addition, although the difference was not significant ($P = 0.051$), the local tumor progression rate tended to be higher in patients aged over 65 years (Table 2). In the multivariate analyses involving the three factors that were found to be significant in the univariate analysis, the hazard ratios (HRs) for tumor size and DCP values for Grades B, C, and D are detailed in Table 3. Only the R grade was found to be a significant independent factor linked to local tumor progression.