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学会案内・お知らせ　告知板　information

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特集

肝細胞癌に併存する結節性病変のうち治療の対象となる病変は

Hepatic nodules detected in patients with hepatocellular carcinoma

久保 正二* 広橋 一裕** 若狭 研一***
Shoji Kubo *Kazuhiro Hirohashi* *Kenichi Wakasa*

●要旨●肝細胞癌症例の多くが肝硬変などを母地とするため，肝内結節性病変がみられることが少なくない。これには大型再生結節（LRN），腺腫様過形成（AH），異型腺腫様過形成（AAH），早期肝細胞癌（eHCC）が含まれるが，その画像診断はしばしば困難である。AHは悪性化の可能性が高く，AAH，eHCCは通常型の肝癌に進行する可能性が高い。AHやAAHは治療の必要性が低く，eHCCはその必要性が高いと考えられている。画像上，乏血性結節は治療の必要性が低いと考えられている。結節の存在部位，数，肝機能に応じて治療法が決定されるが，結節局所のコントロールが基本となる。

key words：早期肝細胞癌，異型腺腫様過形成，腺腫様過形成，大型再生結節，結節内結節

肝細胞癌症例の特徴

本邦における肝細胞癌（肝癌）の多くが肝炎ウイルスなどによる慢性肝疾患に起因するが，この慢性肝炎や肝硬変は高い癌化ポテンシャルをもつ前癌状態と考えられる。このような背景をもつ肝癌の多くは，前癌病変に相当する腺腫様過形成（adenomatous hyperplasia；AH）が発生し，異型腺腫様過形成（atypical AH；AAH）から早期肝細胞癌（early hepatocellular carcinoma）を経て結節内結節型肝細胞癌，さらに肝内転移や門脈侵襲を伴う進行肝癌へと多段階的に進展していく（多段階発育）と考えられている[1][2]。慢性肝疾患を取り扱う臨床の現場において，肝内結節性病変が検出された場合，その質的診断に難渋したり，肝癌治療中に肝内の他部位に結節性病変が検出され，その治療方針決定に迷うことが少なくない。したがって，これら結節性病変の特徴と癌化を含めた病態を把握したうえでの対処が重要となる。

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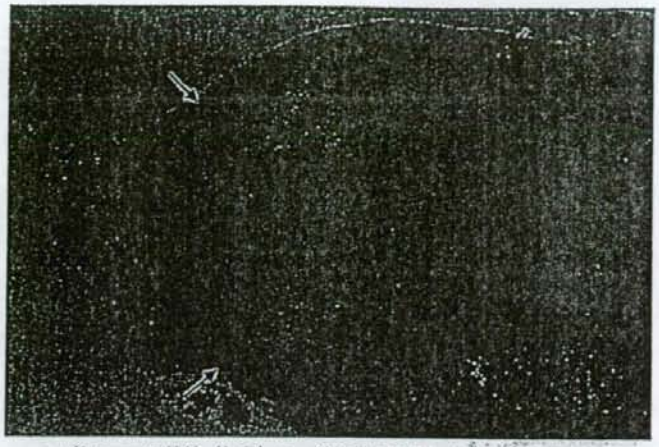
表1 肝細胞癌と肝内結節性病変の分類
（International Working Party）

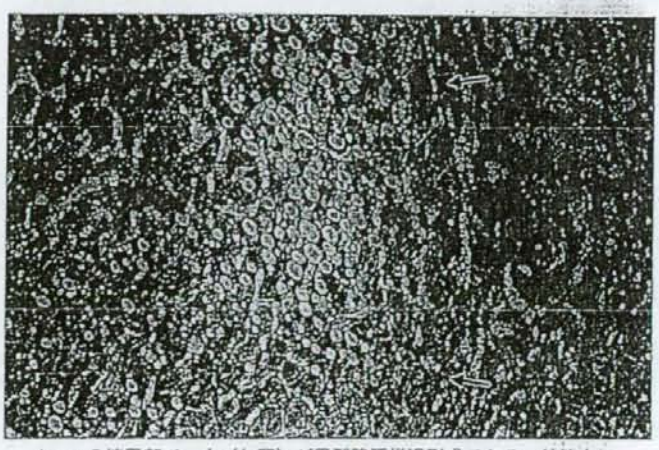
原発性肝癌取扱い規約	International Working Party
大型再生結節	Dysplastic focus
腺腫様過形成	Dysplastic nodule
異型腺腫様過形成	Dysplastic nodule, low-grade
早期肝細胞癌	Dysplastic nodule, high-grade
	Hepatocellular carcinoma

肝細胞癌に併存する結節性病変

肝癌の母地となる肝硬変では大小の再生結節からなる偽小葉構造がみられ，これらのなかには肉眼的に周囲より際立った10mm前後の結節性病変がみられることがある。これら小結節性病変は，『原発性肝癌取扱い規約』[3]では，大型再生結節（large regenerative nodule：LRN），AH，AAHおよび早期肝癌に分類されている（表1）。一方，国際的にはこれら肝硬変にみられる結節性病変の分類は一定ではないものの，現在，もっとも広く用いられている国際分類はInternational Working Party（IWP）による分類[4]である。IWP分類は1994年の国際会議で検討され，1995年に



a：約5mmの結節（矢印）で，結節内に限局した脂肪化を伴っている（対物1倍）



b：aの境界部で，左（矢印）が異型腺腫様過形成である。結節内に脂肪化があり，一部に細胞密度の増加が軽度認められるが，異型性は軽度で，明らかなフロント形成はみられない（対物10倍）

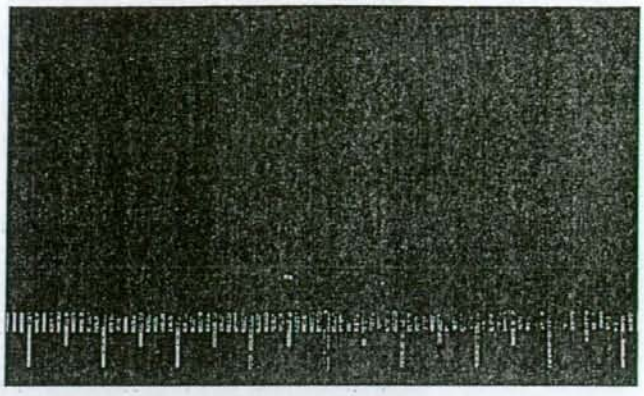
図3　異型腺腫様過形成（AAH）

3. 異型腺腫様過形成（AAH）

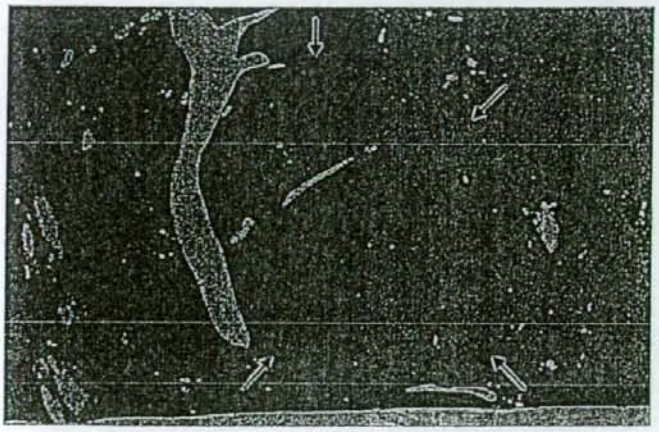
細胞密度の増大が高度な部分を有する，あるいはわずかの構造異型を有する結節で，癌か否かの判定が困難な境界病変（borderline lesion）といえるものである（図3）。

4. 早期肝細胞癌（early HCC）

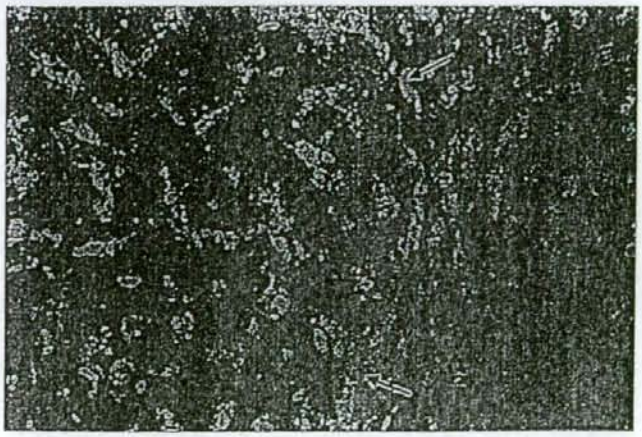
肝癌における早期癌は，『原発性肝癌取扱い規約』[5]においては，肉眼的に小結節境界不明瞭型の癌で，結節内に門脈域，偽小葉間結合織が残存する肝癌が上皮内癌あるいは微小浸潤癌に相当するとみなされている。血行動態的には腫瘍濃染を示さないことがほとんどであり，臨床的にも良好な予後を示すことから早期肝癌と定義されている。組織学的には細胞密度の増大に加え，腺房様あるいは偽腺管構造，索状配列の断裂，不規則化などの構造異型が領域性をもってみられるもの，あるいは間質への浸潤を有するもので，細胞個々の異型は乏しいが，一般に細胞は小型化して，核胞体比が増大する（図4，5）。細胞質では好酸性ないし好塩基性が増強する。通常，細胞密度の増大は周囲肝組織の約2倍以上である。しばしば脂肪化，淡明細胞化を伴う（図6）。癌細胞は膨張性に増殖するにいたっ



a：ホルマリン固定後の切除標本

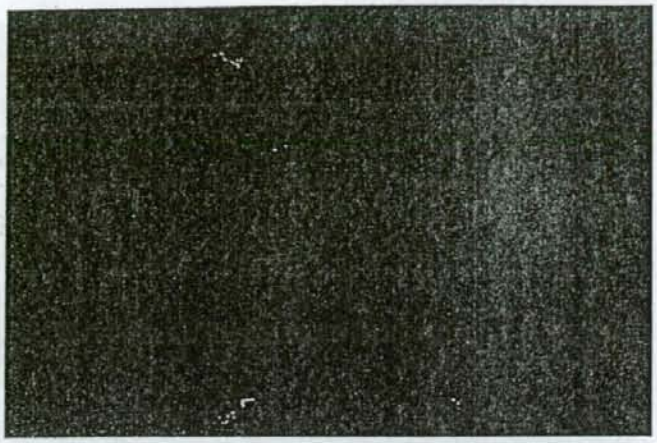


b：約12mmの結節（矢印）で，結節内には門脈域が認められ，ルーペ像での大再生結節や腺腫様過形成との鑑別は困難である（対物1倍）

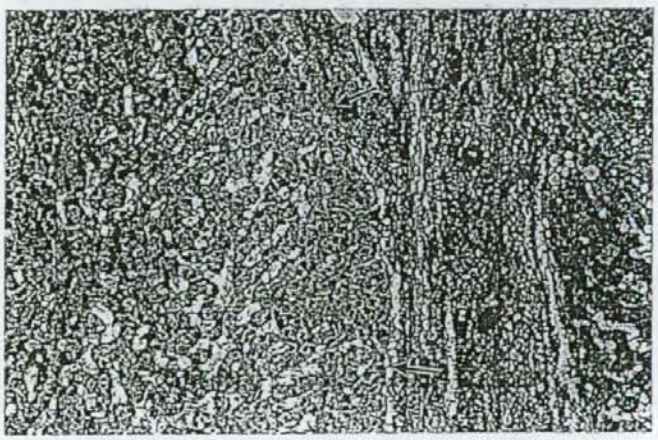


c：aの境界部で，左（矢印）が早期肝細胞癌である。細胞密度の増加があり，肝細胞の染色性の増強，索状構造の明瞭化などがみられ，非腫瘍部肝組織との間に明らかなフロント形成が認められる（対物20倍）

図4　早期肝細胞癌



a：約13mmの結節（矢印）で，結節内には門脈域が認められ，ルーペ像での大再生結節や腺腫様過形成との鑑別は困難である（対物0.5倍）



b：aの境界部で，左（矢印）が早期肝細胞癌である。細胞密度の増加，肝細胞の染色性の増強，索状構造の明瞭化に加えて偽腺管構造が認められる。非腫瘍部との間に明らかなフロント形成が認められる（対物10倍）

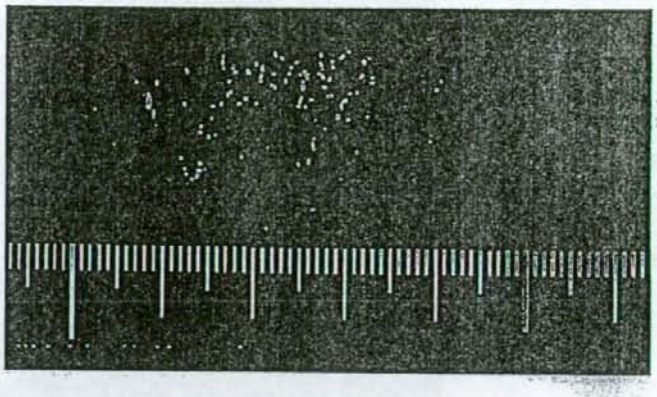
図5　早期肝細胞癌

ていないため，周囲肝組織との境界では癌細胞は隣接する肝細胞索を置換するように増殖し，境界は不明瞭なことが多い。肉眼的には，小結節境界不明瞭型に相当する。

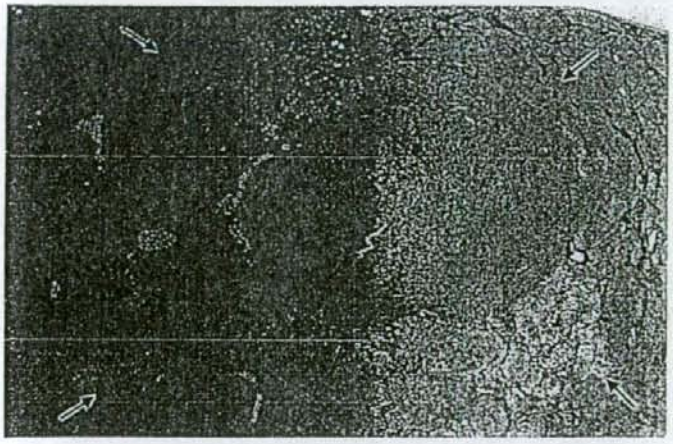
早期肝癌内部による分化の劣る癌組織が境界明瞭，膨張性に存在する際，肉眼的ならびに組織学的に「結節内結節」(nodule-in-nodule) を呈することがあり，早期肝癌の脱分化の形態的表現ともいえる（図7）。

結節性病変の臨床的意義

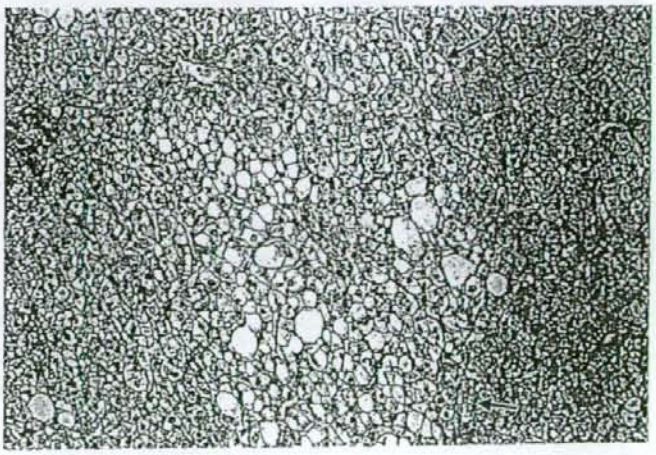
大型再生結節を除く他の3つの小結節病変はAH，AAH，早期肝癌の順に細胞密度が次第に増加する一連の増殖性病変である。臨床的な経過観察や形態的観察により，LRNは悪性化の可能性は低いのに対して，AHはその可能性が高いとされている。とくに，AAHや早期肝癌は通常型の肝癌に進行する潜在的能力の高い病変と理解されている。このようにAHは前癌病変とみなされているが，その根拠として，①硬



a：固定前の切除標本

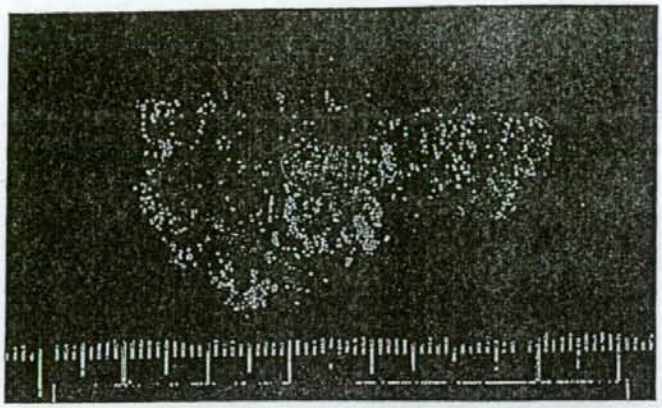


b：約18mm の結節で結節内に限局した脂肪化が認められる（対物0.5倍）



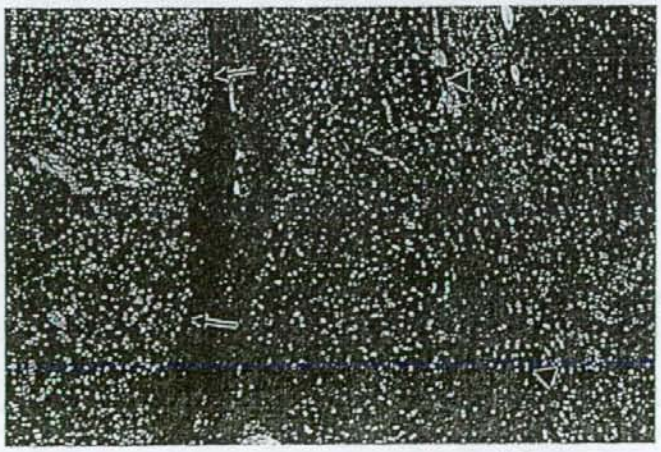
c：a の境界部で左が早期肝細胞癌である。脂肪化に加えて，脂肪化を伴っていない部分においても，細胞質の染色性の変化，細胞密度の増加，核の軽度の腫大があり，フロント形成が認められる（対物10倍）

図６　早期肝細胞癌



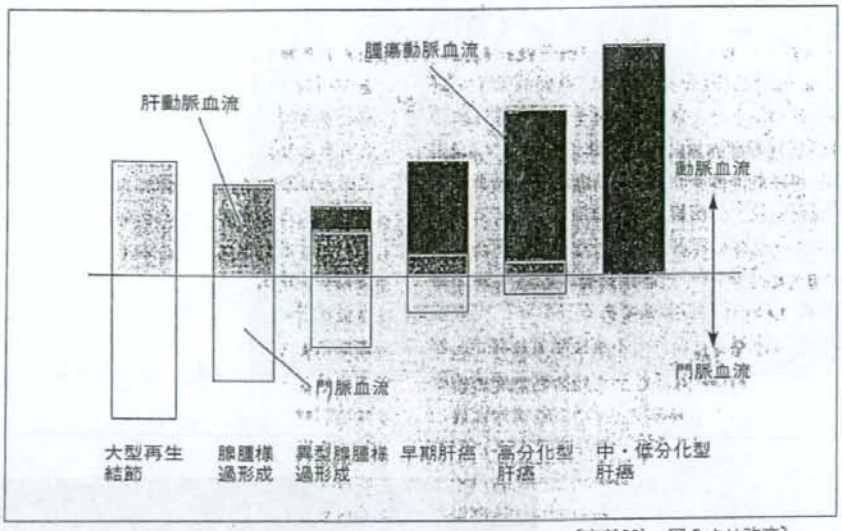
a：ホルマリン固定後の切除標本

b：進行した肝細胞癌を内包する早期肝細胞癌。約18mmの結節（矢印）で，染色性の増強した早期肝細胞癌の内部に多結節融合型の進行した肝細胞癌が認められる（対物0.5倍）



c：進行した肝細胞癌を内包する早期肝細胞癌。図7aの境界部で，左（矢印）の被膜内が進行した肝細胞癌，中央部（矢頭）が細胞質の染色性の増強を伴った早期肝細胞癌，右が非腫瘍部である（対物4倍）

図7



〔文献33)，図5より改変〕

図8　肝内結節性病変の悪性度と血行支配の変化

変肝内に肝癌とAHが高頻度に存在すること[8]，②癌を内包するAHの存在[6,7]，③AHはモノクローナルな増殖を示し，AHとその内部に存在する肝癌とAHが同一のクローンをもつことがあること[8,9]，④臨床的なAHの経過観察による癌への進展例の存在[10~12]などがあげられる。IWP分類[1]においてもdysplastic nodule（AH）は肝細胞のクローナルな増殖という腫瘍性病変の範疇に分類されている。

結節性病変の頻度

当科における肝癌切除例でのAHの検出頻度は454例中20例であったが，切除例であるため肝の一部しか検索し得ていないのに加え，肝硬変併存例は約半数であり，肝硬変症例ではさらに頻度が高いものと考えられる。実際，剖検肝硬変例での検討で約10%と報告されている[9,10]。一方，多中心性発癌を『原発性肝癌取扱い規約』[3]に準じて，複数個の肝癌結節のうち少なくとも1つが高分化型肝癌組織のみで構成されている場合，あるいは複数個の結節がそれぞれ高分化型肝癌組織をもち，それぞれが結節内結節型に多段階発育をしていた場合と定義すると，当科における肝癌切除251例では37例であった[14]。この多中心性発癌の頻度は罹患肝炎ウイルス病態によって異なり，C型肝炎関連肝癌症例においてもっとも頻度が高かった[10]。さらに，C型肝炎関連肝癌症例においても，肝炎活動性や肝線維化の程度が高いほどその頻度は上昇し，強い活動性肝炎を伴う肝硬変症例においては半数近くの症例に多中心性発癌がみられた[15]。中島ら[13]は肝硬変症剖検例や肝切除例の検索から，同時性が剖検例では50%，切除例では15%，異時性が剖検例では37.5%，切除例では11.7%と報告している。

肝内結節性病変の診断

近年，dynamic CT，血管造影下CT，power Doppler超音波検査，造影超音波検査や各種MRIなどの画像診断の進歩により，肝内結節性病変の病理像と画像所見の関連について明らかとなってきた[16~23]。一般に結節の悪性度が高まるに従って結節内門脈血流は次第に減少し，中・低分化型肝癌では完全に欠如する（図8）。結節内動脈支配は周辺再生結節に比べてAAHでは減少するが，その後次第に増加し，中分化型肝癌では周辺肝に比べて著増する。早期肝癌では結節内門脈血流は軽度低下し，動脈血流は周辺肝に比べ低下あるいは同等であるのが典型的であるが，血流パターンはAHやAHHなどの病変や高分化型肝癌と重複する。したがって，中・低分化型肝癌と高分化型肝癌やAHとの鑑別は可能な場合が多いが，高分化型肝癌とAH，AAHの鑑別は困難なことが多い。

また，網内系に取り込まれる超常磁性酸化鉄（superparamagnetic iron oxide，：SPIO）を用いたMRI

の有用性も報告されている[20]。一般に脱分化過程に伴ってKupffer細胞数の低下あるいは機能低下が認められる。古典的な中・低分化型肝癌ではSPIOの腫瘍への取り込みは非腫瘍部に比べ低下しており，AHでは全例，早期肝癌の多くでSPIOの腫瘍への取り込みは非腫瘍部に比べ，同等あるいは増加している。したがって，中・低分化型肝癌と高分化型肝癌との鑑別はある程度可能であるが，高分化型肝癌とAHの鑑別はSPIO造影MRIでは困難である。

このように多段階発育における組織学的連続性・組織学的不均一性のために，AHなどの結節性病変の診断はしばしば困難である。実際，AHのみならず高分化型肝癌ですら，画像診断法のみで検出することはしばしば困難であり，当科における肝癌切除例においても術中超音波検査で初めて検出される病変や切除標本の観察さらには病理検査によって初めて検出される病変が少なくない[5]。ちなみに最近の当科での肝切除例において早期肝癌は13結節みられたが，このうち術前画像診断で検出し得たのは6結節であり，残りの2結節は術中超音波検査によって，5結節は切除標本の検索によって発見された。また，AHおよびAAH 5結節はいずれも術前に検出されず，術中に4結節が，切除標本において1結節が初めて検出された。

肝内結節性病変に対する治療

一般にLRNと診断される病変は治療の必要性がない病変と考えられる。また，AHやAAHは治療の必要性が低いのに対して，早期肝癌はその必要性が高い病変と考えられる。さらに，早期肝癌では転移はきわめて少ないと考えられるのに対して，結節内結節型肝細胞癌では肝内転移や門脈侵襲がみられるため，結節内結節型肝細胞癌あるいは中，低分化型肝癌では肝内進展を考慮した治療の必要性が出てくる。

画像診断から考えると，門脈血流が保たれ，動脈血流の増加がみられない結節は，病理組織学的にはAHなどの前癌病変または早期肝癌と診断される。したがって，これらの結節は連続性をもつ血流動態による診断では良悪性の境界にあると考えられ，治療上のcritical pointと考えられる。乏血性結節は，『科学的根拠に基づく肝癌診療ガイドライン』[20]のように一般には経過観察が提案されている。しかし，乏血性結節であっても，SPIO-MRIの取り込み低下やCTAPにおける欠損像（低吸収域）などの画像的悪性所見がとらえられる場合，将来，高率に多血性肝癌に変化することが知られており，このような結節は注意を要し，治療の必要性も考慮される。一方，結節内動脈血流のみられる結節は基本的に治療対象と考えられる。

AHに対する治療成績についての詳細な報告はみられない。高分化型肝癌についてはその切除例においてもいわゆる転移再発は少ないものの異時性の多中心性発癌が高率にみられることが知られている[21]。多中心性発癌症例では肝内転移巣がなければ単結節症例と治療成績が大きく異なることはなく[10]，癌結節が複数個であっても多中心性発癌と考えられる症例には積極的な治療が望まれる[20]。この際，AH，AAH，早期肝癌は一般に肝内転移をきたすことがないため，結節局所の完全なコントロールが基本となると考えられる。したがって，一括切除にこだわることなく，経皮的治療，術中マイクロウェーブ凝固療法やラジオ波焼灼術，部分切除の組み合わせなど，結節の存在部位，数，肝機能に応じて決定すべきである[20]。なお，異時性多中心性再発に対しては肝癌発生の母地となる肝炎に対する治療が重要である[20]。また，ビタミンK[22]やレチノレイド[23]などの再発抑制効果が期待される。

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Original Article

Effects of lamivudine on outcome after liver resection for hepatocellular carcinoma in patients with active replication of hepatitis B virus

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Aim: Patients with high serum hepatitis B virus (HBV) DNA concentrations are at high risk of tumor recurrence after liver resection for HBV-related hepatocellular carcinoma (HCC).

Methods: Among 24 patients with high serum HBV DNA concentrations who underwent liver resection for HBV-related HCC, postoperative lamivudine therapy was chosen by 14 (lamivudine group). The other 10 patients were controls.

Results: Clinicopathologic findings did not differ between the groups. Tumor-free survival rate after surgery was significantly higher in the lamivudine than the control group ($P = 0.0086$). By univariate analysis, multiple tumors were also a risk factor for a short tumor-free survival. By multivariate analysis, lack of lamivudine therapy and multiple tumors were independent risk factors for a short tumor-free survival. In four patients YMDD mutant viruses were detected after beginning lamivudine administration; in two of them, adefovir dipivoxil was administered because of sustained serum alanine aminotransferase elevations.

Conclusion: Lamivudine therapy improved tumor-free survival rate after curative resection of HBV-related HCC in patients with high serum concentrations of HBV DNA, although careful follow up proved necessary for the detection of YMDD mutant viruses.

Key words: adefovir dipivoxil, hepatitis B virus, hepatocellular carcinoma, lamivudine, liver resection, YMDD mutant virus

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is an etiologic factor for hepatocellular carcinoma (HCC). Although liver resection is an effective treatment for HCC, cancer often recurs even after curative hepatectomy. We previously investigated risk factors for recurrence after resection of HBV-related HCC, finding a high preoperative serum concentration of HBV DNA to be a strong risk factor for HCC recurrence after surgery.[1] On the other hand, the lack of an acute exacerbation of hepatitis and a sustained low serum concentration of HBV DNA after surgery were significantly associated with a low risk of HCC recurrence after resection.[2] We also found substantial serum concentrations of HBV DNA persisting after surgery to be significantly related to short postoperative survival.[2] These findings indicated that biochemical evidence of remission of hepatitis and clearance of viral markers from serum after the operation decrease the likelihood of tumor recurrence.

Recently, a nucleotide analog that inhibits reverse transcriptase, lamivudine, has been developed.[3–5] Lamivudine was found to inhibit the replication of HBV, reduce hepatitis and improve histologic findings in the liver during long-term treatment.[3,6–8] However, lamivudine therapy is not free from problems, such as relapse of hepatitis as a result of the emergence of YMDD mutant viruses.[9–11] Recent studies demonstrated that lamivudine therapy slows the progression of severe liver disease to cirrhosis as well as to HCC.[12,13] However, the effects of lamivudine on outcome after resection of HBV-related HCC are unclear.

In this study we investigated the effects of lamivudine on outcome after resection of HCC in patients with high serum concentrations of HBV DNA.

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METHODS

Patients

FROM NOVEMBER 2000 to October 2005, curative resection of HCC was performed at Osaka City University Hospital in 24 patients seropositive for HB surface antigen (HBsAg) who were negative for antihepatitis C virus antibody and had high serum concentrations of HBV DNA. The patients had not received any lamivudine therapy before the operation. Curative resection was defined as a complete resection of all macroscopically evident tumors. Absence of tumor cells along the parenchymal transection line was confirmed histologically. No remaining tumor was detected in the remnant liver by computed tomography (dynamic study) 3–4 weeks after surgery. The serum concentration of HBV DNA was determined with a transcription-mediated amplification and hybridization protection assay (TMA-HPA) kit, given that serum HBV DNA concentration measured by this method has been shown to be closely related to its hepatitis activity and useful for predicting risk of recurrence after resection of HBV-related HCC.[14,15] In the 24 patients, the serum concentration of HBV DNA was at least 3.7 logarithms of the genome equivalent (LGE) per milliliter, which was considered to represent a high concentration. The patients were told about the previously reported finding that high pre- and postoperative viral concentrations were strong risk factors for HCC recurrence after the operation. Antiviral drug therapeutic effects and side-effects, including the appearance of YMDD mutant viruses, were also explained to the 24 patients. Lamivudine therapy was started in the 14 who then agreed to this therapy and gave their informed consent (lamivudine group), while the control group consisted of the other 10 patients who declined treatment with the drug because of the possibility of adverse events or the necessity of long-term administration of the drug. The 24 patients were followed up until the detection of HCC recurrence or for at least for 6 months in patients without recurrence. The median follow up from operation until the detection of HCC recurrence or the study endpoint (30 April 2006) in this study was 759 days (34–2053). The median follow up for each group was 1117 days (187–2037) for patients receiving lamivudine and 224 days (34–2053) for the controls.

Examination of viral status and active hepatitis

HBsAg and HB envelope antigen (HBeAg) were assayed using an enzyme immunoassay (International Reagents, Kobe, Japan). Anti-hepatitis C virus antibody was assayed using an enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Tokyo, Japan). We used a TMA-HPA kit (Chugai Diagnostics, Tokyo, Japan) to measure serum concentration of HBV DNA. TMA-HPA can quantify 3.7–9.7 LGE/mL (5×10^3–5×10^8 copies/mL). Serum alanine aminotransferase (ALT) activity usually increased immediately following liver resection, then decreased gradually to preoperative activity within 3 weeks. A serum ALT activity at any clinical follow-up assessment (at least every 3 months) of 45 IU/L or less was considered a sustained low ALT activity. The serum concentration of HBV DNA was measured at least twice a year. A serum HBV DNA concentration at any follow up of less than 3.7 LGE/mL was considered a sustained low concentration in the absence of YMDD mutant viruses. YMDD mutant viruses were detected by a reverse hybridization line probe assay (INNO-LiPA HBV DR, Innogenetics, Ghent, Belgium) when serum HBV DNA returned to concentrations above the reference range (≥3.7 LGE/mL) after a decrease to the reference range (<3.7 LGE/mL) with lamivudine therapy.[16,17]

Antiviral treatment

Fourteen patients received lamivudine therapy (100 mg/day), beginning 2 weeks to 2 months after surgery. The period of lamivudine administration was 6 months to 65 months (mean, 32 months). When active hepatitis with a high viral concentration reemerged following proliferation of YMDD mutant viruses, adefovir dipivoxil was administered if patients gave their informed consent. Interferon therapy was carried out in one patient after administration of lamivudine for 6 months, before any appearance of YMDD mutant viruses, to avoid the emergence of YMDD mutant virus.

Pathologic examination

Histologic grading of tumors with respect to differentiation was carried out using the classification of Edmondson and Steiner[18] with certain modifications.[19] When clusters of cancer cells were present in the portal vein, we defined the case as positive for portal invasion. Cancer cells in intracapsular blood vessels, considered branches of the portal vein, also indicated portal vein invasion. When the tumor-free surgical margin was less than 5 mm according to pathologic examination, the margin was defined as positive. Noncancerous hepatic tissues also were examined pathologically. The histologic activity index (HAI) score[20] was used to evaluate the severity of active hepatitis and the degree of fibro-

sis. An HAI score of 0 (based on components 1–3) indicated no activity (grade 0); a score of 1–3, minimal activity (grade 1); a score of 4–8, mild activity (grade 2); a score of 9–12, moderate activity (grade 3); and a score of greater than 12, severe activity (grade 4). The histologic fibrosis score (stage) was determined from component 4 in the HAI scoring. A score of 1 indicated portal fibrous expansion; 2, portal-to-portal septa without architectural distortion; 3, portocentral septa with architectural distortion; and 4, cirrhosis.

Detection of tumor recurrence

Serum α-fetoprotein concentrations were measured every three months. Ultrasonography, computed tomography, magnetic resonance imaging, chest radiography, or a combination of these was performed every three months. When tumor recurrence was suspected on the basis of a tumor marker, radiologic studies, or both, angiography or biopsy was performed to obtain a definitive diagnosis.

Statistics

Student's *t*-test was used to analyze differences in age and tumor size. The Mann–Whitney *U*-test was used to analyze the differences in results of laboratory tests. Fisher's exact test was used to compare categorical data between groups. Tumor-free survival rates were calculated by the Kaplan–Meier method, and the significance of differences in rates between the groups was assessed by the log-rank test. For multivariate analysis, the Cox proportional hazards model with a stepwise method was used. Variables significant at a *P*-value less than 0.1 on univariate analysis were subjected to multivariate analysis. A *P*-value less than 0.05 was considered indicative of significance.

RESULTS

CLINICOPATHOLOGIC FINDINGS IN the lamivudine and control groups are described in Table 1. Age, gender distribution, the proportion of patients with

Table 1 Clinicopathologic findings of patients with and without lamivudine therapy

Findings	Lamivudine therapy		*P*-value
	Yes, $n = 14$	No, $n = 10$	
Age, years, mean ± SD	55 ± 8	55 ± 5	0.993
Gender, men : women	10:4	7:3	>0.999
HBeAg positivity§	11	5	0.204
HBV DNA concentration (LGE/ml)	6.0 ± 1.2	6.0 ± 1.2	0.975
Total bilirubin, mg/dL	0.8 (0.4–1.3)	0.8 (0.4–1.3)	0.656
Albumin, g/dL	3.8 (3.3–4.3)	3.7 (2.6–4.2)	0.426
AST‡, IU/L	44 (35–109)	40 (25–128)	0.578
ALT†, IU/L	53 (25–141)	56 (30–125)	0.558
Child–Pugh classification(A : B)	11:3	8:2	>0.999
α-Fetoprotein, >20 ng/mL	11	7	0.665
Tumor size, cm	2.4 ± 0.7	2.8 ± 1.4	0.329
Multiple tumors	5	5	0.679
Differentiation of main tumor			
Moderately	7	3	0.421
Poorly	7	7	
Portal invasion	4	4	0.673
TMN stage (1:2:3)	2:7:5	3:2:5	0.305
Severity of active hepatitis			
Grade 1, minimal	5	3	>0.999
Grade 2, mild	9	7	
Degree of fibrosis			
Stages 1–3, mild to moderate	8	6	>0.999
Stage 4, cirrhosis	6	4	
Anatomic resection	8	6	>0.999

†ALT, alanine aminotransferase; ‡AST, aspartate aminotransferase; §HBeAg, hepatitis B envelope antigen.
Results of laboratory tests are expressed as medians, with ranges in parentheses.

Table 2 Changes in number of patients with low serum concentration of HBV DNA after surgery

	No. of patients with low serum concentration of HBV DNA (<3.7 LGE/ml)/No. of patients at risk											
	Time after surgery (years)											
	Before	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Control	10/10	1/8	1/4	1/4	1/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Lamivudine	14/14	11/14	8/11	8/11	6/11	4/8	4/8	4/7	4/6	4/5	3/4	1/1

$p = 0.0028$.

HBeAg positivity, viral load, the results of laboratory tests, and Child–Pugh classification[21] did not differ between groups. Type of resection, tumor size, tumor number, differentiation of main tumor, prevalence of portal invasion, cancer stage according to UICC classification,[22] severity of active hepatitis as well as degree of hepatic fibrosis in non-cancerous hepatic tissue showed no difference between groups. No patients had portal invasion detected by preoperative medical imagings. The changes in the number of patients with low serum concentration of HBV DNA and low ALT activity after the operation until the detection of HCC recurrence or the study endpoint in both groups are shown in Tables 2 and 3, respectively. In one patient in the lamivudine group, serum HBeAg changed from positive to negative after the operation.

The tumor-free survival rate was significantly higher in the lamivudine group than in the control group (Fig. 1, $P = 0.0086$). In one patient in the lamivudine group and four patients in the control group, multiple recurrent tumors were detected in the remnant liver. In one patient in the lamivudine group and in one patient in the control group, a single recurrent tumor was detected. By univariate analysis using preoperative factors, multiple tumors was also a risk factor for short tumor-free survival ($P = 0.0036$). By multivariate analysis, lack of lamivudine therapy and multiple tumors were independent risk factors for short tumor-free survival (risk ratio, 18.306; 95% confidence interval, 1.707–196.327; $P = 0.0163$, 16.949; 1.686–166.667; $P = 0.0162$, respectively). Thus, the lack of lamivudine therapy was an independent risk factor for recurrence.

A sustained low serum concentration of HBV DNA was observed in no patient in the control group and in eight patients in the lamivudine group. Although HCC did not recur in the eight patients with sustained low serum concentrations of HBV DNA, HCC recurred in seven of 16 patients with high serum concentrations of HBV DNA (Fig. 2). The proportion of patients with multiple tumors was significantly higher in the seven patients than in nine patients without recurrence. In the lamivudine group, HCC recurred in two patients, who had sustained high serum concentrations of HBV DNA. Sustained normal ALT activity was observed in five patients in the control group and in 10 patients in the lamivudine group. In one patient in the control group, ulsodeoxycholic acid was administered because serum ALT activity was more than 90 IU/L (twice the reference range). In four other patients, no treatment was performed because ALT activity was less than 90 IU/L. Although HCC recurred in two of the 15 patients with sustained normal ALT activity, HCC recurred in five of nine patients with high serum ALT activity (Fig. 3, $P = 0.0346$).

Although three patients in the control group died of HCC recurrence, no patients died in the lamivudine group.

In four patients, YMDD mutant viruses were detected at 10, 11, 21 and 43 months after beginning administration of lamivudine and ALT activity increased in three of the four patients. In two of the three patients with sustained high ALT activity, adefovir dipirvoxil was administered. In one patient, YMDD mutant virus was detected when the HCC recurrence was detected. In the three other patients with YMDD mutant viruses, HCC did not recur.

Table 3 Change in number of patients with low alanine aminotransferase activity after surgery

	No. of patients with low alanine aminotransferase activity (≤45 IU/l)/No. of patients at risk											
	Time after surgery (years)											
	Before	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Control	2/10	5/8	2/4	2/4	2/2	1/1	1/1	1/1	1/1	1/1	0/1	0/1
Lamivudine	6/14	10/14	7/11	7/11	6/11	5/8	5/8	5/7	6/6	5/5	2/4	1/1

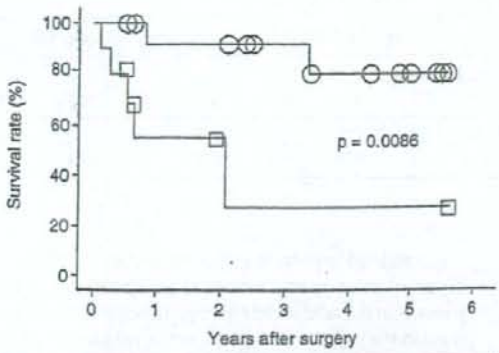

Figure 1 Tumor-free survival rates in lamivudine and control groups. (○), 14 patients with postoperative lamivudine therapy; (□), 10 patients without postoperative lamivudine therapy.

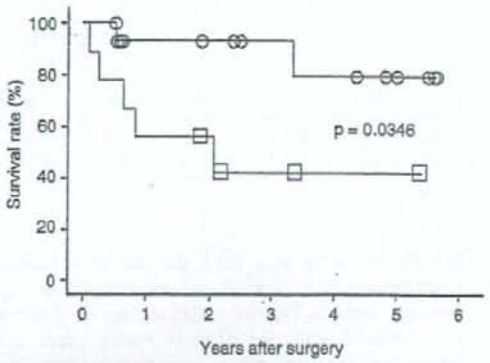

Figure 3 Tumor-free survival rates in patients with and without sustained low serum alanine aminotransferase activity. (○), 15 patients with sustained low serum alanine aminotransferase activity; (□), nine patients with high serum alanine aminotransferase activity.

DISCUSSION

THIS STUDY SHOWED that lamivudine therapy improved the tumor-free survival rate after liver resection for HCC in patients with a high serum concentration of HBV DNA.

This study did not have a randomized prospective design. A well-designed prospective study is difficult to conduct because lamivudine therapy already has been developed as a treatment for chronic hepatitis B and is presently used in clinical practice.[3–8] Despite its status as an established treatment for this form of hepatitis, whether lamivudine therapy is effective for the suppression of tumor recurrence after HBV-related HCC has not been determined. We have reported high preoperative viral concentration and sustained high viral concentration after liver resection to be risk factors for HCC recurrence,[1,2] defining a high-risk patient group. Of 24 patients presently studied, 14 chose lamivudine therapy after explanation of these previous results of liver resection for HBV-related HCC, as well as likely advantages and disadvantages of lamivudine in such a high-risk group. As shown in Table 1, no difference in background characteristics was evident between the lamivudine and control groups. The tumor-free survival rate was significantly higher in the lamivudine group than in the control group, with the absence of lamivudine therapy being an independent risk factor for short tumor-free survival. Thus, this study showed that lamivudine therapy suppressed tumor recurrence after curative resection in these HBV-positive patients.

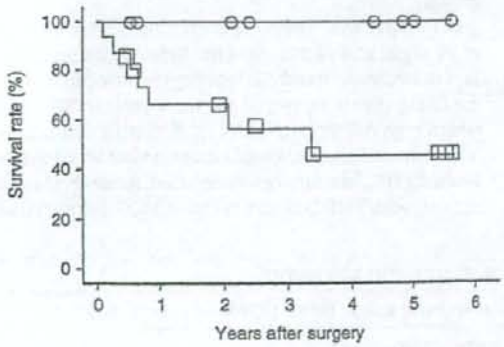

Figure 2 Tumor-free survival rates in patients with and without sustained low serum concentrations of HBV DNA. (○), eight patients with sustained low serum concentration of HBV DNA; (□), 16 patients with high serum concentrations of HBV DNA.

Recurrences after surgery include both intrahepatic metastases from the primary HCC and newly developed HCC (multicentric carcinogenesis). Although the mechanism by which lamivudine therapy suppressed tumor recurrence after surgery was not determined in this investigation, one likely factor underlying the low recurrence rate in the lamivudine group would be a decrease in multicentric carcinogenesis after surgery. Regeneration of hepatocytes amid a background of necrosis during active hepatitis may directly induce DNA mutation, as well as chromosomal rearrangement resulting in genetic instability. Sustained high serum concentrations of HBV and

persistent or intermittent elevation of ALT activity have been linked closely to carcinogenesis in patients with hepatitis B.[23–25] Two recent studies demonstrated that lamivudine therapy reduced the development of HCC in patients with chronic hepatitis B.[12,13] In the present study, HCC did not recur in patients with a sustained low serum concentration of HBV DNA. HCC recurred in only two of 15 patients with sustained normal ALT activity, although HCC recurred in five of nine patients with high serum ALT activity. Thus, sustained serum concentration of HBV DNA and the remission of active hepatitis brought about by lamivudine therapy may decrease multicentric carcinogenesis after surgery.

In our previous study, the percentage of intrahepatic metastases from similarly differentiated main tumors tended to be higher in patients with a high viral concentration than in those with a low viral concentration.[1] HCC thus may behave more aggressively in patients with a high viral concentration, even when histologic differentiation of the tumor is the same; a decrease in viral concentration with lamivudine therapy thus may affect important biologic characteristics of HCC, although lamivudine itself does not have anticancer effects. For example, active hepatitis induces upregulation of adhesion molecules on cells lining hepatic sinusoids, which may enhance the likelihood of metastasis.[26] Remission of active hepatitis in response to lamivudine therapy thus may decrease metastatic potential.

Long-term lamivudine administration is associated with an increasing rate of emergence of drug-resistant viral strains.[9–11] YMDD mutant viruses appeared in four patients in this series. YMDD mutant viruses often cause flare-ups of ALT activity and hepatic decompensation in patients with cirrhosis. In fact, ALT activity increased above the reference range in three of the four patients with YMDD mutant virus. Adefovir dipivoxil was administered in two of the three patients with high ALT activity. In addition to hepatitis exacerbations, viral breakthrough itself may increase the likelihood of the development of HCC,[27] a particular risk in patients with cirrhosis and YMDD mutations.[28] Although HCC did not recur after the appearance of YMDD mutant virus in this series, early identification of YMDD mutant viruses is important. When such mutant viruses are identified, close follow up is necessary to prevent hepatic failure and recurrence of HCC. Several investigators have reported that interferon therapy reduced the incidence of HCC,[29–32] although some others found no decrease.[33,34] Recently, Sun *et al.* reported that interferon therapy improved the overall survival of patients with HBV-related HCC after curative resection, probably by postponing recurrence.[35] Lamivudine appears to be superior to interferon in delaying HCC development.[36] In our series, interferon was administered after lamivudine therapy for 6 months in one patient, who then showed a decreased serum concentration of HBV DNA and ALT activity without appearance of YMDD mutant viruses. A combination of lamivudine and interferon therefore may represent a useful alternative treatment regimen.

This study fell somewhat short of conclusiveness because the number of patients was small. The effects of HBV genotypes on recurrence after surgery also are unclear. The effects of YMDD mutant virus and adefovir dipivoxil on HCC recurrence also should be evaluated. A larger investigation is necessary to clarify the effects of and indications for lamivudine.

In conclusion, lamivudine therapy improved the tumor-free survival rate after curative resection of HBV-related HCC in patients with a high serum concentration of HBV DNA, although careful follow up was necessary for timely detection of YMDD mutant viruses to minimize their adverse effects.

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Original Paper

Risk Factors for Massive Blood Loss during Liver Resection for Hepatocellular Carcinoma in Patients with Cirrhosis

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KEY WORDS:
Liver resection; Hepatocellular carcinoma; Liver cirrhosis; Portal invasion; Hepatic vein involvement; Autologous blood storage

ABBREVIATIONS:
Hepatocellular Carcinoma (HCC); Odds Ratio (OR); Confidence Interval (CI): Central Venous Pressure (CVP); Inferior Vena Cava (IVC)

ABSTRACT

Background/Aims: Liver resection for hepatocellular carcinoma in patients with cirrhosis carries risk of major hemorrhage and sometimes requires blood transfusion. We investigated risk factors for massive blood loss during liver resection and indications for storing blood for autologous intraoperative transfusion.

Methodology: We analyzed clinical records of 100 patients with cirrhosis who underwent liver resection for hepatocellular carcinoma. Autologous blood was stored preoperatively for 19 patients.

Results: Intraoperative blood loss ranged from 5 to 3000mL (mean, 640). Liver resection was performed without transfusion in 67 patients and with autologous blood storage in 17 patients not receiving homologous blood. In the other 16 patients, homologous blood was transfused. Univariate analysis identified youth, large tumors (>4cm), major hepatectomy, portal tumor involvement, hepatic vein involvement, and prolonged operation time as risk factors for massive blood loss; multivariate analysis identified portal involvement and hepatic vein involvement as independent risk factors. Blood loss exceeded 1000mL in the 4 transfused group B patients and 3 of the 4 patients had hepatic vein involvement.

Conclusions: Portal involvement and hepatic vein involvement were risk factors for massive blood loss during liver resection for hepatocellular carcinoma in patients with cirrhosis. Autologous blood storage is indicated in patients with such risk factors.

INTRODUCTION

Despite recent advances in instrumentation and techniques including hepatic inflow occlusion and total vascular exclusion, liver resection is particularly associated with hemorrhage, sometimes requiring blood transfusion. Most hepatocellular carcinomas (HCC) are associated with chronic liver disease including cirrhosis, inducing a coagulopathy. As a result, reported transfusion rate and mean blood loss during liver resection for HCC exceed 30% and 1000mL respectively (1-4). Massive blood loss during liver resection has been reported to be a risk factor for postoperative hepatic failure (1,2,5,6), while homologous blood transfusion carries risks including viral hepatitis and graft-*versus*-host disease. Poor outcomes in patients receiving homologous blood transfusion recently have been reported (7-9), while some investigators have noted efficacy of autologous blood transfusion in liver resection (10-14). As few reports have analyzed risk factors for massive blood loss during liver resection for HCC in patients with cirrhosis, we carried out such a study including the issue of indications for preoperative autologous blood storage.

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METHODOLOGY

From early 1999 to August 2005, we performed liver resection for HCC in 100 patients with cirrhosis. Data from these 100 consecutive patients were analyzed retrospectively. Cirrhosis was diagnosed pathologically using the resection specimens. Subjects consisted of 70 men and 30 women, with a mean age of 65 years (range, 45 to 81 years). Sera from 65 patients were positive for anti-hepatitis C virus antibody alone, 15 patients were seropositive for hepatitis B surface antigen alone, while 1 patient was seropositive for both anti-hepatitis C virus antibody and hepatitis B surface antigen. Sera from 19 other patients were negative for both viral markers.

Before surgery, patients underwent ultrasonography, computed tomography, and angiography. Intraoperative ultrasonography also was performed. The imaging reliably detected portal involvement (portal vein tumor thrombi at the right and left portal veins and the bifurcation of the veins). When the trunk of the right, middle, and/or left hepatic vein was compressed or when tumor thrombi were present in hepatic veins, the hepatic vein was assumed to be

involved. These cases required exposure and dissection or cutting of the hepatic veins. In patients who underwent resection of at least one Couinaud segment (15), an ultrasonic dissector (CUSA Excel, Valleylab, Boulder, CO) was used for hepatic dissection during total or unilateral intermittent clamping of hepatic vascular inflow. In patients who underwent limited liver resection (resection of less than one segment), hepatic parenchyma was dissected with the ultrasonic dissector after coagulation along the dissection line with a microwave tissue coagulator (Microtaze; Heiwa Electronics Industry, Tokyo, Japan).

Operative blood loss was estimated as the sum of blood volumes absorbed by gauze sponges or removed by suctioning. The need for blood transfusion was determined by the surgeons together with anesthesiologists, guided by intraoperative monitoring of blood loss, vital signs, urinary output, and laboratory test results. A restrictive blood transfusion policy during liver resection in patients with cirrhosis, proposed by Makuuchi *et al.* (16), was used.

This study was conducted in accordance with the Helsinki Declaration and the guidelines of the Ethics Committee of our institution. Informed consent was obtained from each patient.

Autologous blood was stored when the conditions below were met. Patients with hemoglobin of at least 11.0g/dL or hematocrit of at least 33%, and without acute infection were offered the option of preoperative autologous blood storage. After informed consent, 400mL (one collection) to 1200mL (three collections) of blood was taken, with the multiple collections carried out at weekly intervals.

A histologic grade for tumor differentiation was assigned using the Edmondson-Steiner classification (17) with modification according to the Liver Cancer Study of Japan (18). Noncancerous hepatic tissues also were examined pathologically. A histologic activity index (19) was used to evaluate the degree of fibrosis (cirrhosis).

Student's *t* test was used to analyze differences in age and tumor size. The Mann-Whitney U test was used to analyze differences in laboratory test results. Fisher's exact test or the chi-squared test was used to compare categoric data between groups. Odds ratio (OR) and 95% confidence interval (CI) were calculated using a conditional regression model. For multivariate analysis, the significant variables ($p<0.1$) were modeled using conditional logistic regression. The adjusted OR and 95% CI for each variable were estimated using the logistic regression coefficient.

RESULTS

Intraoperative blood loss ranged from 5 to 3000mL (mean, 640). Liver resection was performed without blood storage or transfusion in 67 patients (group A). In 17 patients, autologous blood was stored and liver resection was performed with only autologous blood transfusion, if needed (group B); 16 other patients received homologous blood (group C); including 2 patients also receiving autologous blood stored preoperatively. Intraoperative blood loss was 437±374mL (range, 5 to 1800) in group A, 695±445mL (150 to 1855) in group B, and 1432±773mL (355 to 3000) in group C. Of the 16 patients in group C, 11 had blood loses exceeding 1000mL; 5 of 67 patients in group A and 4 of 17 patients in group B had such losses. The proportion of patients with massive blood loss (>1000mL) was significantly higher in group C than in group A or B ($p<0.0001$ and $p=0.0149$ respectively). In group C, homologous blood transfusion was given in 2 patients with anemia, 1 patient with chronic renal failure, and 1 patient with ulcerative colitis, even though blood loss was less than 1000mL. In 10 of the 12 remaining patients in group C, blood loss exceeded 1000mL. Thus, homologous blood often was transfused when blood loss exceeded 1000mL.

TABLE 1 Clinicopathologic and Operative Factors in Patients with Massive vs. Lesser Intraoperative Blood Loss

Factors	Intraoperative blood loss (mL) ≤1000 (n=82)	>1000 (n=18)	*p* value
Age	66.3±8.0	61.4±7.3	0.0204
Gender, male:female	60:22	10:8	0.162
Hemoglobin, g/dL	13.4 (11.1, 15.5)	13.1 (10.9, 15.0)	0.419
Platelet count, x10⁴/mm³	13.2 (7.8, 19.4)	12.0 (6.9, 17.6)	0.131
Prothrombin time (sec)	11.3 (9.8, 12.7)	11.0 (9.3, 11.9)	0.336
Total bilirubin, g/dL	0.9 (0.5, 1.3)	0.8 (0.5, 1.2)	0.708
Albumin, g/dL	3.7 (3.2, 4.1)	3.8 (3.3, 4.2)	0.143
AST, IU/L	56 (33, 100)	56 (32, 89)	0.545
ALT, IU/L	58 (24, 107)	56 (26, 99)	0.851
$ICGR_{15}$, %	17.7 (9.4, 28.0)	15.0 (6.6, 26.5)	<0.0001
AFP elevation (>20ng/mL)	46	12	0.444
Tumor size, cm	2.8±1.6	3.8±2.1	0.0162
Single tumor number	45	11	0.794
Portal involvement	2	4	0.0091
Hepatic vein involvement	8	11	<0.0001
Major hepatectomy	1	5	0.0006
Operation time, min	235±83	373±158	<0.0001

AST: aspartate aminotransferase; ALT: alanine aminotransferase; $ICGR_{15}$: indocyanine green retention rate at 15 min; AFP: α-fetoprotein. For continuous variables, values are means ± SD, or medians followed in parentheses by 10% and 90% percentiles.

In addition to the above transfusion-defined groups, subjects were divided into a group of 18 patients with massive blood loss (>1000mL) and group of 82 patients without massive blood loss (**Table 1**). In patients with massive blood loss, mean age was significantly lower, and mean tumor size was significantly greater, than in patients with less loss. The proportion of patients with a large tumor (4cm or greater in diameter) was significantly higher among patients with massive blood loss than among patients with less loss ($p<0.0001$). The proportion of patients who underwent major hepatectomy (bisegmentectomy) was significantly higher for patients with massive blood loss than for patients with less loss. Operation time was significantly longer in the patients with massive loss.

Proportions of patients with portal tumor involvement and hepatic vein involvement were significantly higher among patients with massive blood loss. In two