座談会

海嶋　確かに夫に関する記入欄というのはないですよね.

荒尾　父子手帳というのは難しいかもしれないけれども，母子手帳の項目に配偶者に関する情報の記入欄を設けることならできると思います．頻度は少ないですが，水平感染プラス垂直感染もあり得るわけで，世の中の男性がこれだけ風疹の予防接種に敏感になっているのですから，生まれてくるかわいい子どもに感染させないという動機づけをしっかりとして，肝炎についても検査を受けてもらうようにする必要があります.

正木　そういう検査をひとつ増やすというのは，非常に重要な提案だと思うのですが，費用の問題などもあるので，貴重な提言ということで厚労省にはまたお話ししたいと思います．肝炎ウイルス検査を受けるきっかけづくりが重要ということですね.内視鏡検査を行うときには，スタンダード・プレコーションは大事だけど，肝炎ウイルス感染の有無を知るきっかけとなりうることも認識しておくと.

荒尾　何かの検査のついでに一緒にやれば多忙な患者さんにも都合がいいというのが私の考えです.

正木　それは重要だと思います.

須田　どのようなケースであれば，肝炎ウイルス検査をしたことについて患者さんに説明するのがよいのでしょうか．陽性であればもちろん結果説明は必須ではありますが，陰性であったときには患者さん全員に伝えていないことが多いと思います．特に，救急外来で診察した患者さんの輸血前検査などで検査を行ったときや，外科手術が必要で手術前にスクリーニング目的に検査を行ったときに多いような気がしています．理想を言えば，検査を行った全ての患者さんに陰性であっても説明をするのが良いのでしょうが，忙しくて伝えられないことがあります．だから，患者さん自身も受検したという認識がないままで，非認識受検という形になってしまうことが多くなってしまうのではないかと思います．私たち医師が「この検査をしました．結果はこのようでした.」と全ての検査において伝えるというのは，日常業務に忙殺されてそこまで手が回らないことが多いのではないのでしょうか．採血して陰性であれば，それを患者さんに伝えてくれるような担当者の方が病院内にいればいいのにと考えてしまいます.

正木　国立病院機構長崎医療センター臨床研究センターの八橋弘先生の調査（八橋弘，病態別の患者の実態把握のための調査および肝炎患者の病態に即した相談に対応できる相談員育成のための研修プログラム策定に関する研究，平成24年度厚生労働科学研究研究報告書，2013年，67）で，特に外科系の先生に「検査しても知らせない傾向がある」ということがわかりました．整形外科で検査をし，実はB型で陽性反応が出たけれども，知らせていなかった結果，その後肝がんが発症し，患者さんに訴えられたという事例もあったそうです．そういう問題にもなりかねないので，陽性なら絶対に伝えないといけませんが，陰性でも「測りましたが，陰性でした」と言っておくと，患者さんに無駄な検診を受けさせなくて済むわけですから，業務が増えるという問題はありますが，やはり医療者側の測った者が伝えるべきだと思います.

須田　今後は積極的に説明していきたいと思います．測る機会が多く，伝えるタイミングも難しいときもありますが，HBs抗原やHCV抗体に関しては，何かあったときにはルーチンとして行うことも多い検査です．患者さんに伝えることによって，患者さんの金銭的負担を減らしたり，医療費そのものを減らしたりすることは可能であると思います.

正木　本日先生方にお集まりいただいて，6年間進められてきた拠点病院事業，肝疾患診療ネットワークの構築も，まだまだ問題点として

残っているということが再認識され，しかしいろいろなご提案もいただき，今後改善できるところがかなりあると感じました．

それでは最後に，本日のディスカッションも踏まえて，お一人ずつお話しいただければと思います．まずは坂口先生，よろしくお願いします．

坂口　最近，肝疾患患者の高齢化が進行しており，受診時の年齢が治療にも大きく影響しています．3剤併用療法を行うときには，3剤併用療法の可能な症例を選択しているのですが，それでも患者さんの年齢の中央値が63歳です．せっかく肝炎ウイルスが陽性だと言われ，かかりつけ医から紹介を受けても，なかなか十分な治療をできない症例もあります．年齢の問題だけではなく，肝硬変まで進行している症例もそうです．

ですが，そうした症例でも，普段の診療はかかりつけ医の先生に診ていただき，画像診断などを当院でさせていただく．肝癌があれば当院で治療させていただくといったように，ネットワークの中での病院の機能分化により，スムーズな診療体系を確立して，自分たちにできるところをしっかりと担っていきたいと思っています．そのためにも，常々，かかりつけ医である開業医の先生方と話し合う機会も設けるようにはしています．

正木　ありがとうございました．荒尾先生，いかがでしょうか．

荒尾　人間ドックもウイルス検診もそうですが，結果を渡されただけで理解できていない患者さんがよく見受けられます．私は産業医も兼務していますが，検診異常者は呼び寄せて「あなたはこの数値がこう悪いから，脂肪肝から肝硬変，肝臓癌合併の危険がありますよ」と指導しています．やはり，丁寧に説明することと本人に納得させることが大事だと思います．

正木　ありがとうございました．須田先生，いかがでしょうか．

須田　今回，参加させていただいて，行政の立場，拠点病院の役割，そしてかかりつけ医の必要性を勉強させていただきました．今回の座談会に参加させていただくにあたって，なぜ肝炎ウイルス検診が周知徹底されないのかということを私なりに考えてみました．まず，自分の両親に，B型肝炎，C型肝炎を知っているか尋ねたところ，「名前を聞いたことはあるけど，どんなものかはよくわからない」という答えが返ってきました．今度は肝硬変や肝がんであれば知っているか尋ねたところ，非常に恐ろしい病気だと認識していました．「肝硬変や肝がんの原因がウイルス性の肝炎なので，肝炎の検診を受けなければいけないのだ」と説明すると，納得してもらえました．このようにB型慢性肝炎，C型慢性肝炎の成れの果てには肝硬変，肝がんが待っているということを患者さんや一般の方々に周知していただくことが，受検するきっかけのひとつになるのではないかと思っています．

正木　ありがとうございました．先生のところも拠点病院ですから，よろしくお願いします．島上先生，いかがでしょうか．

島上　私は石川県の拠点病院ということで，今石川県全体のことも視野に入れて仕事をさせていただいていますが，良質な肝疾患診療ネットワークを築くためには専門医療機関や拠点病院だけが尽力するのではなく，かかりつけ医の先生方や行政の協力が必須であることを痛感しています．先ほどお話しした石川県診療連携や連携非同意者に関する行政によるフォローアップに関しても，細かな点ではかかりつけ医の先生方や行政からのご要望や苦情もお受けしています．しかし，今後も引き続き説明会などを利用して，かかりつけ医の先生方や行政の担当者に対し，肝疾患診療ネットワークの重要性を根気強く説明していきたいと考えています．

石川県の場合は，肝炎ウイルス検診陽性であ

れば，比較的治療に結びつきやすいシステムを構築しているので，先ほどから出ている肝炎ウイルス検診の受検啓発を行い，検診の受診率を少しでも上げたいと考えています．具体的には，特定感染症検査等事業の緊急肝炎ウイルス検査事業として保健所および提携医療機関で実施されている無料の肝炎ウイルス検査を有効利用していきたいと考えています．特に提携医療機関の先生方には，過去に肝炎ウイルス検査を受けていない通院患者さんに対して，この制度を利用して積極的に検査を行っていただくように，最近県の医師会報にも掲載させていただきました．そういう工夫をして少しでも検診の受検率を上げていくということと，あとは県民に肝炎に対する正しい知識を持っていただくように広報活動を行っていきたいと思います．平成23年度の肝炎検査受検状況実態把握事業の報告書によると，普及啓発活動の世代毎の認知度は，テレビやインターネット，新聞などのメディア媒体により異なるという調査結果が出ていましたし，例えばコンビニエンスストアに肝炎ウイルス検査受検を呼びかけるようなポスターを貼っている自治体もあるそうです．1個の媒体に限るのではなく，多くの媒体を使って，肝炎ウイルス検診受検の啓発を行っていくことが必要だと思います．

正木　ありがとうございました．海嶋先生，いかがでしょうか．

海嶋　いろいろと貴重な意見をありがとうございます．先ほどからお話があるように，行政としては，県民の方に肝炎というのがどんな病気なのかを知っていただくことが重要だと思います．肝硬変，肝がんへ進行することもあるということも啓発していくことがまずは我々の仕事だと思いますので，どのように広報していけば一番効果的なのかというのを検証しながらやっていきたいと思っています．

それと肝炎というのは，知っている人は知っているが，知らない人は知らないというように，人によって認知度に極端に差があるので，どのようにして知らない方にも関心を持っていただくかというところにも力を入れていきたいと思います．また，拠点病院の先生方にもご協力いただきながら，一人でも多くの県民の方に「肝炎ウイルス検査を受けてよかったわ」と言っていただけるようがんばっていきたいと思っています．

正木　先生方，本日はどうもありがとうございました．本日お越しいただいた先生方のところは非常に先駆的な自治体も多いし，皆さん非常にモチベーションの高い方々ばかりですが，全国的にそれが普遍化されていなかったり，地域によって温度差があったりするというのが，私が肝炎情報センターに所属している立場で感じているところです．やはり，6年経ったこのシステムがさらにアウトカムを出せるようになるかどうかは，そこにかかっていると思うので，地道に働きかけをしていきたいと思います．住民の方に検診を受けてもらうには，先ほどからお話があるように，効果的な広報活動が重要です．島上先生もおっしゃいましたけれども，やはりインターネットを見られない高齢者も多くいるわけですから，テレビや広報誌を使ったり，定期的に回ってくる新聞に記事を無料で載せてもらったりといった工夫も非常に有効だと思いますので，ぜひとも地道に活動を続けていっていただければと思います．本日は誠にありがとうございました．

著者のCOI (conflicts of interest) 開示：本論文発表内容に関連して特に申告なし

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ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

The Significance of Classifying Microvascular Invasion in Patients with Hepatocellular Carcinoma

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ABSTRACT

Background. Microvascular invasion (MVI) has been recognized as a risk factor for outcome following curative resection in hepatocellular carcinoma (HCC). Because MVI can range from few to many invaded vessels, we evaluated the significance of MVI classification in this study.

Methods. Between January 1995 and December 2010, 207 consecutive patients who underwent curative resection for HCC within Milan criteria were included in this retrospective study. Patients were classified into mild and severe MVI groups based on the number of vessels invaded. This study evaluated whether MVI classification can help to predict recurrence and survival after curative resection.

Results. Of the total 207 patients, 103 (50 %) patients had no detectable MVI, whereas 59 (28 %) had mild MVI, and 45 (22 %) had severe MVI. Recurrence-free survival rates at 2 years for patients without MVI, with mild MVI, and severe MVI were 75.9, 47.2, and 32.7 %, respectively. Patients with severe MVI experienced a high frequency of fatal recurrence, such as multiple tumors, macroscopic vascular invasion, and extrahepatic metastasis after curative resection. Multivariate analysis revealed age, number of tumors, mild MVI, and severe MVI as independent predictors of recurrence-free survival. Disease-specific survival rates at 5 years for patients without MVI, with mild MVI, and severe MVI were 91.5, 70.4, and 51.4, respectively. Multivariate analysis also revealed cirrhosis, tumor size, mild MVI, and severe MVI as independent predictors of disease-specific survival.

Conclusions. We demonstrated that MVI classification can stratify HCC patients by different patterns of recurrence and risk of survival after curative resection.

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Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. Recent advances in imaging procedures and surveillance programs for high-risk patients have led to increased detection of early-stage HCC, resulting in an increase in identification of patients in whom curative resection is possible.[1,2] However, the long-term survival of HCC patients remains unsatisfactory due to the high frequency of intra- and extrahepatic recurrences.[3,4] Vascular invasion (VI) has been recognized as a risk factor leading to early recurrence of HCC.[5–8] Moreover, the fatal recurrence in HCC patients with VI limits additional attempts at various curative therapies, such as liver resection and radiofrequency ablation (RFA), thereby contributing to poor survival.[9,10]

VI is generally classified using either macroscopic or microscopic findings. Macroscopic VI, such as a tumor thrombus in the major portal vein, is known to be a crucial risk factor for survival after liver resection or transplantation in HCC patients and is detectable by various imaging procedures.[11–14] Therefore, the presence of macroscopic VI is usually evaluated before treatment and is an important parameter that is included in the TNM, CLIP, JIS, and BCLC scoring systems and is used to determine treatment

strategies in HCC patients.[10,15,16] In contrast, microscopic vascular invasion (MVI) is difficult to detect before initiation of HCC treatment, even if sophisticated imaging procedures are conducted during patient evaluation. Recently, many studies have reported that the presence of MVI is closely associated with outcome following liver resection or transplantation in HCC patients.[17–21] Our previous study also showed that MVI was an important strong risk factor for recurrence and survival following resection of HCC within the Milan criteria.[22] Therefore, MVI is as important as macroscopic VI and should be evaluated as a risk factor of patients with HCC. Moreover, the current definition of MVI encompasses a wide range of tumor invasion, from one to many microscopic vessels that are contiguous with the tumor. Thus, patients with MVI have been suggested to have a wide range of outcomes after resection. Consequently, the purpose of this study was to evaluate whether the classification of MVI based on number of vessels invaded affects tumor recurrence and survival after resection of HCC.

METHODS

Patients

Between January 1995 and December 2010, 256 patients underwent liver resection at the Kurume University School of Medicine and were diagnosed with HCC by histological findings. The following patients were excluded: (1) patients whose disease did not fulfill the Milan criteria (a single tumor $\leq$5 cm or $\leq$3 tumors each $\leq$3 cm); (2) patients with macroscopic VI; (3) patients with extrahepatic metastasis; (4) patients who underwent noncurative liver resection; and (5) patients who were diagnosed with combined hepatocellular and cholangiocellular carcinoma by histological findings. Of the total 256 patients, 49 patients having one or more of the above criteria were excluded, and the remaining 207 patients were retrospectively enrolled in this study. Patients included 162 males (78 %) and 45 females, with a median age of 66 (range 16–83) years. Overall, 147 patients (71 %) were positive for hepatitis C virus (HCV) infection and 46 patients (22 %) were positive for hepatitis B virus (HBV) infection. Liver cirrhosis was present in 81 patients (39 %). The median tumor size was 25 (range 12–50) mm, and 160 patients (77 %) had a solitary tumor. Various surgical procedures were classified as major or minor resections according to Couinaud's segment classification. Major resections (segmentectomy, sectorectomy, and lobectomy or greater) were performed in 123 patients, whereas minor resections (all other types of resection, including partial hepatectomy and subsegmentectomy) were performed in 84 patients.

Follow-Up and Endpoint

After surgical resection, each patient was followed carefully. Serum biochemistries, alpha-fetoprotein (AFP) levels, and des-gamma-carboxy prothrombin (DCP) levels were measured, and ultrasonography was performed monthly. Contrast-enhanced dynamic computed tomography (CT) was performed every 3 months until 6 months posttreatment and every 6 months thereafter. Magnetic resonance imaging (MRI) was performed as a supplemental examination. Recurrence was diagnosed based on the combined findings of these examinations with appearances typical of HCC. The endpoint of this study was the date of recurrence, death, or last follow-up visit; the closing date was December 2011. The median duration of follow-up was 54.4 (range 9.5–177.8) months.

Histopathological Evaluation

The resected liver specimens were cut into serial 2–3-mm thick slices and fixed in 10 % formalin to facilitate careful gross and histopathological examinations. Each of the liver slices was embedded in paraffin, cut into 4-mm sections, and stained with hematoxylin and eosin. Tumors were examined for maximum tumor size, MVI, intrahepatic micrometastasis, capsular formation, and histologic grade. Noncancerous liver parenchyma was inspected for evidence of cirrhosis. Histological grade was based on the criteria of the Edmondson-Steiner classification and the Liver Cancer Study Group of Japan.[23,24] Intrahepatic micrometastasis was defined as a satellite micronodule in the surrounding liver tissue that is isolated from the main tumor. MVI was defined as microscopic tumor invasion identified in the portal vein and hepatic vein of the surrounding liver tissue that is contiguous with the tumor edge. Moreover, we hypothesize that the extent of MVI may affect tumor recurrence and survival after resection, because MVI encompasses a wide range of tumor invasion. Therefore, in this study, we devised a novel classification of MVI based on number of invaded vessels and divided the patients with MVI into two groups as follows: patients in the mild MVI group had one to five invaded vessels, whereas patients in the severe MVI group had more than five invaded vessels. The number of MVI was counted in each nodule. If patients had multiple tumors, the tumor with the most number of invaded vessels was selected for the classification of MVI. These histopathological evaluations of the resected specimens were retrospectively performed by one experienced pathologist (O.N.). Typical examples of the two MVI types are shown in Fig. 1.

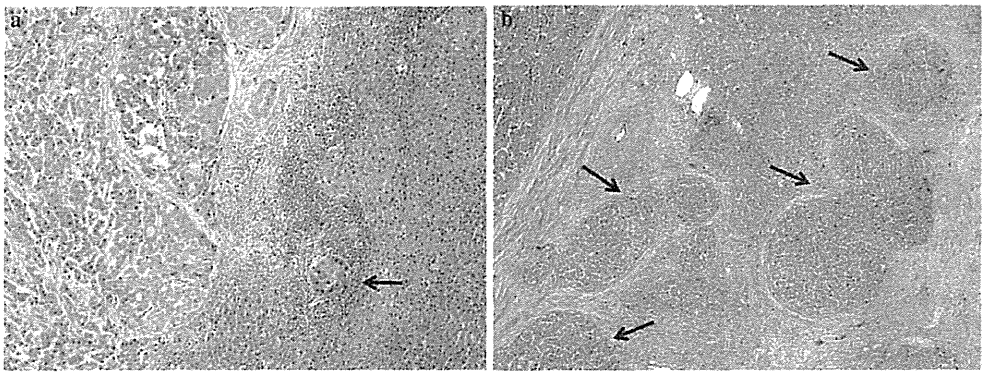

FIG. 1 Typical examples of the two microvascular invasion (MVI) types classified by number of vessels invasion. **a** mild MVI: peritumoral vessel invasion from one to five (hematoxylin–eosin, ×50). **b** severe MVI: peritumoral vessel invasion more than five (hematoxylin–eosin, ×20)

Statistical Analysis

Continuous variables were expressed as median (range). Comparison analysis among MVI grades was performed using the Chi square test for discrete variables and the Kruskal–Wallis test followed by Mann–Whitney *U* test with Bonferroni correction as a post hoc test for continuous variables. Recurrence-free survival and disease-specific survival were determined by Kaplan–Meier analysis, and differences between subgroups were compared with log-rank tests. A cause specific Cox proportional hazards model was used for univariate and multivariate analysis to identify separately any independent variables that were related to recurrence-free survival or disease-specific survival. The variables that were statistically significant by univariate analysis were included in a multivariate analysis. No interaction terms were considered, because the preanalysis showed the nonsignificance for the interaction. Data from these models were expressed as hazard ratio (HR) and 95 % confidence interval (95 % CI). All *P* values were two-tailed, and a level of <0.05 was considered to be statistically significant. Statistical analysis was performed by SPSS software version 20 (SPSS Inc., Chicago, IL, USA).

RESULTS

Comparison of Patient Characteristics Based on Grade of MVI and Predictors of Severe MVI

Clinicopathological characteristics of this study population stratified by grade of MVI are shown in Table 1. Of the total 207 patients, 103 (50 %) patients had no detectable MVI, whereas 59 (28 %) had mild MVI, and 45 (22 %) had severe MVI on pathologic examination. Patients with severe MVI had significantly higher elevated AFP and DCP levels compared with patients without MVI or with mild MVI. Patients with worse MVI grades had larger tumors and a significantly higher prevalence of HCC that was poorly differentiated and had intrahepatic micrometastasis. Other clinicopathological characteristics did not differ significantly among the three groups.

Recurrence-Free Survival and Predictive Factors

Factors associated with recurrence-free survival were evaluated by univariate and multivariate analyses. Univariate analysis showed that age >65 years, HCV infection, HBV infection, elevated DCP level, tumor size >20 mm, presence of multiple tumors, presence of MVI, and presence of intrahepatic metastasis were significant variables affecting recurrence-free survival (Table 2). By multivariate analysis, presence of MVI (mild MVI; hazard ratio [HR]: 1.93, 95 % confidence interval [CI]: 1.25–2.98, $P = 0.003$ and severe MVI; HR: 2.87, 95 % CI: 1.85–4.46, $P < 0.001$), age (>65 years; HR: 1.84, 95 % CI: 1.27–2.65, $P = 0.001$), and number of tumors (2–3; HR: 1.68, 95 % CI: 1.13–2.51, $P = 0.011$) were identified as independent predictors of recurrence-free survival (Table 3). Recurrence-free survival curves of patients stratified by grade of MVI are shown in Fig. 2a. The recurrence-free survival of patients with mild and severe MVI was significantly shorter than that of patients without MVI (no MVI vs. mild MVI, $P = 0.0001$; no MVI vs. severe MVI, $P < 0.0001$; mild MVI vs. severe MVI, $P = 0.1663$).

Pattern and Treatment of First Recurrence after Resection

During the follow-up period, tumor recurrence developed in 122 (54 %) patients, consisting of 48 (47 %) patients without MVI, 38 (64 %) patients with mild MVI, and 36 (80 %) patients with severe MVI. The majority of

TABLE 1 Comparison of patients' characteristics based on grade of microvascular invasion

	No MVI ($n = 103$)	Mild MVI ($n = 59$)	Severe MVI ($n = 45$)	P value
Age, year (range)	65.5 (16–82)	67.0 (34–83)	65.0 (33–81)	0.599
Gender (male/female)	79/24	46/13	37/8	0.754
HCV (positive/negative)	74/29	38/21	35/10	0.319
HBV (positive/negative)	22/81	13/46	11/34	0.917
AST, U/L (range)	43 (15–153)	39.0 (14–137)	41.5 (13–160)	0.838
AFP, ng/ml (range)	11.5 (1–20,764)	10.6 (1.7–5,372)	100.3 (2.8–6,385)†‡	0.001
(≤100/>100)	82/21	45/14	23/22	0.001
DCP, AU/ml (range)	34.5 (10–2,529)	54.5 (10–4,761)	209.5 (13–20,919)†‡	<0.001
(≤100/>100)	77/23	36/23	16/29	<0.001
Tumor size, mm (range)	23.5 (12–50)	26.5 (12–50)†	29 (18–50)†	<0.001
(≤30/>30)	83/20	37/22	25/20	0.002
Number of tumors (single/2–3)	83/20	43/16	34/11	0.505
Histological grade (well/moderate/poorly)	10/92/1	0/52/7	0/33/12	<0.001
Background liver (normal+CH/cirrhosis)	60/43	39/20	27/18	0.61
Intrahepatic micrometastasis (present/absent)	2/101	12/47	23/22	<0.001
Capsular formation (present/absent)	62/41	44/15	25/20	0.09
Type of surgical resection (Major/minor)	57/46	35/24	31/14	0.304

Continuous variables presented as median (range)

MVI microvascular invasion, *HCV* hepatitis C virus, *HBV* hepatitis B virus, *AST* aspartate aminotransferase, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin, *CH* chronic hepatitis, *well* well differentiated, *moderate* moderately differentiated, *poorly* poorly differentiated

† $P < 0.05$ for post hoc test comparison without MVI

‡ $P < 0.05$ for post hoc test comparison with mild MVI

patients without MVI and with mild MVI who underwent resection for recurrence had intrahepatic recurrence with no more than three tumors (more than 50 % patients had a single tumor) and had no macroscopic VI or extrahepatic metastasis. Consequently, 41 (86 %) patients without MVI and 29 (76 %) patients with mild MVI underwent curative treatment for first recurrence, which included resection, RFA, microwave coagulation therapy (MCT), and percutaneous ethanol injection (PEI). In contrast, patients with severe MVI who underwent resection experienced a high frequency of fatal recurrence, including 22 (61 %) patients with multiple intrahepatic tumors, 6 (17 %) patients with macroscopic VI, and 11 (31 %) patients with extrahepatic metastasis. As a result, curative treatment for first recurrence could only be performed in 15 (42 %) patients with severe MVI.

Disease-Specific Survival and Predictive Factors

Of the total 207 patients, 58 patients died during follow-up. Of these 58 patients, 50 patients died from HCC-related causes. Factors associated with disease-specific survival were evaluated by univariate and multivariate analyses. Univariate analysis showed that presence of cirrhosis, elevated AFP level, elevated DCP level, tumor size >20 mm, presence of multiple tumors, presence of MVI, and presence of intrahepatic metastasis were significant variables affecting disease-specific survival (Table 2). By multivariate analysis, presence of MVI (mild MVI; HR: 2.4, 95 % CI: 1.09–5.26, $P = 0.029$ and severe MVI; HR: 6.06, 95 % CI: 2.93–12.53, $P < 0.001$), background liver (cirrhosis; HR: 2.54, 95 % CI: 1.42–4.55, $P = 0.002$), and tumor size (>30 mm; HR: 3.19, 95 % CI: 1.67–8.71, $P = 0.024$) were identified as independent predictors of disease-specific survival (Table 3). Disease-specific survival curves of patients stratified by grade of MVI are shown in Fig. 2b. Patients with worse MVI grades experienced significantly shorter disease-specific survival (no MVI vs. mild MVI, $P = 0.0017$; no MVI vs. severe MVI, $P < 0.0001$; mild MVI vs. severe MVI, $P = 0.0057$).

DISCUSSION

In our previous study, we demonstrated that MVI was a strong risk factor for poor outcome following curative resection in HCC patients within the Milan criteria.[22] The 3-year, recurrence-free survival rates for patients with and without MVI were 27.7 and 62.5 %, respectively. Thus, the presence of MVI was previously known to lead to a high frequency of recurrence of HCC after liver resection in the

TABLE 2 Univariate analyses of recurrence-free survival and disease-specific survival for hepatocellular carcinoma

	Recurrence-free survival HR (95 % CI)	*P* value	Disease-specific survival HR (95 % CI)	*P* value
Gender				
Male	1.26 (0.79–2)	0.325	1.36 (0.64–2.9)	0.426
Age (year)				
>65	1.71 (1.19–2.46)	0.004	1.03 (0.59–1.79)	0.92
HCV				
Positive	1.73 (1.13–2.65)	0.011	1.16 (0.625–2.16)	0.635
HBV				
Positive	0.58 (0.37–0.93)	0.022	0.95 (0.50–1.83)	0.885
AST, U/L				
>50	1.4 (0.97–2.01)	0.069	1.42 (0.82–2.49)	0.215
AFP, ng/mL				
>100	1.36 (0.93–2)	0.115	1.77 (1–3.12)	0.048
DCP, AU/mL				
>100	1.63 (1.14–2.32)	0.008	1.78 (1.02–3.11)	0.042
Tumor size, mm				
>30	1.64 (1.13–2.38)	0.009	2.66 (1.53–4.64)	0.001
Number of tumors				
2–3	1.93 (1.3–2.87)	0.001	1.92 (1.59–3.5)	0.032
Histological grade				
Moderate	1.56 (0.64–3.84)	0.331	2.78 (0.38–20.19)	0.312
Poorly	1.8 (0.64–5.13)	0.268	3.69 (0.43–31.7)	0.234
Background liver				
Cirrhosis	1.21 (0.84–1.75)	0.3	1.85 (1.06–3.22)	0.03
Microvascular invasion				
Mild	2.16 (1.4–3.31)	<0.001	2.78 (1.29–5.98)	0.009
Severe	2.92 (1.9–4.51)	<0.001	6.61 (3.32–13.16)	<0.001
Intrahepatic micrometastasis				
Present	1.98 (1.27–3.07)	0.002	3.47 (1.91–6.30)	<0.001
Capsular formation				
Present	0.87 (0.6–1.27)	0.482	1.09 (0.61–1.94)	0.771
Type of surgical resection				
Minor	1.18 (0.82–1.69)	0.38	1.64 (0.94–2.86)	0.081

HR hazard ratio, *CI* confidence interval, *HCV* hepatitis C virus, *HBV* hepatitis B virus, *CH* chronic hepatitis, *AST* aspartate aminotransferase, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin, *well* well differentiated, *moderate* moderately differentiated, *poorly* poorly differentiated

short term.[5–8] Nevertheless, because MVI encompasses many recurrence patterns ranging from curative to fatal, the differences in recurrence patterns are suggested to be associated with a wide range of outcomes with respect to long-term survival. Therefore, we classified patients into two groups (mild and severe MVI) and evaluated the significance of MVI classification.

In the present study, we showed that both mild and severe MVI were significant independent risk factors affecting recurrence-free survival in HCC patients after curative resection. Moreover, our results showed that both patients with mild and severe MVI had a high frequency of micrometastasis in resected liver specimens. Cancer cell spreading via the portal vein has been generally thought to be the main mechanism for such intrahepatic micrometastasis.[25] Micrometastasis is an important cause of early intrahepatic recurrence after liver resection.[26] Consequently, the identification of MVI as a risk factor for early recurrence after curative resection regardless of MVI grade in this study is very relevant.

In addition, both mild and severe MVI were identified as significant independent risk factors affecting survival in HCC patients after curative resection. These results suggest that a high frequency of early recurrence was the primary contributor to poor survival in patients with mild MVI. Early recurrence of HCC is known to be the major risk factor affecting survival following liver resection.[27,28] Moreover, liver function was also identified as an independent risk factor of survival in the present study. Thus, in patients with mild MVI, repeated recurrence and treatment are thought to decrease liver function, thereby contributing

TABLE 3 Multivariate analyses of recurrence-free survival and disease-specific survival for hepatocellular carcinoma

	HR (95 % CI)	*P* value
Recurrence-free survival		
Microvascular invasion		
Mild	1.93 (1.25–2.98)	0.003
Severe	2.87 (1.85–4.46)	<0.001
Age (year)		
>65	1.84 (1.27–2.65)	0.001
Number of tumors		
2–3	1.68 (1.13–2.51)	0.011
Disease-specific survival		
Microvascular invasion		
Mild	2.4 (1.09–5.26)	0.029
Severe	6.06 (2.93–12.53)	<0.001
Background liver		
Cirrhosis	2.54 (1.42–4.55)	0.002
Tumor size (mm)		
>30	2.41 (1.36–4.27)	0.003

HR hazard ratio, *CI* confidence interval

to poor long-term survival.[28] In contrast, although no significant difference in the frequency of recurrence was observed between patients with mild versus severe MVI, patients with severe MVI experienced significantly shorter survival compared with patients with mild MVI. Thus, this observed difference in survival was likely associated with recurrence pattern and not frequency of recurrence. In this study, patients with severe MVI experienced a higher frequency of fatal recurrence, associated with multiple intrahepatic tumors, macroscopic VI, and extrahepatic metastasis. Such fatal recurrence limits additional attempts at curative therapies in HCC patients with severe MVI.[10] As a result, patients with severe MVI experienced poorer survival compared to those with mild MVI. Consequently, we have shown that MVI classification based on the number of invaded vessels can be used to stratify patients into three distinct groups (without MVI, with mild MVI, and with severe MVI) with different risks of survival after curative resection.

Extrahepatic metastasis contributes to poor survival following liver resection in HCC patients.[4,29] Previous studies proposed that MVI was an independent risk factor affecting extrahepatic metastasis in HCC patients after curative resection.[29,30] In agreement with this, the present results revealed that extrahepatic metastasis occurred in approximately 31 % of HCC patients with severe MVI. Liver transplantation is widely accepted as a therapeutic option in HCC patients, particularly for those with cirrhosis and who fulfill the Milan criteria. Recently, MVI has been proposed as a significant risk factor for recurrence and survival in HCC patients after liver transplantation as well as liver resection.[20,21] Because total hepatectomy is performed in the recipient, extrahepatic metastasis is the primary site of recurrence in HCC patients following liver transplantation. Consequently, the present results suggest that severe MVI is a risk factor for liver transplantation in HCC patients, even if they fulfill the Milan criteria.

Prevention of early recurrence of HCC with MVI is the most important strategy for improving long-term survival in HCC patients after curative resection; however, no adjuvant systemic treatment has previously been reported to show a survival benefit. Sorafenib is an oral multikinase inhibitor that has recently become available for advanced HCC. Randomized phase III placebo-controlled trials demonstrated that sorafenib treatment resulted in a significant survival benefit in patients with advanced HCC and maintained liver function; as a result, sorafenib has become the only standard systemic treatment for advanced HCC.[31,32] Consequently, a prospective trial is required to

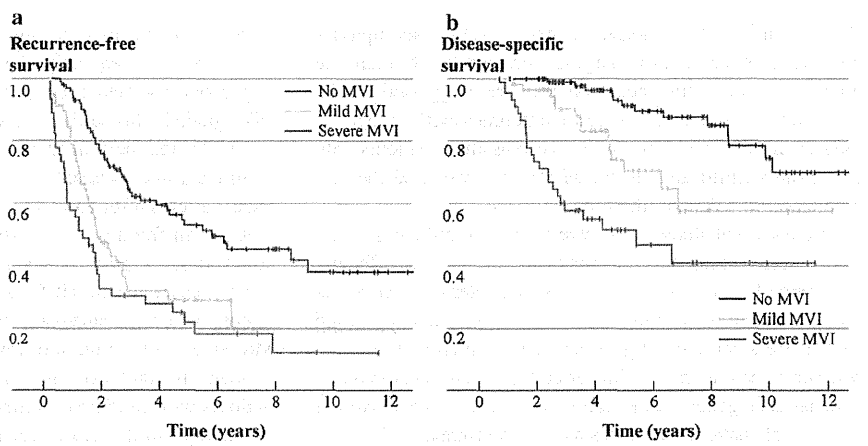

FIG. 2 Recurrence-free survival and disease-specific survival curves of patients stratified by grade of microvascular invasion (MVI). **a** Recurrence-free survival rates at 2 years were 75.9 % in patients without MVI, 47.2 % in patients with mild MVI, and 32.7 % in patients with severe MVI. **b** Disease-specific survival rates at 5 years were 91.5 % in patients without MVI, 70.4 % in patients with mild MVI, and 51.4 % in patients with severe MVI

assess the utility of sorafenib as adjuvant treatment for HCC patients with MVI, particularly because patients with severe MVI experience a high frequency of fatal recurrence after curative resection.

CONCLUSIONS

The present results demonstrated that MVI classification based on number of invaded vessels could stratify different recurrence patterns and risk of survival after curative resection in HCC patients within the Milan criteria. In particular, the presence of severe MVI was found to be associated with high malignant potential of HCC. Therefore, the results of this study suggest that it is important to histologically evaluate the presence of severe MVI after liver resection for determination of strict observation and adjuvant treatment.

DISCLOSURE No commercial interest.

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SHORT REPORT
Changes in hepatitis C virus genotype distribution in Japan

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SUMMARY

Genotypes are associated with the natural course of hepatitis C virus (HCV) infection and response to antiviral therapy for HCV. HCV genotype 1b has been the dominant genotype in Japan, where the prevention of HCV transmission through blood transfusion or nosocomial infection has been established since 1990. The distribution of HCV genotype was investigated based on patient's birth year in 5515 HCV-infected Japanese individuals at three institutions from different areas of Japan. At all three institutions, the proportion of HCV genotype 1b decreased and was <50% in individuals born after 1970. By contrast, the percentage of HCV genotype 2b increased in subsequent birth cohorts after 1920–1929. Significant changes in HCV genotype distribution were observed across Japan regardless of area.

Key words: Birth year, distribution, genotypes, hepatitis C virus.

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. HCV is classified into several genotypes and sub-genotypes with varying prevalence rates in different regions of the world [1]. In Japan, the majority of individuals with HCV infection have genotype 1b [2, 3], which is one of the sub-genotypes resistant to interferon-based antiviral therapy [4]. Before the detection of HCV antibodies was established in 1990, the main modes of HCV transmission had been blood transfusion or nosocomial infection. After the discovery of HCV, prevention of HCV transmission through these routes was established and the number of individuals newly infected with HCV rapidly decreased in Japan [5]. Although decreases in the number of individuals with HCV infection have been reported, changes in the distribution of HCV genotype over time have not been studied. In the present study, we investigate changes in HCV genotype distribution based on the birth year of HCV-infected individuals in Japan. We analysed individuals from three different areas of the country to clarify whether any changes in HCV genotype distribution constitute a nationwide trend.

HCV genotype was analysed in a total of 5515 HCV-infected Japanese individuals at three institutions located in different parts of Japan (Fig. 1): Ogaki

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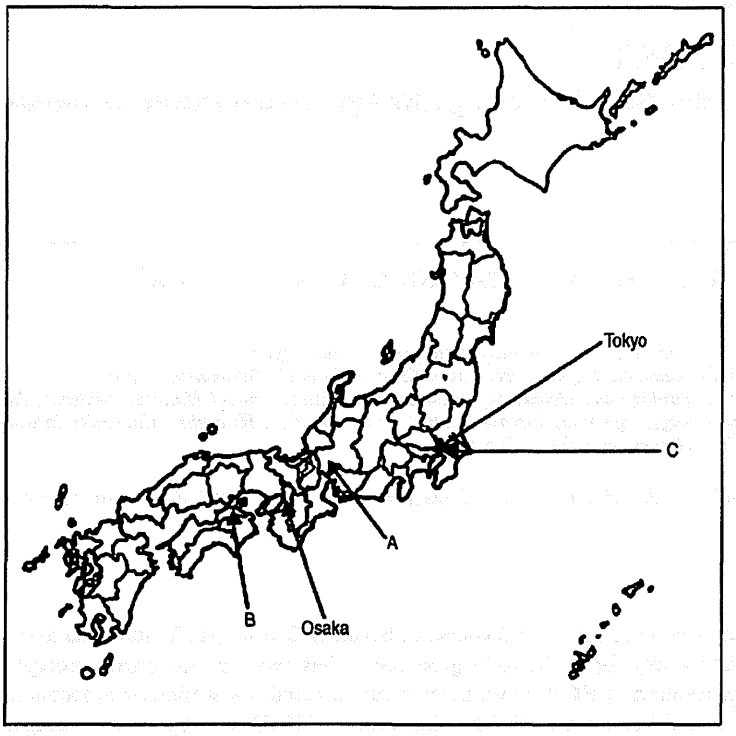

Fig. 1. Location of the three Japanese institutions (liver centres) participating in the study. A, Ogaki Municipal Hospital; B, Kagawa Prefectural Central Hospital; C, Shinmatsudo Central General Hospital.

Municipal Hospital (institution A; Ogaki City, located in central Japan, 3269 individuals), Kagawa Prefectural Central Hospital (institution B; Takamatsu City, located on Shikoku Island, western Japan, 1421 individuals), and Shinmatsudo Central General Hospital (institution C; Matsudo City, near Tokyo, 825 individuals). These individuals received regular follow-up at one of the institutions between 1991 and 2013. HCV infection was confirmed by both positive serum HCV antibody (Architect Anti-HCV; Abbott Laboratories, USA) and serum HCV RNA using a real-time polymerase chain reaction (PCR)-based method (COBAS AmpliPrep/COBAS TaqMan HCV test; Roche Molecular Systems, USA; lower limit of detection, 1·2 $\log_{10}$ IU/ml). HCV genotype was assessed using PCR methods to amplify the core gene sequences using genotype-specific primers [6]. Genotype was classified as 1a, 1b, 2a, 2b, 3a, or mixed. Review and analysis of clinical data including patient's birth year, age, sex, and HCV genotype were approved by the Institutional Review Board of each institution.

Trends for changes in the percentage of each HCV genotype according to birth year were evaluated with the Cochran–Armitage test. All P values were two-tailed, and P <0·05 was considered statistically significant.

All individuals studied were of Japanese ethnicity and no immigrants were included. The HCV-infected individuals comprised of 1790 males and 1479 females with a mean age of 67·1 ± 13·3 years at institution A; 729 males and 692 females with a mean age of 62·0 ± 12·7 years at institution B; and 419 males and 406 females with a mean age of 66·6 ± 13·3 years at institution C. The distribution of HCV genotypes in all patients was: institution A [genotype 1b (64·3%), genotype 2a (27·0%), genotype 2b (7·7%), and other (including genotypes 1a, 3a, and mixed) (1·0%)]; institution B [genotype 1b (62·3%), genotype 2a (26·5%), genotype 2b (10·3%), and other (including genotypes 1a and 3a) (1·0%)]; and institution C [genotype 1b (70·8%), genotype 2a (17·6%), genotype 2b (10·3%), and other (including genotypes 3a and mixed) (1·3%)].

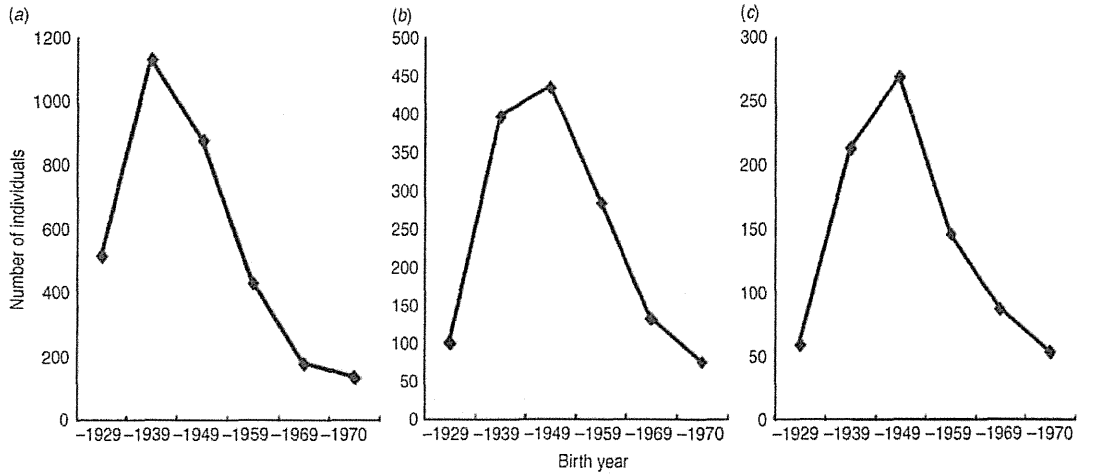

Fig. 2. Changes in the number of hepatitis C virus-infected individuals under follow-up at institutions based on birth year at (*a*) Ogaki Municipal Hospital, (*b*) Kagawa Prefectural Central Hospital, and (*c*) Shinmatsudo Central General Hospital.

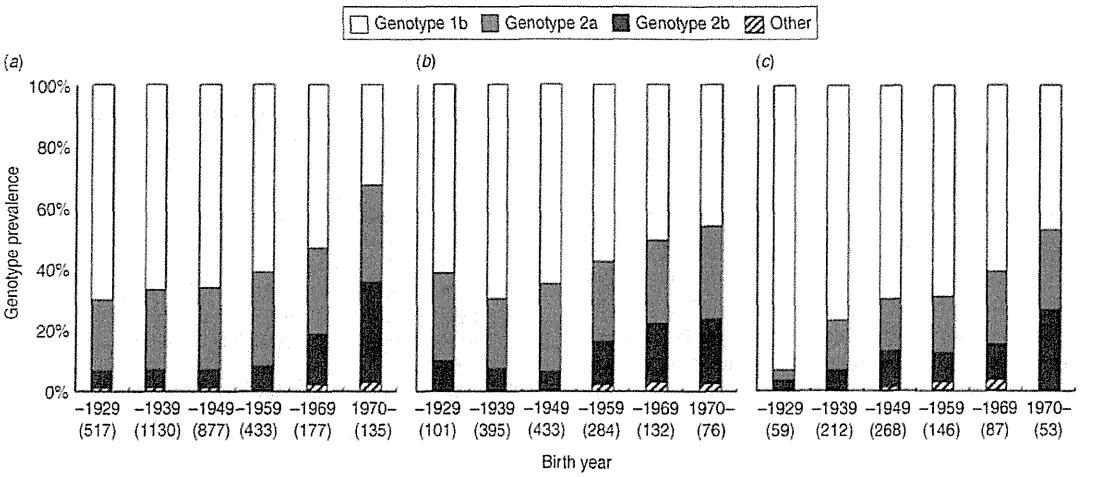

Fig. 3. Changes in hepatitis C virus (HCV) genotype distribution based on birth year of HCV-infected individuals at (*a*) Ogaki Municipal Hospital, (*b*) Kagawa Prefectural Central Hospital, and (*c*) Shinmatsudo Central General Hospital. The number of individuals born during each time period is indicated in parentheses. 'Other' includes genotypes 1a, and 3a, as well as mixed genotypes.

At all three institutions, the number of individuals with HCV infection who were under follow-up at the institutions decreased over time starting with birth years 1930–1939 at institution A and 1939–1940 at institutions B and C (Fig. 2). Figure 3 shows the distribution of HCV genotypes based on patient's birth year at each institution. There is a trend of HCV genotype 1b decreasing over time ($P<0{\cdot}0001$ for all three institutions). The percentage of HCV genotype 1b was <50% in individuals born after 1970. The percentage of HCV genotype 2a was constant except for individuals born before 1929. By contrast, the percentage of HCV genotype 2b increased during the period studied ($P<0{\cdot}0001$ for institutions A and B, and $P=0{\cdot}0004$ for institution C).

This study showed that the HCV genotype distribution changed markedly over time in Japan. The trend was confirmed at three large institutions from different parts of the country and seems to be nationwide. The percentage of individuals with HCV genotype 1, which is a major HCV genotype worldwide [2], is decreasing and is found in <50% of individuals born after 1970. A decrease in the prevalence of patients with HCV genotype 1b has also been reported in the USA and Europe in studies with a smaller number of subjects [7–9].

Several studies have reported that HCV genotypes are different between individuals with HCV acquired through transfusions or medical procedures and individuals with HCV acquired through other transmission route, including intravenous drug use or tattooing [3, 10–13]. In Japan, HCV genotype 1b is associated with the former route of transmission, whereas other genotypes, i.e. genotypes 2a and 2b, are associated with the latter route [13]. With the establishment of methods to prevent HCV transmission during blood transfusions and medical procedures in developed countries, the routes of HCV transmission other than transfusions or medical procedures have become the main routes of HCV infection. Indeed, among 135 individuals with a birth year of 1970 or later at institution A, 29 (21·5%) had a history of intravenous drug use and 33 (24·4%) had a history of tattooing. By contrast, no individuals had a history of blood transfusion. Although the accurate route of HCV transmission was unclear in most individuals in the present study, these changes in the common routes of HCV transmission may be associated with changes in HCV genotype distribution over time. Further studies will be necessary to clarify why the percentage of HCV genotype 2b increased in Japan.

There are several limitations to this study. HCV genotype distribution was analysed based on the birth year of infected individuals instead of the year of HCV infection since there was not enough available information to accurately determine the year of HCV infection. However, results with a large study population from different parts of Japan can be indicative of changes in HCV genotype distribution. The HCV-infected individuals analysed were those who received regular follow-up and may not reflect the HCV genotype distribution of the entire Japanese HCV-infected population. Better nationwide surveillance of HCV that includes data on genotype determination is required to confirm these observed changes in HCV genotype distribution over time.

In conclusion, significant changes in HCV genotype distribution over time were observed in HCV-infected Japanese individuals in various regions of Japan. HCV genotype 1b may no longer be the predominant genotype of HCV in Japan in the future. These changes may also be occurring in other countries throughout the world, especially developed countries. Reappraisal of HCV genotype distribution will be necessary over time. Recent new antiviral drugs for the treatment of HCV, including direct-acting antivirals, mainly target HCV genotype 1 with some exceptions. However, other genotypes may become dominant in various populations of HCV-infected individuals.

DECLARATION OF INTEREST

None.

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CORRESPONDENCE

Noninvasive Diagnosis of Portal Hypertension and Esophageal Varices Through the Identification of Liver Blood Flow Markers

To the Editor:

We read with great interest the article entitled "Use of noninvasive markers of portal hypertension and timing of screening endoscopy for gastroesophageal varices in patients with chronic liver disease"[1] in which the authors, Dr. Annalisa Berzigotti and Prof. Jaime Bosch, describe the usefulness of noninvasive tools for the diagnosis of clinically significant portal hypertension (CSPH, hepatic vein pressure gradient [HVPG] ≥10 mmHg) and esophageal varices (EV).

The most recent guidelines[2] on portal hypertension strongly suggest performing upper endoscopy and, where available, HVPG measurement in all patients with liver cirrhosis. Moreover, endoscopy should be repeated every 2-3 years in patients without esophageal varices and more frequently (according to bleeding risk) in patients with EV. As recognized by Berzigotti et al.,[1] this screening and follow-up program leads to significant healthcare costs and patient discomfort since cirrhosis is, nowadays, frequently diagnosed in a very initial stage when varices are still absent. Therefore, in the near future the selection of high-risk patients represents a clinical challenge for the hepatologist in order to reduce futile examinations, the related costs, and the patients' burden.

We strongly agree with the idea of sparing HVPG measurement and endoscopy in patients with less than 20% probability of CSPH based on the combination of noninvasive tests and to perform it in the remaining patients with higher pretest probability. As the authors correctly point out, a number of noninvasive tests based on liver elastography (alone or combined with other parameters) or on spleen stiffness can help to reliably rule out and diagnose CSPH.

Besides the above-mentioned noninvasive tests, we would like to remember the Indocyanine Green Retention Test (ICG-r15), which is a quantitative function test reflecting liver functional reserve and blood flow. Among patients with initial cirrhosis and well-preserved liver function, ICG-r15 correlates with the presence, degree, and complication of portal hypertension, reflecting the modifications of liver blood flow.

We recently evaluated ICG-r15 as a noninvasive marker of CSPH and EV in a population of 96 consecutive patients with compensated liver cirrhosis of different etiologies[3]; in our study an ICG-r15 <10% correctly ruled out the presence of varices in 26 out 27 patients. Therefore, the good diagnostic performance of ICG-r15 (Table 1) makes it a valid tool for the assessment of PH and EV in cirrhosis patients. Although these results have to be validated in a larger or multicentric population and confirmed by longitudinal analysis, this simple and reproducible test allows an initial stratification of cirrhosis patients.

In conclusion, we definitely agree with the authors on the need to spare unnecessary HVPG and upper endoscopies in this clinical setting and suggest that ICG-r15 might be another test to consider besides those based on transient elastography or liver stiffness, particularly in centers where these technologies are not available.

Table 1. Diagnostic Performance of Indocyanine Green 15 Minutes Retention Test for the Rule-Out of Clinically Significant Portal Hypertension and Esophageal Varices

		Prediction of CSPH (HVPG ≥ 10 mmHg)			Prediction of EV		
	No.	AUROC	Sensitivity	−LR	AUROC	Sensitivity	−LR
ICG-r15[3]	96	0.808	95.9%	0.15	0.859	97.8%	0.042

CSPH: Clinically Significant Portal Hypertension; EV: Esophageal Varices; ICG-r15: Indocyanine Green retention at 15 minutes; AUROC: area under ROC curve; −LR: negative likelihood ratio.

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Postinterferon α-Fetoprotein Elevation and Risk of Hepatocellular Carcinoma Development After Sustained Virological Response: Cause or Results?

To the Editor:

We read with interest the article by Asahina et al.,[1] which clearly demonstrated a higher incidence of hepatocellular carcinoma (HCC) in patients with higher levels of α-fetoprotein (AFP) after interferon-based antiviral therapy. There was a surprisingly high incidence of HCC (almost 50%) in patients who achieved sustained virological response (SVR) but whose postinterferon (IFN) AFP levels were higher than 20 ng/mL.

There are two distinct patterns of HCC development after SVR. In one pattern, HCC develops after the eradication of hepatitis C virus (HCV). This pattern is associated with the residual

potential for hepatocarcinogenesis after SVR, which may be signaled by elevated AFP after IFN. The other pattern involves HCC that was too minute to be detected before and just after IFN treatment, but grew enough to be visualized on imaging studies during post-SVR follow-up. Previous studies of HCC tumor volume doubling time suggest that some minute HCC tumors would take several years to be detected by imaging modalities.[2] Some patients who achieved SVR, therefore, might have had minute, undetectable HCC at the time of SVR. Although the authors described excluding patients with HCC based on imaging studies, such modalities always have limitations in their ability to detect minute HCC (for example, <5 mm in diameter). In particular, the ability of imaging modalities to detect minute HCC was unsatisfactory during the earlier part of the study period (1990s).

Their Fig. 2F suggests a specific feature in the cumulative incidence curves for HCC based on post-IFN AFP levels in patients with SVR. Among patients who did not achieve SVR, the incidence of HCC continued to increase gradually according to the number of years after SVR. This includes patients with high post-IFN AFP levels, whose HCC incidence curves were similar to incidence curves stratified by post-IFN ALT levels. In contrast, in patients with SVR, cumulative HCC incidence curves according to post-IFN AFP levels were different. The incidence of HCC in patients with post-IFN AFP ≥20 ng/mL and with post-IFN AFP ≥10 ng/mL and <20 ng/mL increased rapidly until 3 to 4 years after SVR, with only a few patients developing HCC thereafter. This feature could be due to the detection of preexisting minute HCC after SVR. Was the post-IFN AFP elevation observed in these patients a marker of enhanced hepatocarcinogenesis or an existing HCC?

It will be difficult to determine whether elevated levels of AFP were produced by HCC without visible HCC on imaging studies. It would be interesting to check the fucosylated fraction of AFP (AFP-L3), a more specific marker that has been reported as a marker of minute HCC,[3] if post-IFN AFP-L3 data were available.

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Could Postinterferon Treatment α-Fetoprotein Levels Truly Predict Hepatocarcinogenesis?

To the Editor:

I read with interest the article by Asahina et al.[1] regarding the levels of α-fetoprotein (AFP) and alanine aminotransferase (ALT) after interferon therapy that could predict the occurrence of hepatocellular carcinoma (HCC) in patients with hepatitis C virus (HCV) infection. The authors made a great effort to evaluate the levels of AFP and ALT in a cohort of 1,818 patients after interferon therapy and found that cutoff values for ALT and AFP for prediction HCC development were 40 IU/L and 6.0 ng/mL, respectively. These findings may be helpful for clinicians to closely follow-up high-risk patients to detect early-stage HCC. However, these data may also be misleading and need further clarification.

The levels of AFP and ALT were measured every 1-6 months. It is unknown which timepoint of AFP and ALT levels were selected for calculation. Serum AFP levels usually fluctuate during serial observation. Some patients may present with an AFP >6 ng/mL before therapy, which decreased to <6 ng/mL within a few months after interferon therapy, and became elevated to >6 ng/mL at long-term follow-up. Should these patients be classified as AFP ≧6 ng/mL decreased group or AFP ≧6 ng/mL unchanged group? The mean follow-up period of this study was 6.1 years. The follow-up period in this study has been as long as 20 years. If the interval of AFP measurement was as short as 1 month, too many unnecessary measurements could have been performed. As shown in the results, postinterferon therapy AFP level ≧6.0 ng/mL had a positive predictive value of only 0.262. Consistent with previous observation, this may evoke inappropriate suspicion of malignancy in 74 out of 100 patients with AFP above this cutoff value.[2] A total of 179 patients developed HCC, accounting for 9.8% of the entire cohort of 1,818 patients. Based on the HALT-C trial, patients with persistent elevation of AFP after interferon therapy, only 2% were noted to develop HCC.[3] We believe that a large proportion of patients still did not have elevated AFP levels on detection of HCC occurrence. Thus, the applicability and cost-effectiveness of this policy merits further investigation.

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FULL PAPER

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Keywords: hepatocellular carcinoma; prognosis; prognostic models; biomarkers; AFP; DCP; AFP-L3; bilirubin; albumin

Biomarker-based prognosis in hepatocellular carcinoma: validation and extension of the BALAD model

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Background: The Japanese 'BALAD' model offers the first objective, biomarker-based, tool for assessment of prognosis in hepatocellular carcinoma, but relies on dichotomisation of the constituent data, has not been externally validated, and cannot be applied to the individual patients.

Methods: In this Japanese/UK collaboration, we replicated the original BALAD model on a UK cohort and then built a new model, BALAD-2, on the original raw Japanese data using variables in their continuous form. Regression analyses using flexible parametric models with fractional polynomials enabled fitting of appropriate baseline hazard functions and functional form of covariates. The resulting models were validated in the respective cohorts to measure the predictive performance.

Results: The key prognostic features were confirmed to be Bilirubin and Albumin together with the serological cancer biomarkers, AFP-L3, AFP, and DCP. With appropriate recalibration, the model offered clinically relevant discrimination of prognosis in both the Japanese and UK data sets and accurately predicted patient-level survival.

Conclusions: The original BALAD model has been validated in an international setting. The refined BALAD-2 model permits estimation of patient-level survival in UK and Japanese cohorts.

The key features that influence prognosis in hepatocellular carcinoma (HCC) are now well recognised and can be broadly classified under the headings of tumour-related factors (such as tumour size or multiplicity), those that assess the severity of underlying liver dysfunction (such as conventional liver function tests or the Child–Pugh (C-P) classification (Child and Turcotte, 1964; Pugh *et al*, 1973)) and patient-related factors (such as symptoms or performance status). Several staging systems/prognostic scores that combine a number of these factors have been developed (Okuda *et al*, 1985; Group, 1998; Chevret *et al*, 1999; Leung *et al*, 2002; Kudo *et al*, 2003; Llovet *et al*, 2008) and, to varying degrees, validated and compared (Kudo *et al*, 2004; Marrero *et al*, 2005; Cho *et al*, 2008; Collette *et al*, 2008; Chen *et al*, 2009; Huitzil-Melendez *et al*, 2010; Chan *et al*, 2011). Some simply offer an estimate of prognosis, whereas others aim to indicate the appropriate therapy for specific disease stages (Llovet *et al*, 2008).

In an attempt to develop a more objective staging system, Toyoda *et al* (2006) have described the BALAD model that relies on two liver function tests (Bilirubin and Albumin) and three serological cancer biomarkers (AFP-L3, AFP, and DCP). They have

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shown that it is possible to achieve an excellent degree of discrimination between the proposed risk groups using such objective variables. However, the data analysis approach in the BALAD model utilised dichotomisation of the continuous variables, which raises a number of statistical issues.

In the present study, we aimed to validate the original BALAD model (built on a Japanese Cohort) in a geographically and aetiologically distinct HCC patient data set from the UK. We first confirmed that the variables in the BALAD model were identical to those independently identified in a UK data set, and assessed the discrimination achieved within the proposed prognostic groups. We then, in a collaborative Japan/UK study, took the raw data on which BALAD model was initially derived and applied a more sophisticated statistical method that treats the variables in a continuous manner and does not assume a linear relationship between predictors and outcome. The model developed here not only allows classification of patient risk, as with the original BALAD model, but also provides detailed estimation of patient-level survival in the Japanese cohort, and, with calibration, in UK patients.

A major challenge in applying the BALAD model to the UK population is the great difference in survival compared with the Japanese cohort. This problem is due to the difference in the underlying survivor function that describes hazard in relation to time; hazard could be greatest at diagnosis and then decrease over time or, conversely, the hazard at diagnosis may be low and then increase as time accumulates. Indeed, the hazard may be described by a more complicated, non-linear, and not necessarily monotonic function. To account for such differences, the methods applied in this analysis allowed interrogation of the scale and shape of the baseline hazard function.

The derived model is assessed in terms of discrimination and calibration. To assess discrimination, Harrell's *C*-statistic was measured, as described by Taktak *et al* (2007). This measures the proportion of patient pairs for which the model correctly assigns lower risk to the patient who truly survives longest (i.e. is at least risk). A model with good discriminative performance should have a high *C*-statistic. To assess calibration, graphical methods were used. These assessments compare patient level survival with the predicted values.

MATERIALS AND METHODS

The study comprised two cohorts of patients. The first included 2599 Japanese patients previously reported by Toyoda *et al* (2006) and 319 UK patients, all with HCC (Table 1). The Japanese patients were recruited from five institutions in which a total of 3725 patients were initially diagnosed as having HCC between July 1994 and December 2004, and the UK patients from among 724 patients referred to the Queen Elizabeth, Birmingham, UK, between June 2007 and January 2012. The various aetiologies were classified as hepatitis B virus-related, hepatitis C virus-related, alcoholic-related, and 'other'. The 'other' group comprised patients with hemochromatosis, primary biliary cirrhosis, non-alcoholic steatohepatitis, or cryptogenic cirrhosis. The diagnosis of chronic liver disease was made on the basis of liver biopsy and/or typical clinical and imaging features. The study protocol was approved by the institutional ethics review board at each of the institutions.

Age and gender distributions were similar in the two populations, as was the distribution of liver dysfunction as assessed by the C-P classification (Table 1). However, there were striking differences in aetiological attribution, the Japanese patients having predominantly HCV-related HCC and the UK patients having multiple aetiologies. There were also major differences in disease stage (Table 1) and overall survival between the two cohorts. The median survival for those treated with palliative and curative therapy was 22.6 and 60.7 months for Japanese patients, respectively, with analogous figures for the UK of 13.9 and 27.5 months.

In all patients, the three serological cancer biomarkers of HCC (AFP, AFP-L3, and DCP) were measured at the time of diagnosis, and drugs that would influence the serum DCP levels, such as warfarin and vitamin K, were not taken. A standard operating procedure was applied to all blood collection. Samples were collected in the fasting state, before any treatment. Blood was allowed to clot at room temperature for 1–2 h, centrifuged at 3000 **g** for 20 min and the serum collected and stored at −80 °C until processing. Routine liver and renal function was measured by commercially available methods. Albumin was measured by the bromocresol green method in both UK and Japan. The severity of the liver disease was defined according to C-P classification.

Patients were staged by five systems: TNM 5, TNM 6 (Sobin and Fleming, 1997; Greene *et al*, 2002; UICC, 2002; Sobin *et al*, 2011), CLIP (Group, 1998), JIS or BCLC (Llovet *et al*, 2008), or by Milan criteria (Mazzaferro *et al*, 1996). However, for this analysis that focused on prognosis, we also grouped patients on the basis of whether or not the treatment received was curative or palliative. Curative treatments included transplantation, resection, radio-frequency ablation, and percutaneous ethanol injection. Palliative treatments included transarterial chemoembolisation, any form of chemotherapy, and supportive care. Where patients were listed for transplantation but had transarterial chemoembolisation as initial treatment as a 'bridge' to transplantation, they were classified as having potentially curative therapy. For the purpose of this analysis, UK patients who underwent liver transplantation were excluded, as the survival of this group would not be expected to be influenced by the baseline features included in the model (such as bilirubin and albumin).

Assays of AFP, AFP-L3%, and DCP. AFP, AFP-L3%, and DCP were all measured in the same serum sample. The measurements of hs-AFP-L3% and DCP were achieved by using a microchip capillary electrophoresis and liquid-phase binding assay on a μTASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd, Osaka, Japan) (Kagebayashi *et al*, 2009). Analytical sensitivity of μTASWako i30 is 0.3 ng ml^{-1} AFP, and the percentage of AFP-L3 can be measured when AFP-L3 is over 0.3 ng ml^{-1} (Kagebayashi *et al*, 2009).

Statistical methods

Discrimination. We assessed discriminatory performance using Harrell's *C*-statistic, as described by Taktak *et al* (2007). In brief, this measure reports the number of comparable pairs that are correctly ordered under the risk score. That is, for a pair of comparable patients P_A and P_B, if patient P_A is known to have survived beyond P_B's time of event (death here), then P_A should be subject to a lesser risk than P_B, that is, should be assigned a lower-risk group. This method counts all the correctly ordered pairs from those that are comparable.

Flexible parametric models. Regression analyses utilised flexible parametric models (Royston and Lambert, 2011) that enable fitting of more appropriate baseline hazard functions. The baseline hazard describes risk over time when all covariates take the value zero (rather than the hazard at time zero as sometimes stated), and is described by a restricted cubic spline function (Royston and Lambert, 2011). Here all continuous covariates are centred about their mean, and so the interpretation of the function is the hazard at the mean of all covariates. Traditionally, the baseline hazard is assumed to have a simple constant or monotonic form, as in