Table 1. AST and ALT Levels in Patients with a Sustained Virological Response to Interferon Therapy

	AST(IU/L)	ALT(IU/L)
All cases	19.7 ± 3.0 (17~23)	13.8 ± 3.1 (11~17)
Male	19.8 ± 3 .0 (17~23)	14.4 ± 3.2 (11~18)
Female	12.9 ± 2.9 (10~16)	9.9 ± 3.5 (6~23)
Age		
20y~ (n=12)	17.6 ± 2.9 (15~21)	12.6 ± 3.3 (9~16)
30y~ (n=19)	18.2 ± 2.9 (15~21)	13.4 ± 3.0 (10~16)
40y~ (n=14)	19.8 ± 2.8 (17~23)	14.2 ± 3.5 (11~18)
50y~ (n=34)	20.3 ± 3.5 (17~24)	14.4 ± 2.8 (12~17)
60y~ (n=47)	20.8 ± 2.8 (18~24)	13.9 ± 3.0 (11~17)
70y~ (n=10)	19.2 ± 3.5 (16~23)	13.5 ± 3.8 (10~17)

ALT, alanine aminotransferase ; AST, aspartate aminotransferase
Data expressed as mean ± standard deviation (range)

31.2), and serum total cholesterol, triglyceride and fasting glucose levels were 165.9±30.9 mg/dL (range 82-271), 111.8±58.6 mg/dL (range 39-384), 94±20.8 mg/dL (range 65-258), respectively. Liver histology was F1; 20 cases, F2; 39 cases, F3; 43 cases, F4; 5 cases.

Patients who have fatty liver on ultrasound examination and alcohol intake over 160 g a day and are positive for HBs antigen, autoimmune hepatitis and primary biliary cirrhosis were excluded.

Measurement of AST and ALT

Serial three times of AST and ALT levels were measured every 3 to 4 months over one year after completion of interferon therapy in each patient because we often experience that ALT levels does not normalize soon after HCV RNA becomes negative. Those AST and ALT levels were individually averaged, and then were totally averaged.

Statistical analysis

Fischer's exact tests were used for analysis of ALT and BMI values between groups. A p value of less than 0.05 was regarded as significant.

Results

AST and ALT levels in patients with a sustained virological response to interferon therapy

Overall, AST levels were 19.7±3 IU/L and ALT levels were 13.8±3.1 IU/L, respectively. In male patients, AST and ALT levels were 19.8±3 IU/L and 14.4±3.2 IU/L and in female patients, 12.9±2.9 IU/L and 9.9±3.5 IU/L, respectively. AST level was the highest in the 6th decade and ALT level in the 5th decade (Table 1).

The change of between pre- and post-treatment liver histology in six patients was as follows; F1 to F1, F2 to F1, F2 to F2, F3 to F1, F3 to F2, F4 to F4, respectively.

Distribution of AST and ALT levels in patients with a sustained virological response to interferon therapy

The distribution of serum AST and ALT levels showed normal distribution (Figs. 1, 2).

Discussion

In general check-ups, the serum ALT level is the most commonly used as a laboratory parameter to evaluate the response to various liver medications such as interferon therapy, and for evaluation and follow-up of liver diseases, particularly with hepatitis B and hepatitis C (3, 4). In addition, serum ALT is a surrogate marker for the diagnosis of patients with non-alcoholic fatty liver disease (NAFLD) which is the most common cause of elevated serum ALT levels in otherwise serologically negative patients (5, 6) because of the absence of proper screening tools for NAFLD.

The normal range for serum ALT level was set in the 1950s by Karmen et al. (3) and has changed little since then. Current ULN were set, on average, at 30 to 50 U/L in studies conducted over the past 10 years (7-12); however, normal values may vary greatly among laboratories. This was recently challenged by a research group, who claimed that the true normal values are significantly lower than those listed by kit manufactures, and that an accepted, reliable ULN is needed (4, 13). No such ULN has yet been established in a large-scale population-based study.

Recent studies have shown that serum ALT level can be modulated by a number of factors including age, gender, BMI, fasting blood glucose, and serum triglyceride levels (13, 14). These factors are usually not taken into account when the normal ALT range is determined. Prati and colleagues found that in both men and women, ALT levels correlated strongly with BMI and correlated less robustly with serum triglyceride levels (4). ALT levels correlated directly with cholesterol levels in men and with glucose level and the use of medications, and particularly birth control pills, in women. Next, the authors calculated "healthy" ranges for serum ALT levels in 3,927 donors who had a normal MBI and normal serum cholesterol, triglyceride, and glucose levels and who were not taking medications. The ULN for ALT levels decreased from 40 U/L to 30 U/L in men and from 30 U/L to 19 U/L in women.While other studies suggested new ULN for ALT in a very selected population such as blood donors and the general population (4, 13).

In contrast, our study is unique in its population who achieved SVR to interferon therapy. We often experience that serum ALT level after achieving SVR decreases to almost <20 to 25 IU/L, so we attempted to re-evaluate the suitable ULN for serum ALT in CH-C patients. The results showed that the ULN for serum ALT is 25 U/L which is significantly lower than that listed by the manufacturer of the biochemical test for ALT.

We studied the normal range of ALT levels in 485 age- and sex-matched healthy volunteers, 300 males and 185 fe-

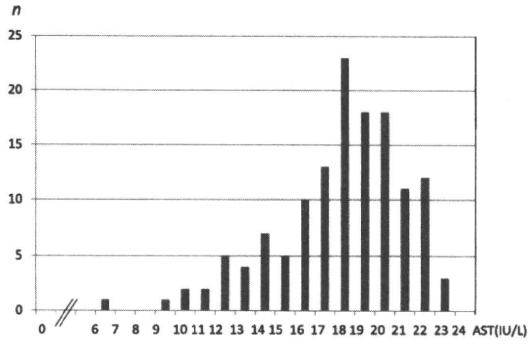

Figure 1. Distribution of AST levels in patients with a sustained virological response to interferon therapy.

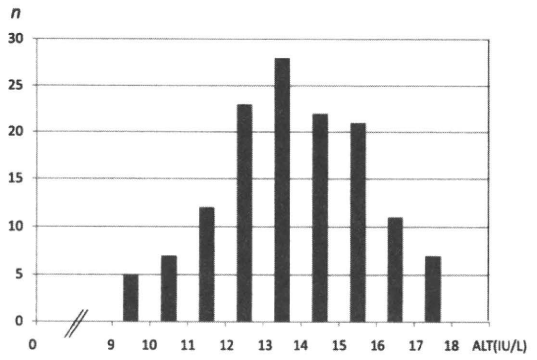

Figure 2. Distribution of ALT levels in patients with a sustained virological response to interferon therapy.

Table 2. Comparison of ALT and BMI Levels between SVR Cases and Healthy Volunteers

	SVR cases			Healthy volunteers		
	n	ALT(IU/L)	BMI	*n*	ALT(IU/L)	BMI
All cases	136	13.8±3.1	22.8±3.4	485	18.2±10.5	22.7±3.1
Male	84	14.4±3.2	23.2±3.4	300	18.6±24.4	23.2±2.8
Female	52	9.9±3.5	23.1±3.8	185	19.5±12.2	22.1±3.3
Age						
20y~	12	12.6±3.3	21.1±1.7	33	13.0±4.9	22.0±2.8
30y~	19	13.4±3.0	23.1±4.2	52	19.1±10.4	23.1±3.2
40y~	14	14.2±3.5	23.2±4.2	39	17.5±11.1	21.8±4.2
50y~	34	14.4±2.8	23.1±3.1	94	20.1±9.7	22.7±2.8
60y~	47	13.9±3.0 *	22.5±3.0	129	20.0±12.6 *	22.9±2.7
70y~	10	13.5±3.8	22.5±2.8	28	12.6±1.4	22.9±2.6

SVR, sustained virological response ; BMI, body mass index; ALT, alanine aminotransferase AST, aspartate aminotransferase. Data expressed as mean ± standard deviation, * $p<0.05$

males, mean age 54.2±9.0 years using 95% percentile, and resulted in 18.2±10.5 IU/L (Table 2). Their BMI was 22.7± 3.1 which was the almost same as 22.8±3.2 in our populations and ALT levels were slightly higher than 13.8±3.1 IU/L in our populations but the difference was not significant (p=0.0504). The reasons are not clear as to why ALT values are slightly higher in healthy volunteers than our populations in each decade, although it is a statistical significant difference, between two groups is recognized in only the 6th decade. It does not seem that fatty liver and alcohol intake have an influence on ALT levels because mean BMI did not different between both groups and patients who drink alcohol over 160 g a day were excluded in both groups. Regarding ALT levels in another cohort of 366 CH-C patients in our hospital, 12 patients (3.3%) had less than 20 IU/L and of which the findings of liver histology of 10 patients were as follows; F0A0; one patient, F1A0; 8 patients and F2A2; one patient.

Thus, this new ULN is useful to consider the indication of the interferon therapy in CH-C with a near normal level of ALT and also to identify whether other causes such as alcohol intake or fatty liver have an influence on the slight elevation of ALT level after achieving SVR.

The major limitations of our study are the method of subject inclusion and the relatively small subject population. However, we obtained significant and clinically useful findings in this study despite these limitations, thus indictating the need for a larger scale evaluation on this issue.

Conclusion

The present study demonstrated that the currently accepted ULN for serum AST and ALT levels are too high. Our new ULN is significantly lower than that given by the manufacturer of the laboratory test currently used and is useful to consider the indication of interferon therapy in CH-C with a near normal level of ALT level. Further studies are needed to evaluate the clinical significance of this finding.

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HCV治療の有効性とIL28B（インターフェロンλ）

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ヒト染色体19番染色体上にコードされているIL28B（インターフェロンλ）遺伝子近傍に存在するSNPs（rs8099917）は，C型慢性肝炎に対するペグインターフェロン・リバビリン併用療法の治療効果を規定するきわめて強力な宿主側因子である．これに加えて，ウイルス側因子の1つであるコア領域70番アミノ酸変異の有無を測定することにより，一層精度の高い治療効果予測が治療開始前に可能となる．さらに，貧血などの有害事象発現に関連するSNPsも徐々に解明されつつあることから，C型慢性肝炎治療において，より個別化されたテーラーメイド医療が実現しつつある．

Ⅰ．C型慢性肝炎治療の現状と治療効果予測因子の重要性

厚生労働省の推計では，わが国には約300～370万人の肝炎ウイルスキャリアが存在し，うち，190～230万人がC型肝炎ウイルス感染者であり，その中で約28万人が慢性肝炎，約9万人が肝硬変・肝臓癌の段階に進行していると見積もられている．C型慢性肝炎に対する現在の標準的治療法はペグインターフェロン・リバビリン併用療法であるが，日本人に最も多いジェノタイプ1型・高ウイルス量の症例（いわゆる難治群）における著効率は一年間投与しても約50％に留まっている[1]．しかも，全治療期間が48～72週間ときわめて長期にわたり，また，特に高齢者ではさまざまな副作用により減量・中断を余儀なくされる治療法である．したがって，治療開始前における治療効果予測がきわめて重要と考えられる．

これまで，その治療効果予測因子として，ウイルス型，ウイルス量，コア領域やNS5A領域のアミノ酸変異などのウイルス側因子に加えて，ペグインターフェロン，リバビリンのアドヒアランス（薬剤因子），年齢，性別，肝線維化進展度，インスリン抵抗性などの宿主側因子の重要性が多数報告されているが，それらの因子を総動員して解析しても治療前効果予測は約60％に留まっていた[2]．

Ⅱ．SNPによるC型慢性肝炎の治療効果予測

一方，2003年のヒトゲノムプロジェクトの成功により，ヒト遺伝子は個人差として約300個に一個，すなわち全体では約1,000万個の遺伝子変異（Single nucleotide polymorphism：SNP）が存在し，このSNPが表現型（外見や性格の違い），各種疾病における病態の相違のみならず，個々の薬剤反応性の強弱や副作用にも大きく関与することが続々と明らかにされている．近年，ゲノムワイドに均一に配置された90万箇所（日本人では62万箇所）のSNPsを一括タイピング（Genome-wide association study：GWAS）することが可能になり[3]，病態進展に多因子が関与すると想定されてきたⅡ型糖尿病[4]，脳血管障害[5]，B型慢性肝炎[6]などにおいて疾患感受性遺伝子の同定が矢継ぎ早に報告されている．

C型肝炎についても米国，オーストラリア，日本，スイスから，自然治癒やペグインターフェロン・リバビリン併用療法への治療反応性に関与するSNPsについての報告が2009年9月以降，立て続けにNatureやNature Genetics，Gastroenterologyなどの一流医学雑誌に報告されている[7～11]．これらSNPs（日本・オーストラリアではrs8099917，欧米ではrs12979860）は第19番染色体上にコードされているIL28B遺伝子の上流（各々～8kb，～3kb）に存在することが明らかとなった．また，リバビリンに起因する貧血の発現は患者の日常生活に大きく影響し，かつ，リバビリンの減量・休薬はアドヒアランスの低下を招き，治療効果を悪化させる．この貧血の発現に関与するSNPs（rs1127354，rs7270101）が第20番染色体上に存在し，inosine triphosphatase（ITPA）遺伝子に関連することが2010年2月にNature電子版[12]に報告されるなど，C型慢性肝炎の治療効果を左右する遺伝子群が宿主側因子として全世界の注目を浴びているところである．

我々は，併用療法を受けた日本人C型慢性肝炎1型患者を，治療成績から「無効群」と「有効群（著効＋再

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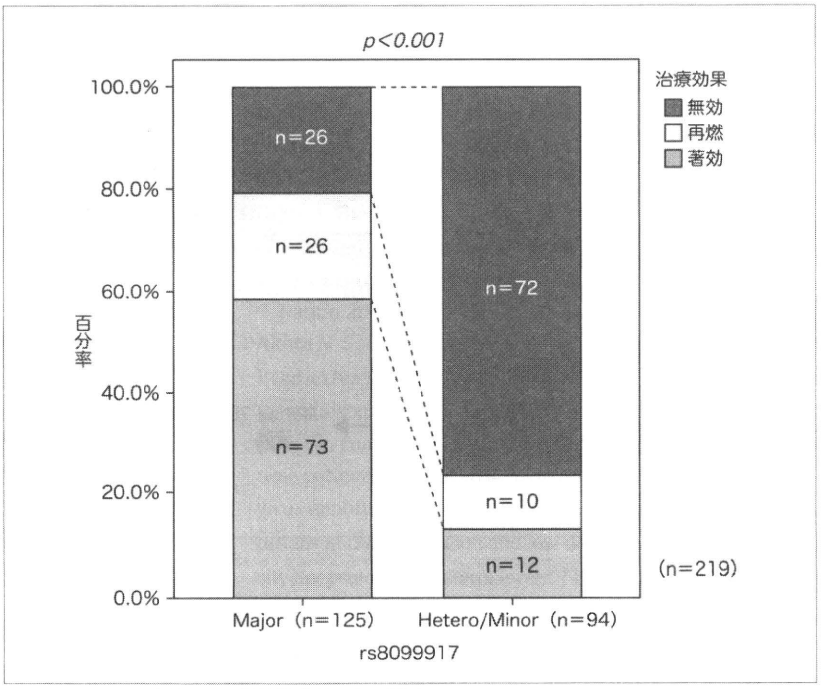

図1　IL28B SNPsとウイルス学的治療効果との関連

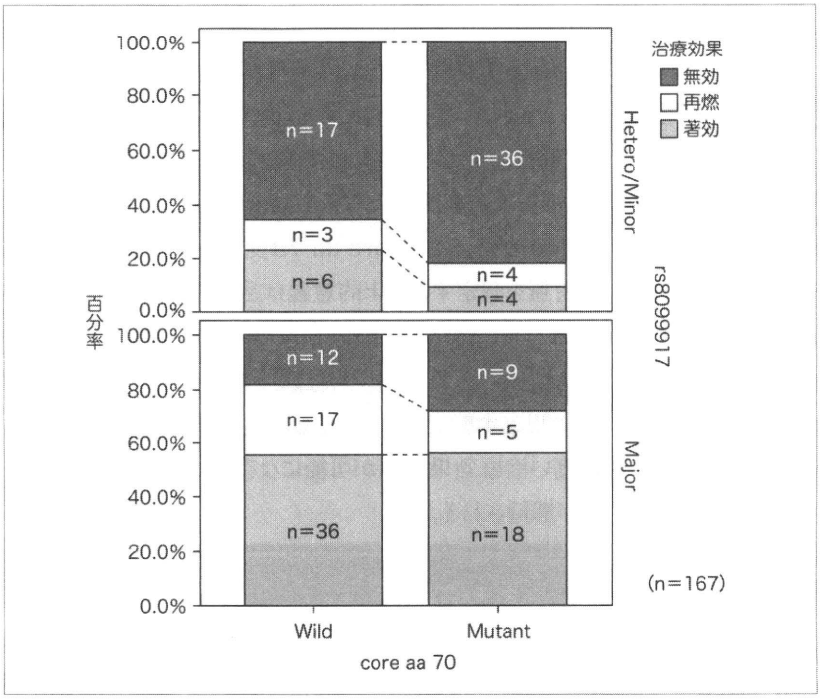

図2　IL28B SNPsとウイルス学的治療効果との関連～core aa 70変異の影響～

燃)」の2群に分けてGWASを行ったところ，IL28B遺伝子周辺に治療無効に関連する有意なSNPsを発見した[10]．その代表的なSNPであるrs8099917（マイナーアリルG）をもつ患者は危険率が約27倍（$P=2.68\times10^{-32}$）で無効となることを見出した．さらに，IL28B遺伝子を含む15.7 kb内にrs8099917と連鎖不平衡の関係にあるSNPsが少なくとも6個存在し，ハプロタイプ解析から，マイナーアリル（リスクアリル）を有する場合に治療無効となるオッズ比は11.1（$P=1.35\times10^{-25}$）にもなった．これまで治療効果に影響するとされてきた年齢，性別，血小板数，治療歴，ALT値，線維化スコア，ウイルス量を加えた多変量解析

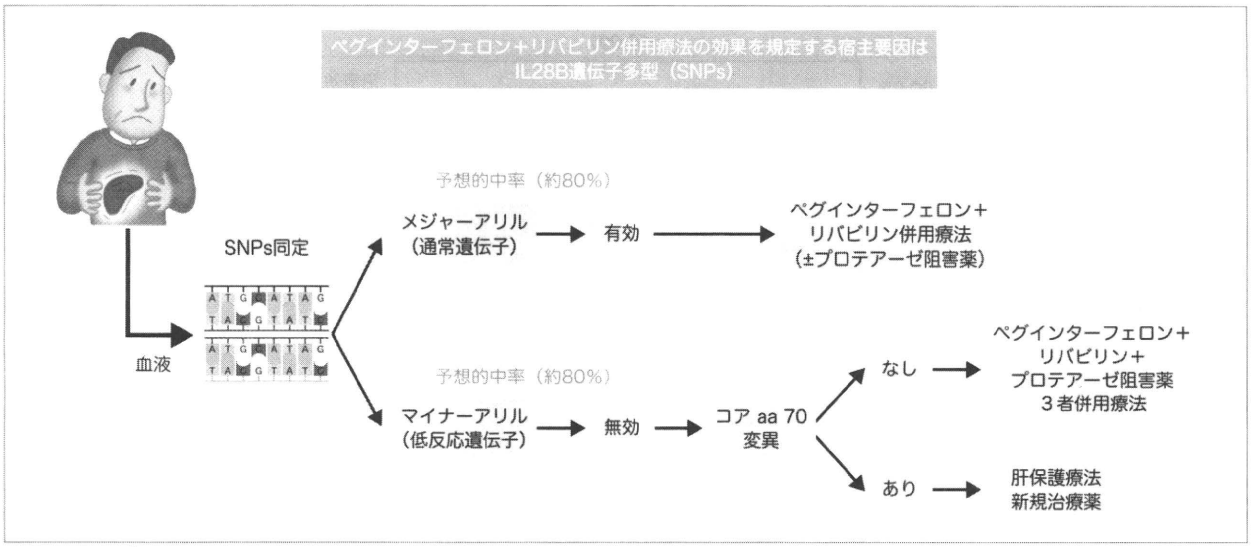

図3　これからのC型慢性肝炎治療のあり方

の結果，rs8099917（マイナーアリルG）と性別（女性）の2つの因子のみが最終的に選択された．

肝炎・免疫研究センターでは治療効果を規定するIL28B SNPs（rs8099917）の臨床的意義を，全く別のC型肝炎患者を対象として検証した．まず，IL28B SNPsの遺伝子型（Major homoかHetero/Minor homoか）とウイルス学的治療効果（著効，再燃，無効）との関連を，ペグインターフェロン・リバビリンのアドヒアランスが80％以上の患者219例でみたところ（図1），Major homoの患者では著効58.4％，再燃20.8％，無効20.8％で有効（著効＋再燃）率が約80％であったのに対し，Hetero/Minor homoの患者では各々，12.8％，10.6％，76.6％と無効率が約80％と逆転していた（P＜0.001）．さらに，ウイルス側因子として治療効果に関与することが報告[13]されているコア領域70番アミノ酸（core aa 70）の変異を測定することができた167例において詳細に検討したところ（図2），core aa 70変異の有無はIL28B SNPがMajor homoの場合には全く影響しないが，IL28B SNPがHetero/Minor homoの場合には，core aa 70に変異があると著効率が23.1％から9.1％に低下する（有効率では34.6％から18.2％に低下する）傾向を認めた（症例数が少ないため有意差はなし）．したがって，IL28B SNPがMajor homoの場合にはcore aa 70変異の有無を測定する臨床的意義は乏しいが，Hetero/Minor homoの場合にはcore aa 70変異の有無を治療前に測定することにより，さらに精度の高い治療効果予測が可能になることが期待される．

III．これからのC型慢性肝炎治療

現時点で我々が提唱しているC型慢性肝炎治療のあり方を図3に示した．

IL28BはIFNλ3（ラムダ）ともよばれ，類似の構造をもつIL28A（IFNλ2），IL29（IFNλ1）とともに，すでに臨床応用されているIFNαとは異なるレセプターを介してインターフェロン・シグナルを伝達し抗ウイルス活性を惹起することが知られている．すでにペグ化IFNλ1とリバビリンを併用する第II相臨床治験が米国で開始されており，副作用が少なく良好なウイルス低下作用を有することが報告されている[14]．GWASにより導き出されたこれらの研究成果の臨床応用がさらに進めば，高齢化した難治例が多数残されているわが国のC型慢性肝炎治療において，より個別化されたテーラーメイド医療の実現が可能になるものと期待される．

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肝臓　50巻12号　738-740（2009）

＜速　報＞

前インターフェロン不応C型慢性肝炎に対する二重濾過血漿交換併用ペグインターフェロン・リバビリン療法の初期効果
—第1報—

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緒言：ペグインターフェロン・リバビリン（PEG・Rib）療法により，Genotype 1b・高ウイルス量の難治性C型慢性肝炎の著効率は改善したが約50％は未だにC型肝炎ウイルス（HCV）の駆除が得られない．この治療抵抗例にはISDR[1)]やCoreの変異と脂質代謝が関与する[2)]ことが最近判明してきた．一方，2007年4月からGenotype 1b・高ウイルス量のC型慢性肝炎に対し二重濾過血漿交換[3)]（Double Filtration Plasmapheresis；DFPP）が保険適応になった．今回我々は，以前インターフェロン治療（IFN）を行ったが一度もHCV陰性化が得られなかった無効例に対し，DFPPとPEG・Ribα2bを併用して治療を行ったので，安全性と初期効果，脂質の変化につき検討した．

対象と方法：対象は，前治療IFN無効3例と前治療PEG・Rib無効6例であり，ISDR変異0が7例・1が1例・3が1例，Core 70番変異なし（wild）3例・変異あり（mutant）6例，Core 91番wild 4例・mutant 5例の全9例である（図中1～9）．DFPPを第1週目に3回，2週目に2回行った．DFPPは一次膜に旭化成クラレメディカル社プラズマフローOP，二次膜にカスケードフローEC-50Wを使用し血漿処理50 mL/kgを目標とした．初回DFPP直後にPEGを注射しRib内服を開始，4回目DFPP直後にPEG 2回目注射を行った．PEGα2a・Rib投与中HCV RNA再上昇の1症例（症例3）のみ途中22週でDFPPを併用した．DFPP前，DFPP開始後2週目，以後1カ月ごとにPEG注射日の早朝空腹でHCV RNA（リアルタイム法）・TG・T-Chol・LDL-Chol・HDL-Cholの測定を行った．

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＜受付日2009年9月1日＞＜採択日2009年11月26日＞

成績：9例ともにDFPP中の副作用は認められず，DFPP直後にPEGを注射することによる副作用の増強も認められなかった．2009年8月末現在，DFPP開始後のPEG・Rib継続期間は症例1から9の順に各々50週，42週，36週，26週，24週，23週，14週，10週，6週である．このうち，症例1，3，4，5，7はDFPP後，各々21週，20週，5週，4週，7週でHCV RNAが陰性化し以後維持している．一方，脂質の変化をみてみると，HCV RNAが消失した症例1，3，4，5，7の5例中TGは5例全例，T-Cholは4例，LDL-Cholは3例でDFPP開始前値より上昇する傾向にあったが，HCV RNAが陰性化していない症例2，6，8，9の4例では上昇していなかった（Fig. 1）．

考案：まずDFPPとPEG・Rib併用に伴う副作用は認められず併用は安全性に施行できると思われた．一方，ISDR変異0または1，Core 70番mutant，Core 91番mutantの症例はPEG・Ribを行っても非常に難治とされ[1)]，今回の9症例は前治療IFN無効および前治療PEG・Rib無効を反映した難治例の集団であった．しかし，9例中5例で24週以内にHCV RNAが陰性化した．特に，症例3，7はISDR変異0・1かつCore double mutantの極めて難治症例とされているにも関わらずHCV RNAの陰性化が認められた．一方，PEG・Rib終了後HCV RNA陰性継続例のみがTG，T-Chol，LDL-Cholが治療終了後に前値より上昇する[5)]と報告がある．DFPPを加えた今回の検討では，DFPPで機械的に除去されて2週間後は一旦低下するものの，HCV RNA消失例ではPEG・Rib終了を待たずにTG，T-Chol，LDL-Cholが上昇していた．DFPP2次膜の穴は約30 nmで物理的にHCVを捕らえるが，HCVはLDLに結合していることから両者はDFPPで同時に除去されていると推測される．DFPPによりHCVの機械的除去と共に，治療早期に脂質代謝が改善されてPEG・Ribが利きやすい環境に変化しているとも推測された．

HCV RNA negative cases

Case	Sex	Age	Prior IFN (effect)	ISDR mutation	Core70 mutation	Core91 mutation
1	F	69	IFN α 2b (NR)	3	wild	mutant
3※	F	44	PEG α 2a・Rib (NR)	0	mutant	mutant
4	F	70	PEG α 2b・Rib (NR)	0	wild	wild
5	F	37	IFN α (NR)	0	mutant	wild
7	F	61	IFN α 2b (NR)	1	mutant	mutant

※DFPP was done at 22 week
(HCV RNA was 6.7 at the start of PEG・Rib)

HCV RNA positive cases

Case	Sex	Age	Prior IFN (effect)	ISDR mutation	Core70 mutation	Core91 mutation
2	F	52	PEG α 2b・Rib (NR)	0	mutant	mutant
6	M	57	PEG α 2b・Rib (NR)	0	mutant	wild
8	F	60	PEG α 2b・Rib (NR)	0	wild	mutant
9	M	74	PEG α 2b・Rib (NR)	0	mutant	wild

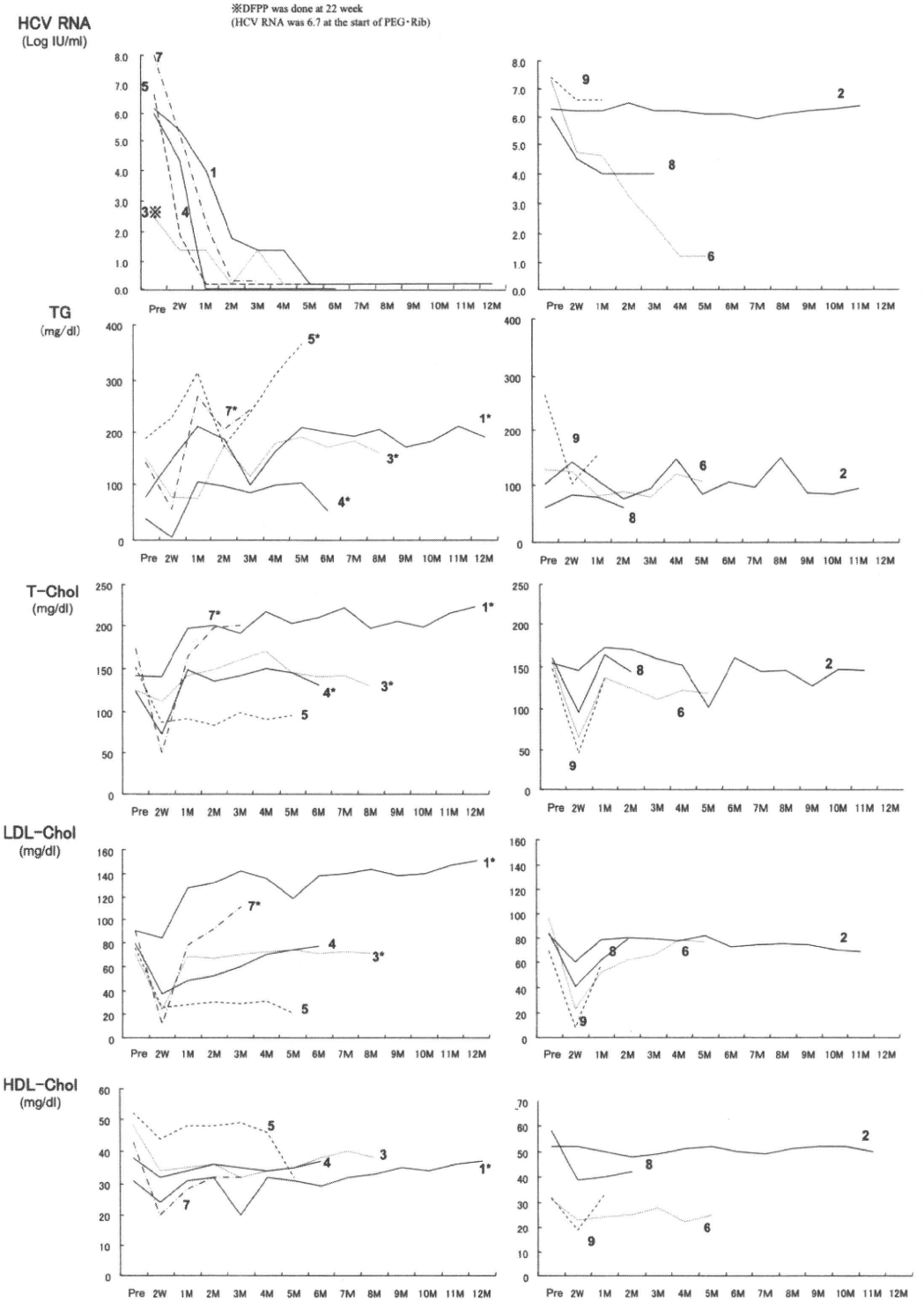

Fig. 1 Change in triglyceride (TG), total cholesterol (T-Chol), LDL-Chol and HDL-Chol levels during double filtration plasmapheresis (DFPP) and peginterferon plus ribavirin (Peg・Rib) combination therapy. *Levels increased after DFPP and higher than those before treatment

索引用語：C型慢性肝炎，二重濾過血漿交換，
ペグインターフェロン・リバビリン療法

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英文要旨

Double filtration plasmapheresis and peginterferon plus ribavirin combination therapy for chronic hepatitis C patients non-responded by previous interferon therapy

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We investigated lipid metabolism in nine patients with chronic hepatitis C virus (HCV), not responded by previous interferon therapy (IFN), undergoing double filtration plasmapheresis (DFPP) and peginterferon plus ribavirin (PEG・Rib) combination therapy. Three patients were non-responder of previous IFN monotherapy and 6 were PEG・Rib. HCV RNA became negative within 24 weeks in 5 out of 9. In the HCV RNA negative group, Triglyceride (TG) and Total-Cholesterol (T-Chol) or LDL-Chol levels increased gradiently after DFPP and were higher than those before treatment, but not in HCV positive group. DFPP plus PEG・Rib combination therapy might not only produce a reduction of HCV but also improve the environment of lipid metabolism effective for PEG・Rib during the early stage of treatment.

Key words: chronic hepatitis C,
double filtration plasmapheresis,
peginterferon plus ribavirin therapy

Kanzo 2009; 50: 738—740

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Differences in prognostic factors according to viral status in patients with hepatocellular carcinoma

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Received October 23, 2009; Accepted February 6, 2010

DOI: 10.3892/or_00000766

Abstract. The number and ratio of both HBsAg- and HCV Ab-negative hepatocellular carcinoma (HCC-nonBC) cases have been steadily increasing in Japan. The aim of this study was to examine the frequency of detection of HCC-nonBC by screening methods and to elucidate the clinical characteristics of HCC-nonBC compared with those of hepatitis C and/or B virus-associated HCC (HCC-virus). We recruited 624 patients with HCC who were diagnosed between 1982 and 2007 at the Department of Gastroenterology and Hepatology, Nagasaki University Hospital. They were categorized into 2 groups as follows: i) 550 were included in the HCC-virus group: positive for HBsAg and/or positive for HCV Ab, and ii) 74 were included in the HCC-nonBC group: negative for both HBsAg and HCV Ab. The follow-up patterns until the initial detection of HCC and the survival rates were analyzed and compared between the 2 groups. Multivariate analysis identified follow-up, alcohol consumption, albumin level, total bilirubin level, α-fetoprotein (AFP) level, and tumor-node-metastasis (TNM) stage as independent and significant risk factors for prognosis. Among the 397 patients with HCC in TNM stage I or II, multivariate analysis identified the cause of liver disease, gender, Child-Pugh score, serum albumin level and TNM stage as independent and significant risk factors for prognosis. We reported that the poor prognoses of patients with HCC-nonBC were attributable to its late detection in an advanced condition due to the absence of a surveillance system for the early detection of HCC. However, in early-stage patients, patients with HCC-nonBC showed significantly better prognosis than those in the HCC-virus group.

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Key words: hepatocellular carcinoma, viral hepatitis

Introduction

Primary liver cancer is the most common cancer of the liver, accounting for approximately 6% of all human cancers. It is estimated that half a million cases of this disease occur worldwide each year, making primary liver cancer the fifth most common malignancy in men and the ninth in women (1-6). Hepatocellular carcinoma (HCC) accounts for 85 to 90% of primary liver cancers, (7) and the age-adjusted HCC mortality rate has increased over the past few decades in Japan (8). Similarly, a trend in increasing incidence rates of HCC has been reported for several developed countries in North America, Europe and Asia (9,10). HCC often develops in patients with liver cirrhosis caused by hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption or nonalcoholic fatty liver disease. HCV is the predominant causative agent of HCC in Japan (11-14). However, it has been reported that the number and ratio of both HBsAg- and HCV Ab-negative HCC (HCC-nonBC) have been steadily increasing in Japan (15,16).

The prognosis for patients with HCC is still poor. Surgical resection and liver transplantation are the standard treatment methods available. Radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) have recently been recognized as effective methods of achieving complete tumor necrosis in small HCCs (17); however, the chances of curative treatment are often limited by several features of HCC. HCCs usually grow to a large size before symptom manifestation. Bilobar or multifocal tumors are common, and the incidence of associated cirrhosis is high, being over 80% in most cases (18-20). Transcatheter arterial chemoembolization (TACE), which is considered to be an ineffective method of achieving complete necrosis of HCCs, also depends on the above factors (21). Early detection of HCC by α-fetoprotein (AFP) and/or imaging screening has been implemented in many countries to increase the chances of successful intervention and to improve survival (22-26).

The aim of this study was to examine the frequency of detection of HCC-nonBC by screening methods and elucidate the differences in the clinical characteristics between non-B, non-C HCC and hepatitis C and/or B virus-associated HCC (HCC-virus).

Patients and methods

Patients and study groups. We recruited 624 patients with HCC who were diagnosed between January, 1982 and December, 2007 at the Department of Gastroenterology and Hepatology, Nagasaki University Hospital. Informed consent was obtained from all patients. The diagnosis of HCC was based on AFP levels; results of imaging techniques such as ultrasonography (USG), computerized tomography (CT), magnetic resonance imaging (MRI) and hepatic angiography (HAG); and/or liver biopsy. The diagnostic criteria included characteristic liver biopsy findings, elevated AFP (≥20 ng/ml) and neovascularization on HAG and/or CT.

Sera were stored at -80˚C until they were used for the following assays. The diagnosis of chronic hepatitis C virus (HCV) infection was based on the presence of HCV Ab (microparticle enzyme immunoassay; Abbott Laboratories) and HCV RNA as detected by polymerase chain reaction. The diagnosis of chronic HBV infection was based on the presence of HBsAg (enzyme-linked immunosorbent assay; Abbott Laboratories); the serum AFP level was measured by radioimmunoassay (Abbott Laboratories). The history of alcohol intake was noted from medical records; habitual drinking was defined as an average daily consumption of an amount equivalent to 80 g of pure ethanol for a period of more than 10 years.

The patients were categorized into 2 groups as follows: i) HCC-virus group (550) comprising patients positive for HBsAg and/or positive for HCV Ab and ii) HCC-nonBC group (74) comprising patients negative for both HBsAg and HCV Ab. We analyzed and compared the 2 groups for age distribution, gender ratio, body-mass index, alcohol intake, serum AFP level, tumor-node metastasis (TNM) stage of hepatocellular carcinoma tumors at the time of initial detection, Child-Pugh score, follow-up pattern until the initial detection of HCC and the survival rates.

Follow-up. All patients were categorized into 2 groups: the follow-up group included 365 (58%) patients with subclinical HCC diagnosed by screening; the non-follow-up group consisted of 259 (42%) patients who were diagnosed at our hospital owing to the appearance of symptoms indicative of HCC. AFP levels and liver function were assessed every 3 to 6 months, and USG or CT imaging was performed every 3 to 12 months over a period of at least 12 months prior to the diagnosis of HCC in patients of the follow-up group. The non-follow-up group patients presented with clinical symptoms such as abdominal pain, discomfort, nausea or weight loss which led to the evaluation and diagnosis of HCC.

Treatment modalities. Patients diagnosed with HCC were assessed for surgery on the basis of the extent of lobar involvement and liver function status. The extent of lobar involvement was evaluated by a combination of USG, CT, MRI and HAG. Patients were considered unfit for resection when they met the following criteria: i) bilobar involvement, ii) evidence of tumor infiltration into the main portal vein or thrombosis of the vein, iii) evidence of extrahepatic metastases, iv) Child's grade C cirrhosis or v) poor cardiac and respiratory statuses. If the patients were deemed unfit for operation or refused to undergo operation, PEI therapy was the second choice of treatment offered to such patients with HCCs <3 cm in diameter. The remaining patients without main portal vein thrombosis or extrahepatic metastasis were advised to undergo TACE irrespective of the size and number of tumors.

After initial treatment, AFP levels and liver function of the patients were assessed every 1 to 3 months, and USG imaging was performed every 3 to 6 months during the follow-up period. Patients suspected to have HCC recurrence were further evaluated by CT and/or MRI. The assessment of treatment for recurrent HCC was based on lobar involvement and liver function status as described for the initial treatment. RFA or liver transplantation to treat HCC was started at our institution in 2002; none of the patients were treated by these methods between 1982 and 2001. Furthermore, none of the subjects in our study received either of these treatments for recurrent HCC during the follow-up period.

Statistical analysis. The time of survival was measured from the time of the diagnosis of HCC to the time of death or until the time of preparation of the manuscript. The data were analyzed by the Mann-Whitney test for continuous ordinal data, and the Chi-square test with Yates' correction and Fisher's exact test were performed for intergroup comparisons to determine the association between 2 qualitative variables. The survival rate was analyzed using the Kaplan-Meier method, and the differences between the survival probability curves were tested using the log-rank test. The independent risk factors associated with the rate of survival were estimated by the non-time-dependent stepwise Cox regression analysis. The standard error was calculated based on the binomial model to estimate the response rate. A value $P<0.05$ was considered statistically significant. Data analysis was performed with SPSS version 16.0 software for Windows.

Results

Patient characteristics at enrollment. We diagnosed 624 patients with HCC during the study period. Patient characteristics at the time of diagnosis of HCC are presented in Table I. The underlying causes of HCC were determined to be as follows: 120 (19%) patients were positive for HBsAg, 411 (66%) were positive for HCV Ab, 19 (3%) were positive for both HBsAg and HCV Ab and 74 (12%) were negative for HBsAg and anti-HCV.

Comparison of clinical characteristics and survival between patients with and without hepatitis virus infection. The patients were divided into 2 groups: the HCC-nonBC group (74 patients) and the HCC-virus group (550 patients); the characteristics of each group were compared (Table I). There were no significant differences in gender, BMI, Child-Pugh score, prothrombin time, or albumin and total bilirubin levels. However, there were significant differences between the 2 groups in terms of median age ($P=0.001$), habitual drinkers ($P=0.015$), TNM stage ($P=0.030$), AFP ($P=0.002$) and follow-up group ($P=0.010$). The HCC-nonBC group had a lower proportion of patients who were followed up when compared to those of the HCC-virus group.

Table II indicates the results of univariate and multivariate analyses of the prognosis factors for HCC using the

Table I. Comparison between HCC patients with and without virus infection.

	All patients		HCC-nonBC		HCC-virus		P-value
Total	624		74		550		
Median age, years	65	(13)	70	(6)	64	(12)	0.001
Gender (%)							
Male	478	(77)	54	(73)	424	(77)	
Female	146	(23)	20	(27)	126	(23)	NS
BMI	22.4	(4.2)	23.1	(6.0)	22.3	(4.8)	NS
Alcohol consumption (%)							
Not excessive	497	(80)	51	(69)	446	(81)	
Excessive	127	(20)	23	(31)	104	(19)	0.015
Follow-up (%)							
Follow-up group	365	(58)	33	(45)	332	(60)	
Non-follow-up group	259	(42)	41	(55)	218	(40)	0.010
Child-Pugh score	6	(1)	5	(2)	6	(2)	NS
Hepatitis virus							
HBsAg (+)/HCV Ab (-)	120	(19)	0	(0)	120	(22)	
HBsAg (-)/HCV Ab (+)	411	(66)	0	(0)	411	(75)	
HBsAg (+)/HCV Ab (+)	19	(3)	0	(0)	19	(3)	
HBsAg (-)/HCV Ab (-)	74	(12)	74	(100)	0	(0)	-
TNM stage (%)							
I	158	(25)	11	(15)	147	(27)	
II	239	(38)	30	(40)	209	(39)	
III	142	(23)	20	(27)	122	(22)	
IV	85	(14)	13	(18)	72	(12)	0.030
Laboratory data							
Albumin (g/dl)	3.7	(0.8)	3.8	(0.9)	3.7	(0.8)	NS
Prothrombin time (%)	80	(22)	85	(22)	80	(22)	NS
Total bilirubin (mg/dl)	1.0	(0.8)	0.9	(0.7)	1.0	(0.8)	NS
AFP (ng/ml)	51	(446)	16	(290)	59	(452)	0.002

Data are median (IQR) or frequency (%). NS, not significant.

Cox proportional hazards model. Univariate analysis revealed that 9 of 12 factors (male, excessive alcohol intake, Child-Pugh score ≥7, albumin <3.7 g/dl, prothrombin time <80%, total bilirubin ≥1.1 mg/dl, AFP ≥52 ng/ml, TNM stage III or IV, and the follow-up group) significantly affected the survival rate in patients with HCC. Multivariate analysis identified follow-up (follow-up group, relative risk 0.71), alcohol consumption (excessive drinker, relative risk 1.32), albumin (<3.7 g/dl, relative risk 1.37), total bilirubin (≥1.1 mg/dl, relative risk 1.53), AFP (≥52 ng/ml, relative risk 1.44), and TNM stage (III or IV, relative risk 2.50), as independent and significant risk factors (P=0.002, 0.043, 0.046, <0.001, 0.001 and <0.001, respectively) for prognosis.

Comparison of clinical characteristics and survival between patients with and without hepatitis virus infection in those patients with TNM stage I or II. Characteristics of patients with TNM stage I or II at the time of HCC diagnosis are presented in Table III. No significant differences were observed in gender, habitual drinkers, BMI, TNM stage, prothrombin time, or total bilirubin level. However, there were significant differences in the median age (P<0.001), Child-Pugh score (P=0.012), albumin level (P=0.009), AFP (P<0.001) and follow-up group (P=0.010).

Table IV indicates the results of univariate and multivariate analyses of the prognosis factors for HCC using the Cox proportional hazards model. Univariate analysis revealed that 6 of 12 factors (male, Child-Pugh score ≥7, albumin <3.7 g/dl, AFP ≥52 ng/ml, TNM stage II and HCC-nonBC) significantly affected the survival rate in HCC patients. Multivariate analysis identified HCC-nonBC (HCC-nonBC, relative risk 0.55), gender (male, relative risk 1.58), Child-Pugh score (≥7, relative risk 1.47), albumin (<3.8 g/dl, relative risk 1.62) and TNM stage (stage II, relative risk

Table II. Univariate and multivariate analyses of prognostic factors for HCC in the 624 patients.

Variable		Univariate analysis		Multivariate analysis	
		P-value	Relative risk (95% CI)	P-value	Relative risk (95% CI)
Age (years)	≥65	0.058	0.82 (0.67-1.01)		
Gender	Male	0.003[a]	1.46 (1.14-1.88)	0.800	1.28 (0.97-1.68)
BMI	≥25	0.177	0.84 (0.65-1.08)		
Alcohol consumption	Excessive	0.011[a]	1.37 (1.08-1.75)	0.043[a]	1.32 (1.01-1.72)
Follow-up	Followed up	<0.001[a]	0.63 (0.52-0.77)	0.002[a]	0.71 (0.56-0.89)
Child-Pugh score	≥7	<0.001[a]	2.10 (1.70-2.59)	0.134	1.30 (0.92-1.82)
Albumin (g/dl)	<3.7	<0.001[a]	1.98 (1.62-2.43)	0.046[a]	1.37 (1.01-1.85)
Prothrombin time (%)	<80	0.002[a]	1.37 (1.12-1.68)	0.959	0.99 (0.78-1.27)
Total bilirubin (mg/dl)	≥1.1	<0.001[a]	1.67 (1.36-2.05)	<0.001[a]	1.53 (1.22-1.92)
AFP (ng/ml)	≥52	<0.001[a]	1.83 (1.49-2.24)	0.001[a]	1.44 (1.16-1.79)
TNM stage	III or IV	<0.001[a]	3.02 (2.45-3.72)	<0.001[a]	2.50 (2.00-3.13)
Etiology of liver disease	HCC-nonBC	0.139	0.77 (0.54-1.09)		

CI, confidence interval.

Table III. Comparison between HCC in TNM stage I or II patients with and without virus infection.

	All patients		HCC-nonBC		HCC-virus		P-value
Total	397		41		356		
Median age, years	65	(13)	72	(13)	65	(13)	<0.001
Gender (%)							
Male	288	(73)	27	(66)	261	(73)	
Female	109	(27)	14	(34)	95	(27)	NS
BMI	22.3	(4.0)	23.7	(5.2)	22.3	(3.9)	NS
Alcohol consumption (%)							
Not excessive	328	(83)	31	(76)	297	(83)	
Excessive	69	(17)	10	(24)	59	(17)	NS
Follow-up (%)							
Follow-up group	268	(68)	21	(51)	247	(60)	
Non-follow-up group	129	(32)	20	(49)	109	(40)	0.019
Child-Pugh score	6	(2)	5	(1)	6	(2)	0.012
Hepatitis virus							
HBsAg (+)/HCV Ab (-)	70	(18)	0	(0)	70	(20)	
HBsAg (-)/HCV Ab (+)	274	(69)	0	(0)	274	(77)	
HBsAg (+)/HCV Ab (+)	12	(3)	0	(0)	12	(3)	
HBsAg (-)/HCV Ab (-)	40	(10)	40	(100)	0	(0)	-
TNM stage (%)							
I	158	(40)	11	(15)	147	(27)	
II	239	(60)	30	(40)	209	(39)	NS
Laboratory data							
Albumin (g/dl)	3.8	(0.7)	4.0	(0.6)	3.8	(0.8)	0.009
Prothrombin time (%)	82	(22)	87	(20)	80	(21)	NS
Total bilirubin (mg/dl)	0.9	(0.6)	0.8	(0.4)	1.0	(0.7)	NS
AFP (ng/ml)	32	(222)	9	(32)	36	(254)	<0.001

Data are median (IQR) or frequency (%). NS, not significant.

Table IV. Univariate and multivariate analyses of prognostic factors for HCC in patients with TNM stage I or II.

Variable		Univariate analysis		Multivariate analysis	
		P-value	Relative risk (95% CI)	P-value	Relative risk (95% CI)
Age (years)	≥65	0.514	0.91 (0.69-1.20)		
Gender	Male	0.039[a]	1.40 (1.02-1.94)	0.008[a]	1.58 (1.13-2.21)
BMI	≥25	0.062	0.71 (0.50-1.02)		
Alcohol consumption	Excessive	0.083	1.36 (1.96-1.93)		
Follow-up	Followed up	0.270	0.85 (0.64-1.13)		
Child-Pugh score	≥7	<0.001[a]	2.04 (1.52-2.73)	0.041[a]	1.47 (1.02-2.11)
Albumin (g/dl)	<3.8	<0.001[a]	2.04 (1.56-2.68)	0.007[a]	1.62 (1.15-2.30)
Prothrombin time (%)	<82	0.083	1.27 (0.97-1.67)		
Total bilirubin (mg/dl)	≥0.9	0.067	1.30 (0.98-1.72)		
AFP (ng/ml)	≥32	<0.001[a]	1.64 (1.26-2.16)	0.065	1.31 (0.98-1.74)
TNM stage	II	0.004[a]	1.52 (1.14-2.01)	0.004[a]	1.53 (1.14-2.04)
Etiology of liver disease	HCC-nonBC	0.020[a]	0.51 (0.29-0.90)	0.048[a]	0.55 (0.30-0.99)

CI, confidence interval.

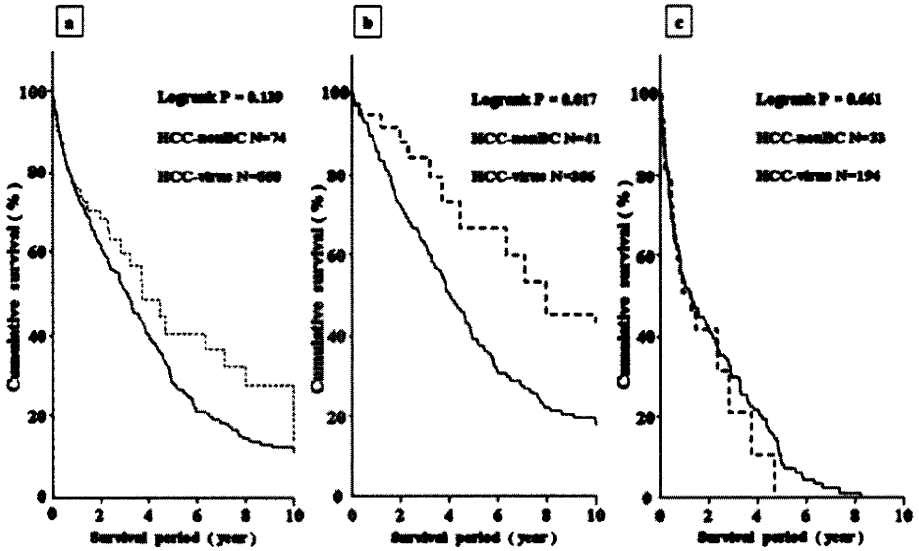

Figure 1. The cumulative survival rate in HCC patients without hepatitis virus infection (HCC-nonBC, dashed-line) and in HCC patients infected with hepatitis virus (HCC-virus, thin line) according to the TNM staging system.

1.53), as independent and significant risk factors (P=0.048, 0.008, 0.041, 0.007 and 0.004, respectively) for prognosis.

Patient survival. Overall, the median survival of all 624 patients was 1.84 years. No significant difference was detected in the survival rate between patients with and without hepatitis virus infection (Fig. 1a). When patients were classified according to the TNM stage, patients in the HCC-nonBC group with TNM stage I or II had a higher cumulative survival rate than those in the HCC-virus group (Fig. 1b; P=0.017). Patients who had TNM stage III or IV and HCC-nonBC and HCC-virus patients did not show significant differences in survival rates (Fig. 1c).

Discussion

The age-adjusted mortality rate for HCC has increased over the past few decades in Japan (27). However, the majority of patients are still diagnosed at an advanced stage and so have a short survival time after diagnosis. Patients with chronic HBV and/or HCV infection complicated by cirrhosis should be monitored with ultrasonography, CT or MRI of the liver to detect tumors at an early stage. In 58% of our patients, the tumors were detected on follow-up. Patients in the follow-up group had smaller tumors at the time of diagnosis and were more likely to be eligible for treatment. In addition, there was a significant improvement in survival rates among the

follow-up group (24-26,28-32). We recognized that the 2 groups of patients could not be evaluated in a prospective study, and improved survival in the follow-up group patients may be owing to the effect of lead-time bias. Nevertheless, our data corroborate those of previous studies indicating that follow-up may have increased rates of early detection and eligibility for curative treatment, which may in turn translate to improved survival.

In the TNM stages I and II, patients with HCC-nonBC had a better prognosis than those with HCC-virus. This difference may be explained as follows. HCC secondary to liver cirrhosis is less frequent in patients with HCC-nonBC than in those with HCC-virus (12). Patients with HCC-nonBC are less likely to progress to liver cirrhosis (33). However, in the TNM stage III and IV, the patients with HCC-nonBC had a similar prognosis to those with HCC-virus. The percentages of advanced stage HCC and non-follow-up patients were significantly higher in the HCC-nonBC group than in the HCC-virus group. Taken together, these results indicate that the prognosis of patients with HCC-nonBC is linked to the follow-up studies for detecting HCC.

A large proportion of people infected with HCV, HBV or both have latent cancer. Therefore, it is essential that HCC is detected at an early stage in individuals who harbor chronic HCV or HBV infections. In this study, more than 80% of patients had HCC associated with HBV and/or HCV; therefore, the target population for the surveillance of HCC must be easily identifiable. However, the incidence of hepatitis virus associated with HCC will decrease in Japan (15,34,35) because of the following reasons. In Japan, the population of individuals infected with chronic HCV is rapidly aging (36,37), and chronic HBV infection has been preventable since the licensing of the hepatitis B vaccine in 1982. In fact, primary tumors in 12% of our patients with HCC were negative for both HBsAg and HCV Ab. Of these, non-alcoholic fatty liver disease (NAFLD) may be a cause of HCC. Bugianesi *et al* suggested that liver disease was caused by NAFLD in 23/641 (4%) patients with HCC (38). However, it will be difficult to select patients for the screening of HCC, who are negative for both HBsAg and HCV Ab.

HCC surveillance for patients eligible for imaging tests is usually performed at 6-month intervals. Additionally, a combined imaging test and a serological test such as AFP or des-γ carboxy prothrombin is a sensitive method to detect HCC (29,39). The target population for the surveillance of HCC may not be easily identified in Japan. It has been reported previously that more than 60% of patients in the follow-up group had HCCs measuring less than 3 cm in diameter (26). It is possible that 12-month intervals for the imaging test were reasonable to ensure the detection of treatable tumors in patients with HCC.

In summary, the poorer prognosis of patients with HCC-nonBC was attributable to its late detection in an advanced condition, owing to the lack of a surveillance system for early detection of HCC. However, among early-stage patients, those with HCC-nonBC showed a significantly better prognosis than those with HCC-virus. To conclude, we suggest that the entire population of Japan should be tested using imaging techniques at least every 12 months along with an abdominal examination.

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