表1　統計解析とデータマイニングの相違

統計解析：多変量解析	データマイニング
限られたサンプルから仮説を検証する．	膨大なデータから網羅的に高速探索し，精緻に解析して仮説を発見する．
仮説検証型	仮説発見型
近似的な定式化	厳密な定式化
特定の線形関数	任意の非線形関数
$y=f(x)=a+bX$	$y=f(x)=\sum_{i=1} wi*\exp\{(x-c)**2/2\sigma**2\}$

これら治療を受ける場合の1回あたりの医療費は，薬剤費用だけで200万円から300万円とかなりの高額である．治療期間は半年から1年間と長く，また，稀ながらも重篤な副作用を伴う治療でもある．治療効率，医療経済，治療の安全性の観点から，C型慢性肝炎に対するIFN治療効果を，事前にできるだけ正確に予測する必要性は極めて高い．

厚生労働科学研究，肝炎緊急対策事業，標準的治療困難例の治療に関する研究班（主任研究者：八橋弘）では，2000年から2002年の3年間に全国22箇所の国立病院機構の肝疾患専門医療施設において，IFN治療を受け治療効果が明確となった1049症例のC型慢性肝炎患者を対象に，IFN治療後のウイルス駆除の可否を目的因子として，ウイルスの量と型，患者の年齢，性差，IFNの種類，リバビリン併用の有無，IFN総投与量，IFN投与期間などを説明因子としてDm解析を行った．その結果，HCVの型，HCV-RNA量，IFNの総投与量，IFN製剤の選択などが治療効果に影響を及ぼす因子として抽出され，それぞれの因子を組み合わせることで具体的なウイルス駆除確率，治癒確率を明示したアルゴリズム（決定木法）を作成することができた（図1）．このアルゴリズムに従って，個々の患者のもつ各説明因子をYes，Noとたどることで，容易に治癒確率を計算することができる．またIFNの総投与量やIFN製剤の選択は，人為的に選択，操作できる因子であり，本解析結果を参考にしながら個々の治療法を選択することで，より高い治療効果を目指すことも可能となる．

3. C型慢性肝炎IFN治療関連遺伝子に関する網羅的探索研究

ゲノム研究は，配列決定の段階からバイオインフォマティクスを駆使して配列と疾患感受性，薬剤感受性との関連を網羅的に探索する段階へ移行しつつあり，これらの遺伝子情報解析にはウエットとドライの協調と融合が必要であることが指摘されている．前者は多くの企業の参入により順調に開発が進行しつつあるも，後者に関しては扱う情報量が多く，従来の統計解析手法では，組合せ爆発が発生する可能性が示唆され，実用時間内で処理可能なSNPの個数には限界があることが指摘されている．ポストゲノム時代の今，膨大な遺伝子地図情報と臨床データとの関連から，有効な法則性を効率的に発見する強力な情報処理技術の開発が急務であると言われている．一方，現時点でのSNP研究の多くは，単変量解析により，1つの遺伝子を吊り上げて機能解析を行うか，ハプロタイプの同定，臨床データとの関連を，遺伝統計学を用いて解析するまでにとどまっており，遺伝子ネットワークの探求までには至っていない．

一方，C型慢性肝炎のIFN治療効果に影響を及ぼす因子は，大きく，ウイルス因子（ウイルスの型と量），薬剤因子（IFNの種類，投与量，投与期間，リバビリン併用の有無など），宿主因子（患者の年齢，性，肝線維化の程度，薬剤感受性の遺伝的要因，個人の資質，遺伝多型：SNPなど）の3つの要素に分類される（図2）．C型肝炎IFN治療効果に関して，ウイルス因子は極めて重要な因子であることは明らかとなっているも宿主因子に関しては断片的な解析が行われているに過ぎない．今までにMxA[1]，IL10[2,3]，IRF1[4]のプロモーター領域のSNPやハプロタイプ，IFNAR1（IFNレセプター）のマイクロサテライト配列[5]がIFN治療効果に関係したと報告されているも，いずれも少数のSNPを統計学的手法で解析したものである．最近PegIFNとリバビリンの併用療法が可能となり，従来のIFN単独治療法に比較して格段とIFN治療効果は向上した．高ウイルス症例でも治癒させうることが可能となった現在，治療効果予測因子としてのウイルス因子の重要性は以前に比して低下し，相対的に個々の症例の薬物応

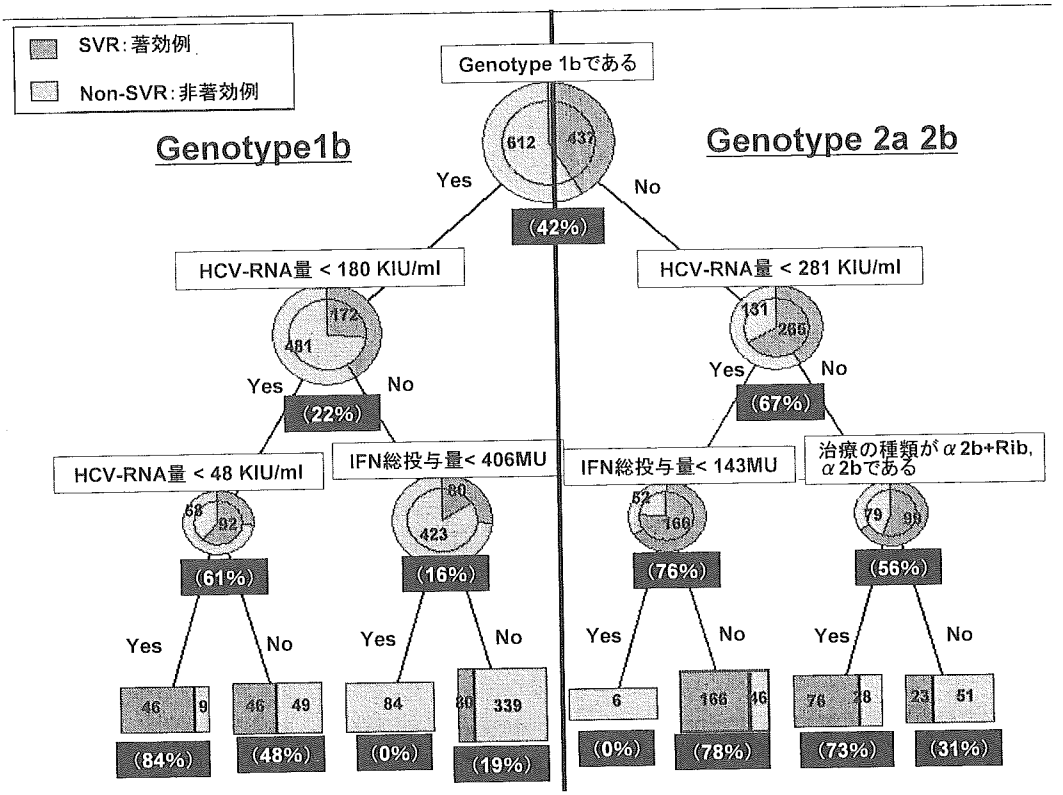

図1　データマイニングによるC型慢性肝炎(N＝1049) IFN治療効果予測モデルの構築，IFN単独・コンセンサスIFN・IFNα2b/リバビリン療法例でのウイルス駆除率(SVR%)算出のアルゴリズム(決定木法)．HCV-RNA定量はオリジナル法による．

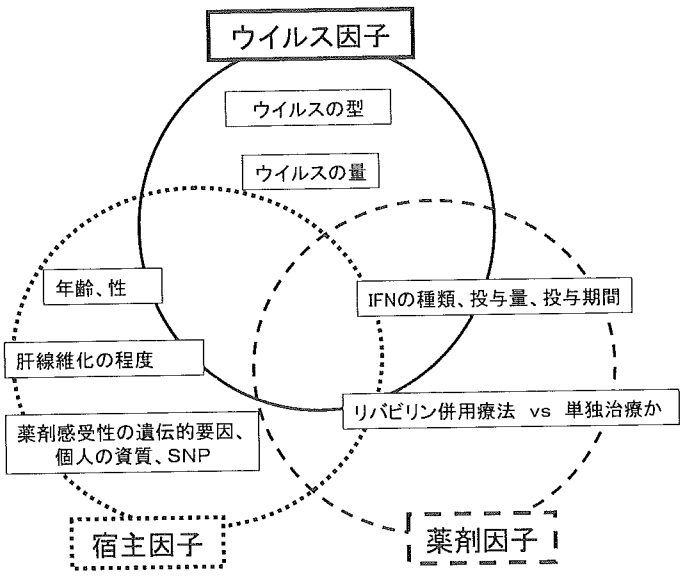

図2　C型慢性肝炎IFN治療効果に影響を及ぼす因子

答性の遺伝子の評価，宿主因子の重要性が高まりつつある．

我々はIFN治療に関連する遺伝子マップからSNPを網羅的に探索し，Dmを用いてIFN治療関連遺伝子の

ネットワークの解明をすることを試みている．その結果，120症例という少数例で，269という限られたSNP数，ゲノム情報ではあるも，C型慢性肝炎IFN治療関連遺伝子に関するSNPの組み合わせによって，具体的な治癒期待率が容易に算出可能であることを確認したので紹介する．

対象は，IFN治療を行ったC型慢性肝炎患者120例．ウイルス因子による治療効果の影響をできるだけ排除する目的で，この120症例は全例治療前HCV-RNA量が100 KIU/m*l*以下の低ウイルス症例を選択した．すなわち，ウイルス学的にはIFN治療効果が期待できるも，IFN単独治療でウイルス駆除に成功した著効(SVR)65例と，無効例(Non-SVR)の55例が対象である．IFN治療に関連する16遺伝子(IFN-AR1, IFN-AR2, JAK1, TYK2, JAB(CIS), STAT1, STAT2, ISGF3G(IRF9), IRF1, IRF2, TAP1, TAP2, LMP7, PKR, MxA, IL-10)の中から269箇所のSNPを同定し，インベーダ法(大塚TRC研究所)及びダイレクトシークエンス法を用いて決定した．Dm解析は，IBM Intelligent Miner II Ver.8（日本IBM)を用いた．

統計解析結果では，単点解析ではIRF2(111, 115), MxA(200, 304, 202), IFNAR1(369, 355)が有意であった．多点解析として各染色体における全ての解析SNP間の連鎖不平衡係数(D', r2乗)の算出，連鎖不平衡解析ブロック内で推定されるハプロタイプの頻度分布比較解析では，IRF2(111-T/115-G, 111-C/115-T))をもつハプロタイプTG, CAがnonSVRで有意であった(表2)．

SNPの組み合わせという観点で269個のSNPの組み合わせパターンを計算したところ，10の161乗の組み合わせパターンとなり，高だか269個という限られたSNP数の組み合わせ解析でも，統計学的手法を用いての情報処理は不可能であることが判明した．

次にDmによる決定木法解析を行うと，数分間で，図3のようなアルゴリズムを作成することが可能であった．具体的には，RF2(111-nonT/T), MxA(200-nonA/C), TAP2(160-nonG/G)の3条件を満たすと21例中21例(100%)がSVR, IRF2(111-T/T), IFNAR2(447-nonT/T), IRF2(131-A/T), IFNAR2(392-nonG/G)の4条件を満たすと23例中3例(13%)しかSVRにならなかった．Dm解析によって，3つないし4つのSNPの組み合わせで，100%近く治療効果が期待できる集団と，10%前後しか治癒が期待できない集団に判別することが可能であった．

遺伝病とは異なり個々の遺伝子が形質に与える影響が弱い一般的な疾患でのSNP解析では多点解析が必須である．今までのSNP解析に関する研究は，主に疾患原因遺伝子を探索する目的で，数千人から数万人規模の集団を対象に，遺伝統計学の手法を用いて，1つの遺伝子を吊り上げ同定した後，その遺伝子の機能解析を行うというプロセスで研究が進められてきた．しかしながら薬剤感受性の遺伝的要因，遺伝子ネットワークを解明し，治療効果を予測するためには，1つのSNPの発見やハプロタイプを見つけるだけでは不十分であり，多点解析を行い，その情報のからみを解きほぐす，SNPパターンの組み合わせの解析が必要である．Dmは，SNPの組み合わせ解析，特に薬剤感受性の解明や

表2　IFN著効(SVR)群・無効(Non-SVR)群間での統計解析結果

単点解析(単変量解析)				P値<0.05
G.S.		position	P値	OR(95%C.I.)
IRF 2	111	intron	0.011	2.59(1.24—5.42)
	115	intron	0.031	2.25(1.07—4.75)
MxA	200	5'UTR	0.016	2.61(1.18—5.74)
	304	5'flanking	0.044	2.22(1.02—4.84)
	202	5'flanking	0.034	2.39(1.08—5.28)
IFNAR 1	369	intron	0.041	3.39(1.00—11.5)
	355	intron	0.044	1.74(1.01—2.99)
多点解析(各染色体における全ての解析SNP間の連鎖平衡解析ブロック内で推定されるハプロタイプの頻度分布比較解析)				
IRF 2	111/115	T/G vs others	0.021	1.93(1.09—3.41)
		C/A vs others	0.031	2.03(1.08—3.84)

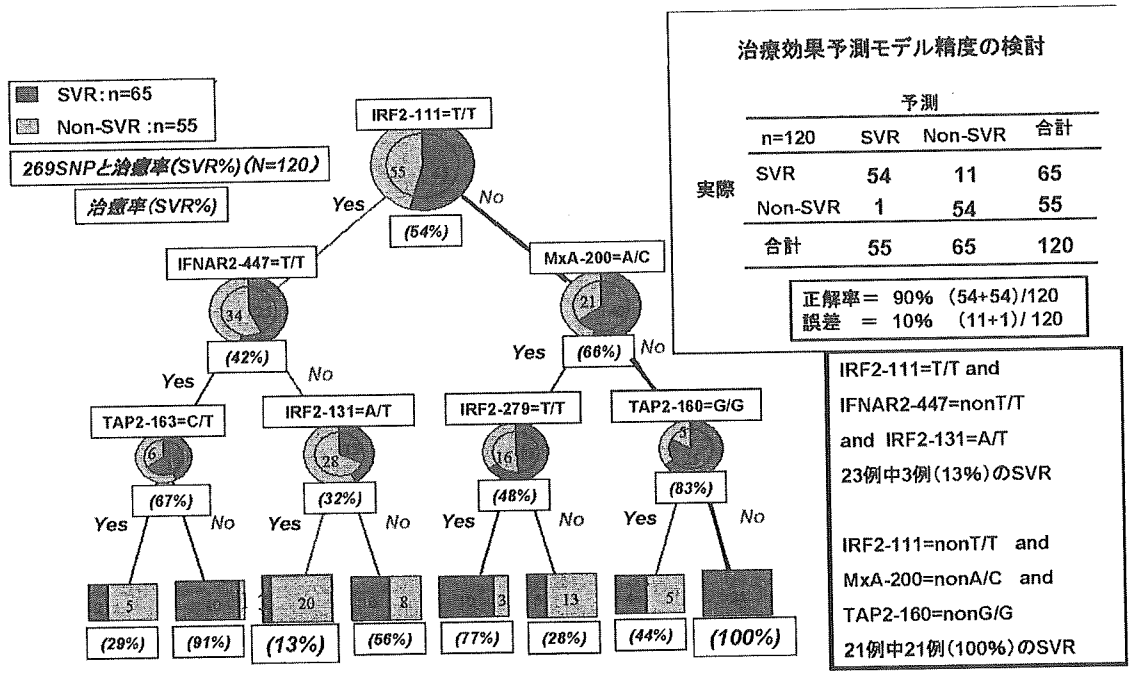

図3　IFN 治療に関係する遺伝子多型(SNP)の組み合わせによる治療効果予測：データマイニング解析

治療効果予測を目的とする場合には，膨大な情報処理，精緻な解析の観点から，極めて有用な情報解析方法と言えよう．

4. おわりに

これからのC型肝炎に対するIFN治療では，ウイルス因子，薬剤因子，宿主因子など質の異なる複数の因子を総合的に評価することで，個々の症例の治療効果を予測し，治療適応，治療法を決定すべきであり，その実現化が切望されている．いわゆるオーダメード医療，個の医療，質の高い医療を展開するために我々がなすべきことは何なのか，Dmというツールを活用しながら，今後も模索してゆきたいと考えている．

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医学と薬学
54巻5号・2005年11月
54(5)：677-682，2005

高感度HCV-Core抗原定量試薬の基礎的検討と臨床的有用性

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はじめに

C型慢性肝炎に対する抗ウイルス療法として，1992年1月にインターフェロン（IFN）単独療法が保険適応となり，現在まで広く用いられてきた。しかし，その当時のIFN単独療法の成功率は満足できるものではなく，C型慢性肝炎患者全体の30～35%の症例でC型肝炎ウイルス（HCV）が駆除できるにすぎなかった。近年，IFNと併用することにより抗ウイルス効果を高める経口薬（リバビリン）や新しいIFN（コンセンサスIFN，PEG-IFN）の効果が発表され，期待が高まっている。

これまでIFN治療の効果判定および治療の経過観察として，血中のHCV-RNA量を測定することが有用とされている[1)]。現在，一般臨床に用いられるHCV定量法には，b-DNA probe法，AMPLICOR monitor法，HCV-Core抗原測定法の3種類があり，測定感度，信頼される定量範囲などに若干の違いがみられる。前2者はHCV-RNAの定量であるため，検体の保存条件や測定者の熟練が測定結果に影響する[2)]。後者はHCVのCore領域を構成する蛋白を直接測定するものである。HCV-Core抗原量はHCV-RNAの定量法とよく相関すること，HCVウイルス粒子にはCore抗原がHCV-RNAと一定の比率で含まれ，ウイルス量を反映していること，genotype 2においても良好に検出されること，操作が簡便で安定して測定できることが報告されている[3)~6)]。最近，従来のHCV-Core抗原測定法（蛍光酵素免疫法：FEIA）よりも前処理操作が簡便で高感度な

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Fundamental evaluation and clinical efficacy of high sensitive HCV-Core antigen measurement assay

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Key words：HCV-Core抗原，HCV-RNA，C型肝炎，IFN/リバビリン併用療法

表1　同時再現性　(fmol/L)

測定回数	Sample A	Sample B	Sample C
1	255	4,008	15,741
2	245	4,095	17,266
3	229	4,024	15,078
4	225	4,517	15,379
5	222	3,976	17,521
6	240	3,816	15,688
7	251	4,004	17,510
8	202	4,028	17,411
9	236	4,139	15,300
10	247	4,003	16,663
Mean	235.2	4,061	16,355.7
SD	16.05	180.83	1,013.89
CV (%)	6.8	4.5	6.2

HCV-Core抗原測定試薬(ルミスポット '栄研' HCV抗原)が開発された[7)~9)]。今回われわれは，この試薬を用いて，測定系の基礎的検討を行うとともに，C型慢性肝炎IFN/リバビリン併用療法における高感度HCV-Core抗原測定の臨床的有用性について検討した。

I．対象および方法

1．対　象

対象は，血液センターでスクリーニングされ第2世代HCV抗体(PHA法)陽性と判定された供血者182例と，2000年1月から2002年12月の3年間，全国22カ所の国立病院機構肝疾患専門医療施設に登録されたC型慢性肝炎IFN治療導入症例のうちIFN/リバビリン併用例317例(genotype 1b：236例，non-1b：81例)で，いずれも凍結保存血清検体を用いた。

2．方　法

1）HCV-Core抗原測定

HCV-Core抗原の測定は，ルミスポット '栄研' HCV抗原(栄研化学)(以下本キット)を用い，全自動化学発光酵素免疫測定装置「LS-2000」(栄研化学)にて測定した。検体中のウイルス粒子の抽出ならびにHCV抗体を失活させるために，検体に1/2容量の前処理液を添加し，56℃で30分間のインキュベーションを行った血清を前処理済み試料として測定に用いた。測定は，HCV-Core抗原に特異的なモノクローナル抗体を用いた2ステップサンドイッチ法を原理として測定した。HCV-Core抗原濃度(モル濃度)の決定は，キャリブレーターの発光強度から検体の発光強度を補正後，検量線情報をもとにして検体中のHCV-Core抗原濃度を算出した。なお，測定結果は，20 fmol/L未満をHCV-Core抗原陰性，20 fmol/L以上をHCV-Core抗原陽性と判定した。

2）統計解析

測定結果の統計学的解析は，Mann-Whitney検定，χ^2検定を用い，$p<0.05$を有意と判定した。

II．結　果

1．基礎的検討

1）再　現　性

同時再現性については，HCV-RNA陽性血清3検体を用いて，それぞれHCV-Core抗原を10回連続測定した(**表1**)。変動係数(CV)は4.5～6.8%の範囲内と良好な再現性が得られた。

日差再現性については，別のHCV-RNA陽性血清2検体を用いて，6日間測定した。強陽性検体(Sample E)での変動係数は5.3%，中陽性検体(Sample D)では9.1%であった(**表2**)。

2）希釈直線性

HCV-RNA陽性血清2検体を正常プール血清にて，それぞれ4,096倍まで等間隔希釈し，HCV-Core抗原量を測定した(**図1**)。1～4,096倍の希釈系列で良好な直線性が得られた。

2．IFN/リバビリン併用療法治療前の治療効果予測

C型慢性肝炎患者317例にIFN/リバビリン併用療法を行い，その治療前血清中のHCV-Core抗原量と治療効果との関連性についてgenotype別で検討した(**図2，3**)。IFN/リバビ

表 2　日差再現性　(fmol/L)

測 定 日	Sample D	Sample E
1	8,835	15,505
2	7,291	15,254
3	7,570	14,024
4	8,712	15,264
5	7,626	13,665
6	7,211	15,298
Mean	7,874.17	14,835
SD	715.42	780.96
CV (%)	9.1	5.3

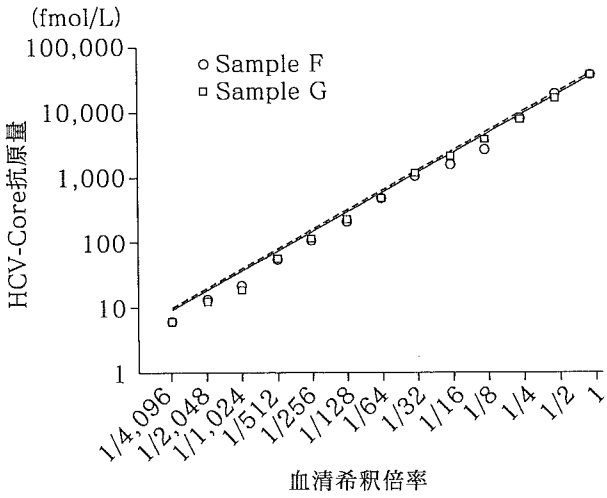

図 1　希釈直線性

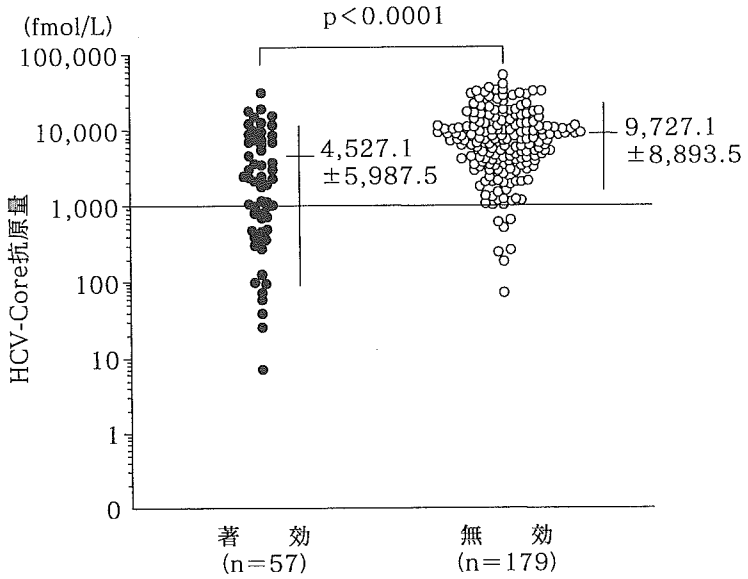

図 2　Genotype 1b における治療前 HCV-Core 抗原と IFN/リバビリン治療効果 (n=236)

リン併用療法の治療効果判定は，治療終了後6カ月目の時点で血中 HCV-RNA 陰性を著効 (SVR)，それ以外を無効 (Non-SVR) とした。

Genotype 1b では，IFN/リバビリン併用療法無効例患者の治療前 HCV-Core 抗原量は 72.8～51,704 fmol/L (平均値±標準偏差：9,727.1±8,893.5 fmol/L) に分布しているのに対し，著効例患者の HCV-Core 抗原量は 7～30,209 fmol/L (平均値±標準偏差：4,527.1±5,987.5 fmol/L) となり，HCV-Core 抗原量は SVR 群に比べて Non-SVR 群で有意に高値であった ($p<0.0001$)。

Non-1b では IFN/リバビリン併用療法無効例患者の治療前 HCV-Core 抗原量は 31～25,198 fmol/L (平均値±標準偏差：5,267.8±7,128.6 fmol/L) に分布しているのに対し，著効例患者の HCV-Core 抗原量は 0～18,615 fmol/L (平均値±標準偏差：3,814.3±4,006.0 fmol/L) となり，SVR 群と Non-SVR 群との間に統計学上 HCV-Core 抗

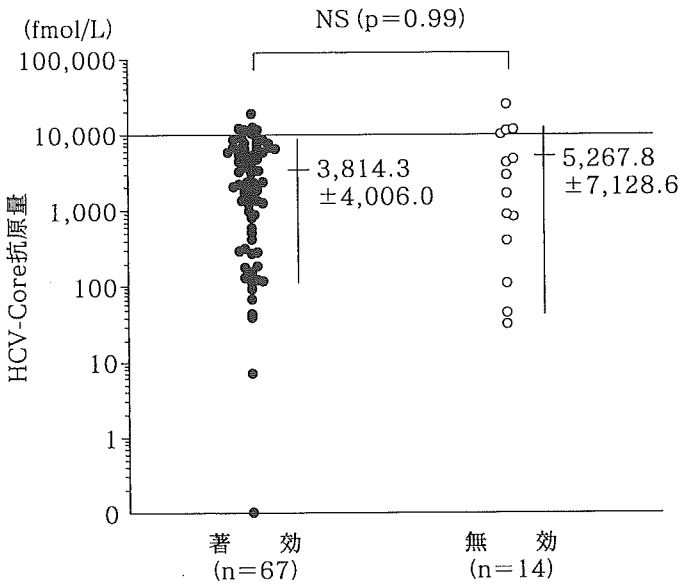

図 3　Genotype Non-1b における治療前 HCV-Core 抗原と IFN/リバビリン治療効果（n=81）

表 3　C 型慢性肝炎，治療前 HCV-Core 抗原量層別の IFN/リバビリン併用療法の治療効果（317 例）

HCV-Core 抗原量 (fmol/L)	Genotype 1b（n=236）著効例/対象例（著効率：%）	Non-1b（n=81）著効例/対象例（著効率：%）
10,000≦	8/66 (12%)　p<0.0001	6/10 (60%)　p=0.0353
1,000～9,999	27/141 (19%)　p=0.0001　p<0.0001	38/42 (90%)
100～999	15/21 (71%)	17/21 (81%)
<100	7/8 (88%)	6/8 (75%)
合　計	57/236 (24%)	67/81 (83%)

原量と治療効果との関連はなかった。

さらに，本キットで IFN/リバビリン併用療法治療前の治療効果予測の指標を明らかにする目的で HCV-Core 抗原量を層別し，Genotype 別 SVR 率を検討した（**表 3**）。Genotype 1b では，100 fmol/L 未満では 88%，100～999 fmol/L では 71%，1,000～9,999 fmol/L では 19%，10,000 fmol/L 以上では 12%の SVR 率で，高ウイルス量になるにつれ SVR 率の低下がみられた。HCV-Core 抗原量 1,000 fmol/L を境界に有意に治療効果が低下する結果が得られた。

一方，Non-1b では，100 fmol/L 未満では 75%，100～999 fmol/L では 81%，1,000～9,999 fmol/L では 90%，10,000 fmol/L 以上では 60%の SVR 率であった。1b とは異なり，Non-1b では，ウイルス量層別に著効率の差がみられないものの，唯一，10,000 fmol/L を境界として有意差が認められた。

III. 考　察

HCV-Core 抗原の免疫学的測定は，1992 年に Takahashi ら[10]によって初めて報告され，

1996年にKashiwakumaら[4]は検体をポリエチレングリコール処理により遠心濃縮，さらにアルカリ処理によるCore抗体の失活とCore抗原の抽出を行う方法を報告している。この処理済み検体を試料として2種のモノクローナル抗体でCore抗原をサンドイッチし検出するFEIAが実用化された。しかし，先の2法は遠心処理，アルカリ処理といった方法を使用しているが，操作が煩雑であり，検出感度も必ずしも十分とはいえなかった。Aoyagiら[11][12]は，この抗体の解離と不活化をより簡便に行える方法を考案し，さらに高感度にHCV-Core抗原を測定する化学発光酵素免疫測定法（CLEIA）を報告している。本キットは，この方法を利用しHCV-Core抗原を全自動化学発光酵素免疫測定装置「LS-2000」により測定する試薬である。

測定系の基礎的検討，片山ら[8]の報告と同様，本法は，同時再現性，日差再現性ともCV値が10%以内と良好で安定した測定系と考えられた。また，希釈直線性試験では，正常プール血清で4,096倍まで等間隔希釈した結果，6～36,000 fmol/Lの範囲内で良好な直線性が認められ，本試薬を用いることにより，6 fmol/Lまでの定量性があるものと考えられた。

本試薬によるHCV-Core抗原陽性，陰性のカットオフ値は，20 fmol/Lに設定することが適切であるとされている[8]。このように本キットは，20～100,000 fmol/Lと広い測定範囲を有しており，今回検討したC型慢性肝炎317例においても最大値は51,704 fmol/Lで，測定範囲の上限を超えた検体はみられなかった。

また，C型慢性肝炎に対するIFN治療において治療前の血中HCV-RNA量は治療効果と密接に関連しており，HCV-RNA量が少ないほどIFNが効きやすいことがすでに報告されている[13]。今回は，IFN/リバビリン治療前317症例の検討では，genotype別に治療効果予測をHCV-Core抗原量層別で行ってみた。Genotype 1bでは，1,000 fmol/Lが難治例の基準，Non-1bでは10,000 fmol/Lが基準となると考えられた。

以上のことより，本キットによるHCV-Core抗原量の測定は，測定の簡便性，測定値の安定性，HCVウイルス量を広範囲に定量可能であるという点，治療効果を高率に予測できる点で，臨床的有用性の高い測定系であると考えられた。

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Increasing hepatitis C virus-associated hepatocellular carcinoma mortality and aging: Long term trends in Japan

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Received 13 June 2005; received in revised form 10 November 2005; accepted 28 November 2005

Abstract

Background: The incidence of hepatocellular carcinoma (HCC) in Japan has been increasing. The aim of the study was to determine the epidemiological trends in HCC mortality in Japan.
Methods: We reviewed the medical records of all patients whose death was caused by liver disease between 1981 and 2000 at two hospitals. The courses of death were separated based on presence or absence of HCC when death ensued. Additionally, cohorts of patients with HCC were analyzed in 5-year time periods.
Results: The number of deaths from hepatitis C virus (HCV)-associated HCC steadily increased 2.6 times from 49 to 128 during observation period. The mean age at death from HCV-associated HCC from 1996 to 2000 was significantly higher than that in the period from 1981 to 1985 ($p < 0.0001$).
Interpretation: Deaths from HCV-associated HCC increased from 1981 to 2000, consistent with the aging of the population in Japan.

Keywords: Hepatitis C virus; Hepatocellular carcinoma; Epidemiological

1. Introduction

Hepatocellular carcinoma (HCC) affects approximately half a million people each year worldwide, making it the fifth most common malignancy in men and the ninth most common in women [1–7]. Recently, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia [1–9], and the incidence of primary liver cancer in Japan has been increasing over the past four decades [10,11]. HCC often develops in patients with liver cirrhosis caused by hepatitis C virus (HCV), hepatitis B virus (HBV) or excessive alcohol consumption.

Of the hepatitis viruses that cause HCC, HCV is more common than HBV in Japan [12–15]. Although the age-adjusted incidence rates of HCC have been increasing during the period of rising HCC mortality, the temporal and demographic features of survival for HCC patients in Japan are unknown. Hence, we have analyzed these trends over time, using information from two independent databases that deal with HCC in Japan.

2. Patients and methods

We reviewed the medical records of all patients who died from liver disease and received medical care between 1981 and 2000 at the Liver Disease Center, National Nagasaki Medical Center and at The First Department of Internal

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doi:10.1016/j.hepres.2005.11.007

Medicine, Nagasaki University School of Medicine. A total of 1001 patients were studied. All the patients were followed-up after diagnosis until death in one of the two hospitals and we were able to confirm their date of death and that death had occurred after severe liver disease.

All patients were entered into this study because sera were stored at −80 °C. These sera were used to assay HBV or HCV infection. A diagnosis of chronic HCV infection was based on the presence of anti-HCV antibody and HCV-RNA detected by polymerase chain reaction (PCR), whereas diagnosis of chronic HBV infection was based on the presence of hepatitis B surface antigen (HBsAg) or anti-hepatitis B core antigen (anti-HBc) reactivity. Diagnosis of HCC was based on histological findings or on characteristic images in dynamic computed tomography, dynamic magnetic resonance imaging and hepatic angiography. Demographic information, including age at death, sex and year of death, was collected from the patients' chart. Excessive alcohol consumers (an alcohol consumption of >50 g/day for 5 years) were not including in this study.

The courses of death were separated into those occurring with or without HCC when death ensued. Additionally, the patients with HCC were analyzed in 5 yearly intervals (1981–1985, 1986–1990, 1991–1995 and 1996–2000). Patients were classified according to 5-year age groups, and by HBV or HCV infection, and the number of patients in each age group with HBV- or HCV-associated HCC was calculated in each time period.

The SAS computer program for Windows was used to perform statistical analysis of the data, using analysis of variance (ANOVA).

3. Results

A total of 1001 patients died at the Liver Disease Center, National Nagasaki Medical Center and at The First Department of Internal Medicine, Nagasaki University School of Medicine from 1981 to 2000. The patients with HBV-associated HCC were 73.7% (210 of 285) in HBV-related disease and the patients with HCV-associated HCC were 84.5% (381 of 451) in HCV-related disease. There were 653 patients with HCC died. The mean time during followed-up were 2.5 years. The proportion of patients diagnosed with HBV-associated HCC was 32% (210 of 653), whereas 58% (381 of 653) had HCV-associated HCC, and an additional 2% (12 of 653) had HCC associated with both viruses (Table 1).

Table 1
Course of death from 1981 to 2000

	HBV	HCV	Overlap	Others	Total
HCC (%)	210 (32)	381 (58)	12 (2)	50 (8)	653 (100)
Chronic liver failure	47	35	1	36	119
GI bleeding	8	17	1	13	39
Other disease	3	5	0	16	24
Acute liver failure	10	1	3	19	33
Other cancer	7	12	0	114	133
Total (%)	285 (28)	451 (45)	17 (2)	248 (25)	1001 (100)

HCC, hepatocellular carcinoma; GI bleeding, gastrointestinal bleeding; HBV, hepatitis B virus; HCV, hepatitis C virus; overlap, both HBV and HCV positive; other, both HBV and HCV negative.

From 1981 to 2000, 210 patients died of HBV-associated HCC, whereas 381 died of HCV-associated HCC. Table 2 shows the number and the mean age at death from HBV- or HCV-associated HCC during the 5-year periods 1981–1985, 1986–1990, 1991–1995 and 1996–2000. The number of deaths from HBV-associated HCC was not changed within the range from 49 to 58 during the four 5-year periods: 54 (1981–1986), 49 (1986–1990), 49 (1991–1995) and 58 (1996–2000), and the mean age at death was not also statistically significantly different among the periods: 55.4 ± 9.9 (1981–1985), 55.6 ± 10.3 (1986–1990), 55.5 ± 10.6 (1991–1995) and 59.3 ± 10.2 (1996–2000). In contrast, the number of deaths from HCV-associated HCC steadily increased 2.6 times from 49 to 128 during same observation period: 49 (1981–1986), 90 (1986–1990), 114

Table 2
Mean age of KBV associated HCC deaths

Year	1981 – 1985	1986 – 1990	1991 – 1995	1996 – 2000	total
Number	54	49	49	58	210
Mean age (y.o.)	55.4	55.6	55.5	59.3	56.8
SD	9.9	10.3	10.6	10.2	10.3

NS, NS, NS, NS, NS, NS

Mean age of HCV-associated HCC deaths

Year	1981 – 1985	1986 – 1990	1991 – 1995	1996 – 2000	total
Number	49	90	114	128	381
Mean age (y.o.)	60.0	63.0	64.1	67.0	64.3
SD	8.1	7.0	7.2	7.9	7.8

NS, NS, 0.0267, 0.0176, 0.0016, < 0.0001

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; S.D., standard deviation; NS, not significant.

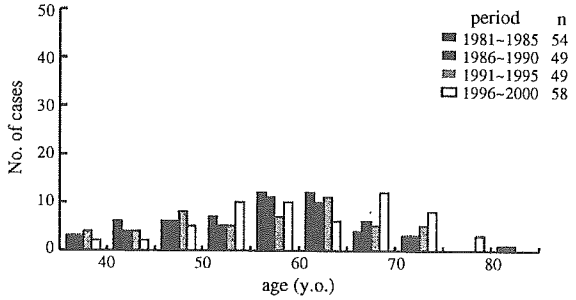

Fig. 1. Age distribution of the total number of deaths from hepatitis B virus-associated hepatocellular carcinoma from 1981 to 2000. There was no change of number of patients and age distribution of patients who died from hepatitis B virus-associated hepatocellular carcinoma during the four time periods.

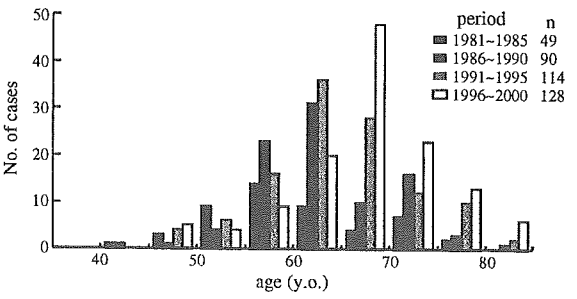

Fig. 2. Age distribution of the total number of deaths from hepatitis C virus-associated hepatocellular carcinoma from 1981 to 2000. The number of death from HCV associated HCC has increased 2.6 times during recent 20 years and this increase was provided by a close association with older shift of age distribution.

(1991–1995) and 128 (1996–2000). In addition, the mean age at death from HCV-associated HCC also increased over time. The mean age at death from 1996 to 2000 (67.0 ± 7.9 years old) was significantly higher than that from 1981 to 1985 (60.0 ± 8.1) ($p < 0.0001$), 1986 to 1990 (63.0 ± 7.0) ($p = 0.0016$) and 1991 to 1995 (64.1 ± 7.2) ($p = 0.0267$), respectively.

Fig. 1 shows the age distribution for deaths from HBV-associated HCC during the four 5-year periods. There was no change of number of patients and age distribution for deaths from HBV-associated HCC during these periods. In contrast, Fig. 2 shows the age distribution for deaths from HCV-associated HCC during the four 5-year periods. The number of patients with HCV-associated HCC aged more than 60 years in 1981–1985, 1986–1990, 1991–1995 and 1996–2000 were 22, 61, 88 and 110 patients, respectively. Fig. 2 indicated that the number of death from HCV associated HCC has increased during recent 20 years and this increase was provided by a close association with older shift of age distribution.

Table 3 shows the age distribution of HCC deaths in 5-year period (1981–1985, 1986–1990, 1991–1995 and 1996–2000). The number of patients with HCV-associated HCC obviously had an increase in the ratio of patients aged more than 60 years ($p < 0.0001$): 18.6% (1981–1985), 37.9% (1986–1990), 51.2% (1991–1995) and 54.4% (1996–2000). There was a significant difference of age distribution in the patients with HCV-associated HCC between aged more than and less than 60 years old in each 5-year period ($p < 0.0001$). In contrast, there was no difference in the age distribution of patients with other types of during these periods.

Fig. 3 shows the ratio between HCV-associated deaths and HBV-associated HCC deaths in 5-year period (1981–1985, 1986–1990, 1991–1995 and 1996–2000). The ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau during the observation period: 0.9 (1981–1985), 1.8 (1986–1990), 2.3 (1991–1995) and 2.2 (1996–2000) (1981–1985 versus 1991–1995, $p = 0.0030$; 1981–1985 versus 1996–2000, $p = 0.0042$). Above all, the ratio of patients aged more than 60 years old increased during the observation period: 1.1 (1981–1985), 3.0 (1986–1990), 4.2 (1991–1995) and 3.8 (1996–2000) (1981–1985 versus

Table 3
Age distribution of HCC deaths in 5-year period

Age (y.o.)	1981–1985, no. (%)	1986–1990, no. (%)	1991–1995, no. (%)	1996–2000, no. (%)		p-Value
HBV						
<60	34 (28.8)	29 (18.0)	28 (16.3)	29 (14.4)	]	NS
>60	20 (17.0)	20 (12.5)	21 (12.2)	29 (14.4)		
HCV						
<60	27 (22.9)	29 (18.0)	26 (15.1)	18 (8.9)	]	<0.0001
>60	22 (18.6)	61 (37.9)	88 (51.2)	110 (54.4)		
Overlap						
<60	1 (0.9)	3 (1.9)	2 (1.2)	1 (0.5)	]	NS
>60	0	2 (1.2)	0	3 (1.5)		
Other						
<60	5 (4.2)	2 (1.2)	4 (2.3)	2 (1.0)	]	NS
>60	9 (7.6)	15 (9.3)	3 (1.7)	10 (4.9)		
Total	118 (100)	161 (100)	172 (100)	202 (100)		

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; overlap, both HBV and HCV positive; other, both HBV and HCV negative.

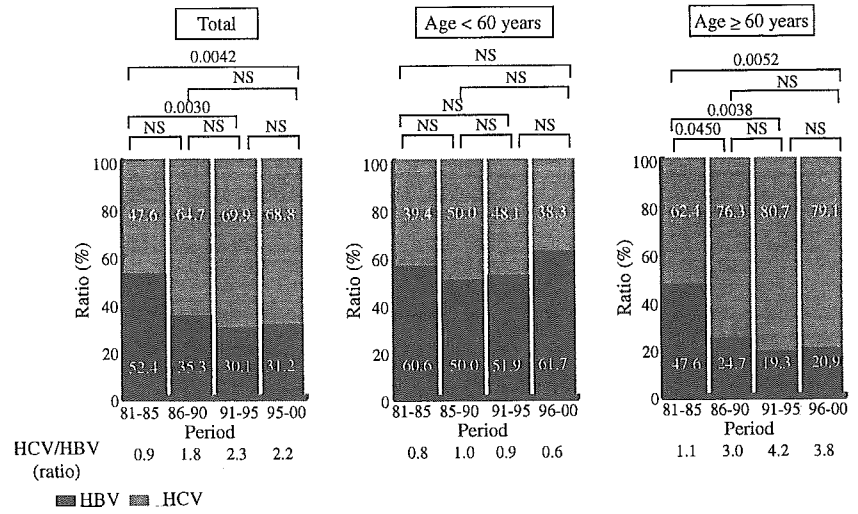

Fig. 3. Ratio between hepatitis C virus-associated hepatocellular carcinoma deaths and hepatitis B virus-associated hepatocellular carcinoma from 1981 to 2000. The ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau during the observation period.

1986–1990, $p=0.0450$; 1981–1985 versus 1991–1995, $p=0.0038$; 1981–1985 versus 1996–2000, $p=0.0052$). In contrast, there was no difference in the ratio of patients aged more than 60 years old of during these periods.

4. Discussion

HCC accounts for approximately 6% of all human cancers. It is estimated that half a million cases occur annually worldwide, making HCC the fifth most common malignancy in men and the ninth in women [1–7,9]. The age-adjusted mortality rate from HCC has increased over the past decades in Japan [16], and in the current study more than 90% of deaths from HCC were HBV- and/or HCV-related and the number of deaths from HCV-associated HCC apparently increased 2.6 times from 1981 to 2000, and the mean age of deaths from HCV-associated HCC also significantly rose. During the same period, the number and the age distribution of deaths from HBV-associated HCC remained unchanged. The increase in the number of deaths from HCV-associated HCC seemed to be closely associated with the shift of age distribution of HCV infected population between 1981 and 2000. Although our data had the limitations of applying the findings from two hospitals to a general population, Kiyosawa described that deaths due to HCC in Japan have continued to increase in males, particularly in those older than 60 years of age between 1982 and 2003. This also suggests that the average age of diagnosis of HBV-related HCC was similar in all three time periods. In contrast, the average age of patients with HCV-related HCC rose from 61.6 years in 1982 to 63.1 years in 1990 and 67.8 years in 2003 [11]. The research group for population-based cancer registration in Japan described that incidence of HCC in Japan have continued to increase and reached a plateau in males and female from 1975 to 1999. Above all, the age distribution incidence and incident rate of HCC reached a peak older than 65 years old in males and female [17]. And, this study suggested that the ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau from 1981 to 2000, especially more than 60 years old. Where did these findings and difference of HCC development between HCV and HBV, which were considered to be both oncogenic virus after long-term persistent infection with inflammation and fibrotic change in the liver but popular hepatitis virus infections in Japan, come from?

The simple reason may be explained as follows. From 1981 to 2000, mortality from a variceal hemorrhage in cirrhotic patients has declined [9,18]. Long term nutritional supplementation with oral branched-chain amino acids has been useful in the prevention of progressive hepatic failure, and improvement of surrogate markers and perceived health status in advanced cirrhosis has occurred [19,20]. Additionally, many new treatments and techniques have been introduced for HCC, including transcatheter arterial embolization, percutaneous ethanol injection therapy, microwave coagulation therapy, radiofrequency ablation, systemic chemotherapy and advance surgical techniques. However, these advances of medical treatment cannot explain the difference between HBV-associated HCC and HCV-associated HCC.

Alternatively, well considered reasons of the recent rapid increase of the number of patients who died from HCV-associated HCC in Japan, were shown in the current two studies. First, Hamada et al. recently reported that the majority of HCC patients develop HCC when they are aged over 60 years old, regardless of the timing of HCV infection. This result was obtained by the long-term observation of the patients infected by post-transfused HCV infection [21]. This also suggests that HCC has increased among patients over 60 years old with HCV infection and such phenomenon has never been observed nor reported till now in patients with HBV infection.

Second, the chronically HCV-infected population is aging in Japan. Yoshizawa et al. reported that age-specific prevalence rates for the presence of anti-HCV antibody among ~300,000 voluntary blood donors from Hiroshima in 1999 clearly increased with the age, reaching the highest rate of 7% in individuals who were more than 70 years old [11,22]. In a word, HCV infected people become older with years in Japan and they were regarded as a high risk for HCC. Then, the number of deaths from HCV-associated HCC has been increased recent 20 years in Japan.

El-Serag et al. reported that an increase in the number of cases of HCC affecting mainly younger age groups has occurred in the United States (U.S.) over the past two decades [23,24]. HCV infection accounts for most of the increase in the number of cases of primary liver cancer [4,6,7,9,25], while the rates of primary liver cancer associated with alcoholic cirrhosis and HBV infection have remained unchanged [4,6,9]. Tanaka et al. reported that HCV was introduced into the U.S. population around 100 years ago and was widely disseminated between 1954 and 1978 [26]. Most HCV-infected patients in the U.S. were born between 1940 and 1965 [27,28], and are therefore younger than HCV-infected Japanese patients. Hence, the burden of disease associated with HCV infection will probably increase in the U.S. during the next 10–20 years, as has occurred in Japan, as this cohort reaches an age at which complications of chronic liver disease typically occur [1–7,26]. The current study suggests that increased HCV-associated HCC will occur in the U.S. over the next two to three decades.

In conclusion, we found that the number of patients with HCV-associated HCC in Japan has increased, consistent with aging of the population, but the number of patients with HBV-associated HCC has remained unchanged over the last 20 years.

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Liver International 2006: 26: 90–96

Liver International
DOI: 10.1111/j.1478-3231.2005.01200.x

Clinical Studies

Measurement of hepatitis B virus core-related antigen is valuable for identifying patients who are at low risk of lamivudine resistance

Tanaka E, Matsumoto A, Suzuki F, Kobayashi M, Mizokami M, Tanaka Y, Okanoue T, Minami M, Chayama K, Imamura M, Yatsuhashi H, Nagaoka S, Yotsuyanagi H, Kawata S, Kimura T, Maki N, Iino S, Kiyosawa K, HBV Core-Related Antigen Study Group. Measurement of hepatitis B virus core-related antigen is valuable for identifying patients who are at low risk of lamivudine resistance.
Liver International 2006: 26: 90–96. © Blackwell Munksgaard 2005

Abstract: *Objective:* The clinical usefulness of hepatitis B virus core-related antigen (HBVcrAg) assay was compared with that of HBV DNA assay in predicting the occurrence of lamivudine resistance in patients with chronic hepatitis B. *Patients:* Of a total of 81 patients who were treated with lamivudine, 25 (31%) developed lamivudine resistance during a median follow-up period of 19.3 months. *Results:* The pretreatment positive rate of HBe antigen, or pretreatment levels of HBVcrAg or HBV DNA did not differ between patients with and without lamivudine resistance. Levels of both HBVcrAg and HBV DNA decreased after the initiation of lamivudine administration; however, the level of HBVcrAg decreased significantly more slowly than that of HBV DNA. The occurrence of lamivudine resistance was significantly less frequent in the 56 patients whose HBV DNA level was less than 2.6 log copy/ml at 6 months of treatment than in the remaining 25 patients. The cumulative rate of lamivudine resistance was as high as 70% within 2 years in the latter group, while it was only 28% in the former group. Lamivudine resistance did not occur during the follow-up period in the 19 patients whose HBVcrAg level was less than 4.6 log U/ml at 6 months of treatment, while it did occur in 50% of the remaining patients within 2 years. *Conclusion:* These results suggest that measurement of HBV DNA is valuable for identifying patients who are at high risk of developing lamivudine resistance, and that, conversely, measurement of HBVcrAg is valuable for identifying those who are at low risk of lamivudine resistance.

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Key words: chronic hepatitis B – HBV core-related antigen – HBV DNA – lamivudine resistance

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Received 19 June 2005,
accepted 8 August 2005

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Lamivudine, a nucleoside analogue that inhibits reverse transcriptases, was first developed as an anti-viral agent against human immunodeficiency virus (HIV). It was later also found to be effective against hepatitis B virus (HBV) because HBV is a member of the Hepadnaviridae family of viruses, which use reverse transcriptases in their replication process (1, 2). Lamivudine was found to inhibit the replication of HBV, reduce hepatitis, and improve histological findings of the liver in long-term treatment (3–5). Furthermore, it has been shown that lamivudine treatment improves the long-term outcome of patients with chronic hepatitis B (6, 7). However, there are a number of problems with lamivudine therapy, such as relapse of hepatitis because of the appearance of YMDD mutant viruses and the reactivation of hepatitis after discontinuation of the treatment (8–11).

The concentration of HBV DNA in serum decreases and usually becomes undetectable during lamivudine administration, but it rapidly increases when HBV becomes resistant to lamivudine. Thus, the measurement of HBV DNA is useful for monitoring the anti-viral effects of lamivudine. However, a negative result of HBV DNA in serum does not necessarily indicate a good outcome of lamivudine therapy, because lamivudine resistance may occur even if HBV DNA levels remain undetectable during therapy (11–13). Recently, a chemiluminescence enzyme immunoassay (CLEIA) was developed in our laboratory for the detection of hepatitis B virus core-related antigen (HBVcrAg) (14, 15). The assay reflects the viral load of HBV in a similar manner to that used in assays, which detect HBV DNA. HBVcrAg consists of HBV core and e antigens; both proteins are transcribed from the precore/core gene and their first 149 amino acids are identical (16–18). The HBVcrAg CLEIA simultaneously measures the serum levels of hepatitis B core (HBc) and e (HBe) antigens, using monoclonal antibodies, which recognize common epitopes of these two denatured antigens. In the present study, we analyzed the clinical significance of the HBVcrAg assay in monitoring the anti-viral effects of lamivudine treatment.

Patients and methods

Patients

A total of 81 patients with chronic hepatitis B, who received lamivudine therapy, were enrolled in the present study. These were 58 men and 23 women with a median age of 49 years (range 24–79 years). The 81 patients were selected retrospectively from six medical institutions in Japan (Shinshu University Hospital, Toranomon Hospital, Nagoya City University Hospital, Kyoto Prefectural University Hospital, Hiroshima University Hospital, National Nagasaki Medical Center). Eight to 25 patients who met the following three criteria were selected consecutively in each institution: the first, a daily dose of 100 mg lamivudine was administered for at least 6 months in a period from 1999 to 2004; the second, histologically confirmed for chronic hepatitis without liver cirrhosis; and the third, serum samples at several time points available for testing. All patients were naive for lamivudine therapy. Chronic hepatitis B was defined as positive hepatitis B surface (HBs) antigen for more than 6 months with elevated levels of serum transaminases. The HBV genotype was A in two patients, B in three and C in 76. Serum HBV DNA was detectable in all patients, and HBe antigen was positive in 51 (63%) of the 81 patients just before lamivudine administration. The median follow-up period was 19 months with a range from 6 to 50 months. Follow-up of patients ended when lamivudine administration was discontinued. Written informed consent was obtained from each patient.

The occurrence of lamivudine resistance was defined as a rapid increase in serum HBV DNA levels with the appearance of the YMDD mutations during lamivudine administration. Using this criteria, resistance appeared in 27 (33%) of the 81 patients. The median period from the start of lamivudine administration to the occurrence of resistance was 12 months with a range from 4 to 37 months.

Serological markers for HBV

HBs antigen, HBe antigen and anti-HBe antibody were tested using commercially available enzyme immunoassay kits (Abbott Japan Co., Ltd., Tokyo, Japan). Six major genotypes (A–F) of HBV can be detected using the method reported by Mizokami et al. (19), in which the surface gene sequence amplified by polymerase chain reaction (PCR) is analyzed by restriction fragment length polymorphism. The YMDD motif, that is, lamivudine resistant mutations in the active site of HBV polymerase, was detected with an enzyme-linked mini-sequence assay kit (HBV YMDD Mutation Detection Kit, Genome Science Laboratories Co., Ltd., Tokyo, Japan) (20).

Serum concentration of HBV DNA was determined using Amplicor HBV monitor kit (Roche, Tokyo, Japan), which had quantitative range from 2.6 to 7.6 log copy/ml. Sera containing

over 7.0 log copy/ml HBV DNA were diluted 10- or 100-fold with normal human serum and re-tested to obtain the end titer.

Serum concentrations of HBVcrAg were measured using the CLEIA method reported previously (10, 11). Briefly, 100 μL serum was mixed with 50 μL pretreatment solution containing 15% sodium dodecylsulfate and 2% Tween 60. After incubation at 70 °C for 30 min, 50 μL pretreated serum was added to a well coated with monoclonal antibodies against denatured HBc and HBe antigens (HB44, HB61 and HB114) and filled with 100 μL assay buffer. The mixture was incubated for 2 h at room temperature and the wells were then washed with buffer. Alkaline phosphatase-labeled monoclonal antibodies against denatured HBc and HBe antigens (HB91 and HB110) were added to the well, and the mixture was incubated for 1 h at room temperature. After washing, CDP-Star with Emerald II (Applied Biosystems, Bedford, MA) was added and the plate was incubated for 20 min at room temperature. The relative chemiluminescence intensity was measured, and the HBVcrAg concentration was determined by comparison with a standard curve generated using recombinant pro-HBe antigen (amino acids, 10–183 of the precore/core gene product). The HBVcrAg concentration was expressed as units/ml (U/ml) and the immunoreactivity of recombinant pro-HBe antigen at 10 fg/ml was defined as 1 U/ml. In the present study, the cutoff value was tentatively set at 3.0 log U/ml. Sera containing over 7.0 log U/ml HBVcrAg were diluted 10- or 100-fold in normal human serum and re-tested to obtain the end titer.

Statistical analysis

The Mann–Whitney *U*-test and Wilcoxon signed-ranks test were utilized to analyze quantitative data, and Fisher's exact test was used for qualitative data. A log-rank test was used to compare the occurrence of lamivudine resistance. Statistical analyses were performed using the SPSS 5.0 statistical software package (SPSS, Inc., Chicago, IL). A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Table 1 shows a comparison of the clinical and virological backgrounds of the 27 patients who showed lamivudine resistance and the 54 patients who did not. Median age, gender distribution and median follow-up period did not differ between the two groups, and the positive rate of HBe antigen was similar. Both HBV DNA and HBVcrAg levels at the beginning of lamivudine administration were similar between the two groups; however, both HBV DNA and HBVcrAg levels at 6 months after the start of lamivudine administration were significantly lower in the lamivudine resistance negative group than in the positive group. ALT level was normal at the beginning in eight (15%) of the 54 patients without lamivudine resistance and in two (7%) of the 27 patients with it ($P > 0.2$).

Table 1. Comparison of the clinical and virological backgrounds of patients who showed lamivudine resistance and those who did not

Characteristics	Appearance of lamivudine resistance: Negative ($n = 54$)	Positive ($n = 27$)	*P*
Age (years)*	47.0 (24–79)	50.6 (34–67)	0.140†
Gender (male %)	74%	67%	>0.2‡
Follow-up period (months)*	16 (6–50)	21 (9–43)	>0.2†
HBV genotype (A/B/C)	2/2/50	0/1/26	>0.2‡
HBe antigen (positive %)	59%	70%	>0.2‡
ALT (IU/ml)*			
Initial	85 (22–713)	95 (20–1140)	>0.2†
At 6 months	27 (11–115)	30 (15–92)	>0.2†
HBV DNA (log copy/ml)*			
Initial	7.0 (3.5–9.1)	7.3 (4.2–9.2)	>0.2†
At 6 months	<2.6 (<2.6–4.8)	3.3 (<2.6–6.6)	<0.001†
HBVcrAg (log U/ml)*			
Initial	6.2 (<3.0–8.8)	7.3 (4.4–9.1)	0.073†
At 6 months	5.2 (<3.0–6.7)	5.8 (4.7–8.4)	<0.001†

HBe antigen, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; HBVcrAg, HBV core-related antigen. *Data are expressed as median (range). †Mann–Whitney *U* test. ‡χ^2-test.

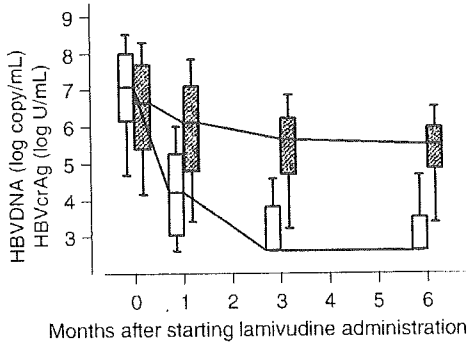

Fig. 1. Changes in the median levels of hepatitis B virus core-related antigen (HBVcrAg) and hepatitis B virus (HBV) DNA during lamivudine administration. The box plots show the 10th, 25th, 50th, 75th and 90th percentiles, with the open boxes indicating HBV DNA and shaded boxes indicating HBVcrAg. The median amount of decrease from the baseline in HBVcrAg levels was significantly smaller (Wilcoxon signed-ranks test) than that in HBV DNA level at 1 (2.80 log copy/ml vs. 0.27 log U/ml, $P < 0.001$), 3 (3.60 log copy/ml vs. 0.83 log U/ml, $P < 0.001$) and 6 months (3.90 log copy/ml vs. 1.15 log U/ml, $P < 0.001$) after the initiation of lamivudine administration.

Figure 1 shows changes in HBV DNA and HBVcrAg levels during lamivudine treatment in all patients. The level of HBV DNA decreased rapidly and became undetectable at 3 months after treatment was initiated. On the other hand, although HBVcrAg levels decreased continuously, the median amount of decrease from the base-line was significantly lower than that in HBV DNA levels at 1, 3 and 6 months after starting lamivudine administration (Wilcoxon signed-ranks test, $P<0.001$ at all analyzed points in time).

Changes in HBV DNA and HBVcrAg levels during lamivudine administration are compared in Fig. 2 between the 27 patients who showed lamivudine resistance and the 54 patients who did not. Serum HBV DNA levels were found to decrease rapidly and become undetectable within 6 months in 45 (83%) of the 54 patients without lamivudine resistance. On the other hand, only 11 (41%) of the 27 patients with lamivudine resistance showed a similar rapid decrease, and the HBV DNA levels of the remaining patients stayed above the detection limit during the follow-up period. HBVcrAg levels decreased but did not reach levels lower than 4.7 log U/ml (5000 U/ml) in the 27 patients with lamivudine resistance. In 19 (35%) of the 54 patients without lamivudine resistance, on the other hand, the levels decreased to levels below 4.7 log U/ml within 6 months after the start of lamivudine administration. The level of HBVcrAg increased rapidly as did the level of HBV DNA when lamivudine resistance occurred.

The occurrence of lamivudine resistance was significantly less frequent in the 56 patients whose HBV DNA level was less than 2.6 log copy/ml at 6 months after the initiation of treatment than in the remaining 25 patients (Fig. 3). The cumulative occurrence of lamivudine resistance was as high as 70% within 2 years in the latter group, while it was only 28% in the former group. There was no occurrence of lamivudine resistance during the follow-up period in the 19 patients whose HBVcrAg levels were less than 4.6 log U/ml at 6 months after the initiation of lamivudine therapy (Fig. 3). On the other hand, lamivudine resistance occurred in 50% of the remaining patients within 2 years.

Discussion

The HBVcrAg assay is a unique assay, which measures the amounts of e and core antigens

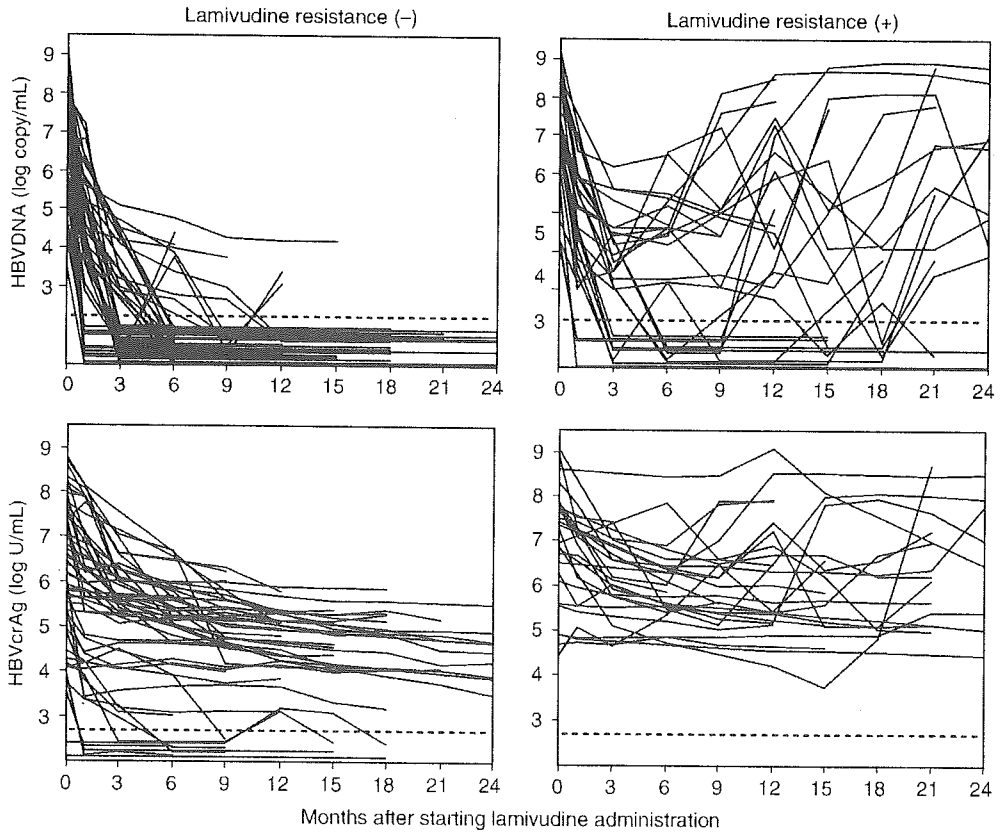

Fig. 2. Comparison of changes in serum hepatitis B virus (HBV) DNA and serum HBV core-related antigen (HBVcrAg) levels between patients who showed lamivudine resistance and those who did not. The broken lines indicate the detection limit of each assay.

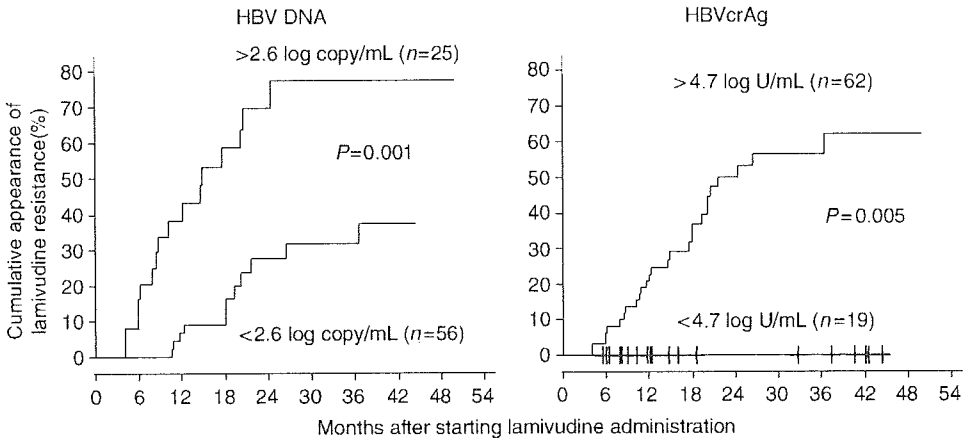

Fig. 3. Comparison of the cumulative occurrence of lamivudine resistance between patients who showed hepatitis B virus (HBV) DNA levels of less than the detection limit (2.6 log copy/ml) at 6 months after starting lamivudine administration and those who did not (left figure), and similarly between patients who showed HBV core-related antigen (HBVcrAg) levels of less than 4.7 log U/ml and those who did not (right figure).

coded by the core gene of the HBV genome with high sensitivity and a wide quantitative range. Serum HBVcrAg levels reflect the viral load in the natural course because these levels correlate linearly with those of HBV DNA (14, 15). On the other hand, the character of HBVcrAg is somewhat different from that of HBV DNA in patients undergoing anti-viral therapies such as lamivudine. That is, HBVcrAg levels decrease significantly more slowly than those of HBV DNA after the initiation of lamivudine administration.

HBV is an enveloped DNA virus containing a relaxed circular DNA genome, which is converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells (18, 21–23). The cccDNA molecules serve as the transcriptional template for the production of viral RNAs that encode viral structural and non-structural proteins. Reverse transcription of the viral pregenomic RNA and second-strand DNA synthesis occur in the cytoplasm within viral capsids formed by the HBV core protein. Because lamivudine, a nucleoside analogue, inhibits reverse transcription of the pregenomic RNA, it directly suppresses the production of HBV virion. Thus, serum HBV DNA levels decrease rapidly after the initiation of lamivudine administration. On the other hand, the production of viral proteins is not suppressed by lamivudine because the production process does not include reverse transcription. Furthermore, it has been reported that the amount of cccDNA, which serves as a template for mRNA, decreases quite slowly after starting the administration of nucleoside analogues (24–26). Thus, it is reasonable that serum HBVcrAg levels decrease much more slowly than HBV DNA levels after the initiation of lamivudine therapy.

Significant markers that can predict the presence or absence of lamivudine resistance are clinically valuable because the emergence of this resistance and the subsequent recurrence of hepatitis are fundamental problems in lamivudine therapy. Serum markers that reflect the activity of HBV replication have been reported to be associated with the occurrence of lamivudine resistance (11, 12, 27, 28). However, neither the pretreatment existence of HBe antigen nor pretreatment levels of HBV DNA or HBVcrAg were found to be significant markers in the present study. These results may reflect a weak association between the pretreatment activity of HBV replication and the occurrence of lamivudine resistance (13, 29). Changes in HBV DNA and HBVcrAg levels after starting lamivudine administration clearly differed between patients with and without lamivudine resistance. Thus, HBV DNA and HBVcrAg levels at 6 months after starting lamivudine administration were analyzed to determine whether these levels might serve as predictive markers; both were found to be significantly lower in patients without lamivudine resistance at the tested point in time. Furthermore, patients who showed higher levels of HBV DNA and HBVcrAg at 6 months after the initiation of treatment were significantly more likely to develop lamivudine resistance than those who showed lower levels.

We believe that the measurement of HBV DNA levels is useful to identify patients who are at high risk for lamivudine resistance because as many as 70% of patients who were positive for HBV DNA at 6 months after starting lamivudine