表2 B型慢性肝炎に対するIFN4週投与と24週投与の比較検討

	投与終了時	投与終了6ヵ月後
ALT正常化		
S群	2/26(7%)	3/23(13%)
L群	10/25(40%) *	8/25(32%)
H群	20/25(80%) **	9/25(36%)
HBV-DNA陰性化		
S群	5/26(19%)	3/23(13%)
L群	6/23(26%)	5/20(25%)
H群	11/22(50%)	8/23(35%)
Seronegative		
S群	2/25(8%)	1/25(4%)
L群	4/25(16%)	6/22(27%)
H群	7/25(28%)	6/24(25%) ***
L＋H群	11/50(22%)	12/46(26%)
Seroconversion		
S群	2/25(8%)	1/25(4%)
L群	4/25(16%)	4/22(18%)
H群	6/25(24%)	5/24(21%)

*$p < 0.02$, **$p < 0.0001$, ***$p < 0.05$

の必要な症例には有用な治療法と考えている.

2. 4週連日投与

西口[3]はわが国のIFN 4週投与の成績をまとめ，投与終了1年後，2年後のHBe抗原陰性化率はそれぞれ29%，55%，HBe抗原抗体seroconversion率は12%，29%で自然経過よりも高率であるとしている．われわれ[4]もHBe抗原陽性例23例(男性16例，女性7例，平均年齢36.3歳)に対してIFNα-2a 9MU 3日間連日投与後，18MU 25日間連日投与の計477MUの投与を行った．成績は投与終了1年後のHBe抗原陰性化率50.0%，ALT正常化率36.8%およびHBV-DNA陰性化率41.2%であった．4週投与においても1回投与量高用量の製剤を用いることによって，6ヵ月投与と同等の総投与量を投与でき，また，対象に若年例が多かったことが，良好な成績が得られた要因と考えている.

3. 長期24週投与

2000年4月より24週の長期投与が可能となった．24週投与の最大の利点は投与期間中にHBe抗原抗体seroconversionが生じる可能性が高く，投与終了後の急性増悪の出現を防止できることである．4週投与との比較として大阪大学旧第1内科関連病院肝臓グループで行ったIFNの長期投与試験の成績[5]を紹介する．IFN投与スケジュールはS群600万単位4週間連日投与，L群300万単位4週間連日投与後，週3回20週投与，H群600万単位4週間連日投与後，週3回20週投与の3群間の比較試験である．治療成績は**表2**に示すようにALTの改善率，HBV-DNAの消失率とも4週投与(S群)に比較して24週投与(L群，H群)の方が，投与直後および投与6ヵ月とも高率であった．また，HBe抗原陰性化率，HBe抗原抗体seroconversion率でも，4週投与(S群)に比較して24週投与(L群，H群)の方が高率であり，24週の長期投与の有効性が高いと考えられた．西口の国内治療成績の集計でも，投与終了6ヵ月後のHBe抗原陰性化率は4週投与，24週投与でそれぞれ11%，28%と長期投与の有効性が確認されている．欧米では6ヵ月投与が標準投与法であるが，Wongら[6]の比較対照試験の集計でも投与終了後6ヵ月の時点でのHBe抗原陰性化率33%と，自然経過例12%に比し有意に高率であったとしている.

Ⅲ．これからのIFN治療

1．IFN・ラミブジン併用治療

HBe抗原陽性例に対するIFN治療は24週の長期投与でも，満足できる成績は得られていない．また，ラミブジン治療においてもHBe抗原陽性のHBV-DNA高値例ではHBe抗原陰性化率は低率である．われわれの施設ではHBe抗原陽性例に対するIFN・ラミブジン併用治療とIFN単独治療の比較検討を行っている．併用治療のプロトコールはIFNα6MU2週連投後22週週3回投与とラミブジン100mg連日投与を同時に投与開始し，24週後よりはラミブジン単独投与を継続するものである．現在，治療成績を解析中であるが，併用群においてMeriganのType Ⅰ効果にあたるHBs抗原陰性化例を2例(genotype AおよびC)認めている．HBステージⅠb，Ⅱbはともに HBe抗原陽性期であるが，われわれはこの時期においても大半の例はpre C mutantの出現が確認され，また，IFNはpre C wild，pre C mutantのいずれの株にも同等に有効であることを報告[4]した．ラミブジンはpre C mutant株に対してより強い抗ウィルス効果が得られる[7]こと，また，ラミブジンのYMDD変異株はIFN前投与あるいは併用群において出現率が低率であること(自験例，未発表)を考慮すると，ステージⅠb，Ⅱb群にはラミブジン単独よりもIFNとの併用がより有効ではないかと考えている．Schalmら[8]，Serfatyら[9]もIFN/ラミブジン併用治療の有効性について報告している．

2．ペグIFN治療

C型慢性肝炎には国内で2003年12月より週1回投与のペグIFNα-2a治療が保険適用になったが，B型慢性肝炎に対しても海外ではペグIFNα-2aを用いたトライアルが行われている．まず，Cooksleyら[10]はHBe抗原陽性例に対するphase Ⅱ studyを実施し，従来のIFNα-2a製剤に比しペグIFNα-2a製剤は有意に有効性が高いと報告している．また，Marcellinら[11]はHBe抗原陰性例に対してペグIFNα-2a単独，ペグIFNα-2a/ラミブジン併用およびラミブジン単独の比較試験を行い，ペグIFNα-2a単独群およびペグIFNα-2a/ラミブジン併用群はラミブジン単独群に比し，投与終了後6ヵ月でのHBV-DNA陰性化率，ALT正常化率はともに高率であったと報告している．

3．HBe抗原陰性例に対するIFN治療

HBe抗原陰性例に対するIFN治療の有用性に関しては一定の見解が得られていないが，積極的に使用を勧める報告[12][13]は少なく，わが国では現在保険適用になっていない．HBe抗原陰性例はHBステージのⅢとⅣにあたるが，臨床的治癒の状態であるステージⅣは治療の必要はなく，pre C mutantの増殖が持続するステージⅢが抗ウィルス治療の対象となる．ステージⅢの平均年齢は53歳[1]で，大半の症例が40歳以上である．ラミブジン治療ではHBe抗原陰性例の方がYMDD変異株出現率が低く治療効果も良好で，アデホビルも使用可能となったことから，HBe抗原陰性例に対してはラミブジン治療が第一選択と考えられる．IFN治療としてはペグIFN長期投与に期待したいところである．

Ⅳ．IFN治療例の長期予後と肝発癌

最後にIFN治療例の長期予後について述べる．1981年8月より1992年12月までにIFN治療を開始したB型慢性肝炎102例(平均観察期間7.3年)を対象に長期予後と肝癌発癌について検討した[14]．102例の性別は男性64例，女性38例，平均年齢はそれぞれ34.3歳，35.7歳で，使用したIFNの総投与量は6.8MU～1284MU(平均143.6MU)であった．投与終了後12～15年後(平均年齢約50歳)の累積HBe抗原陰性化率は90%，累積ALT正常化率は80%と80%の症例は臨床的治癒の状態とな

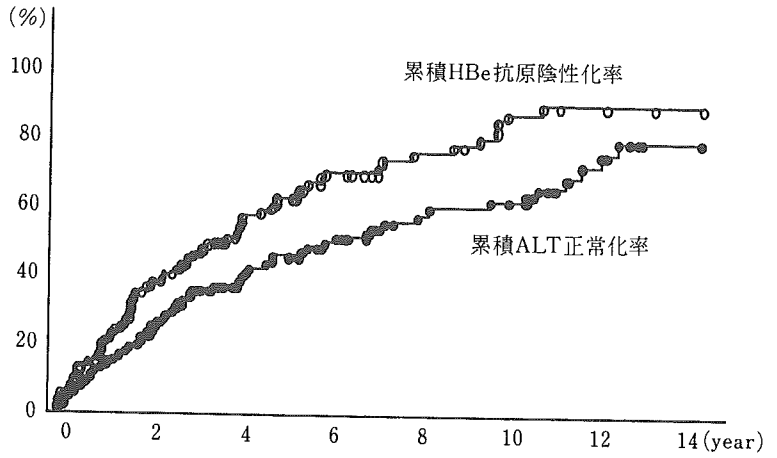

図3 累積 HBe 抗原陰性化率と累積 ALT 正常化率(カプラン・マイヤー法)

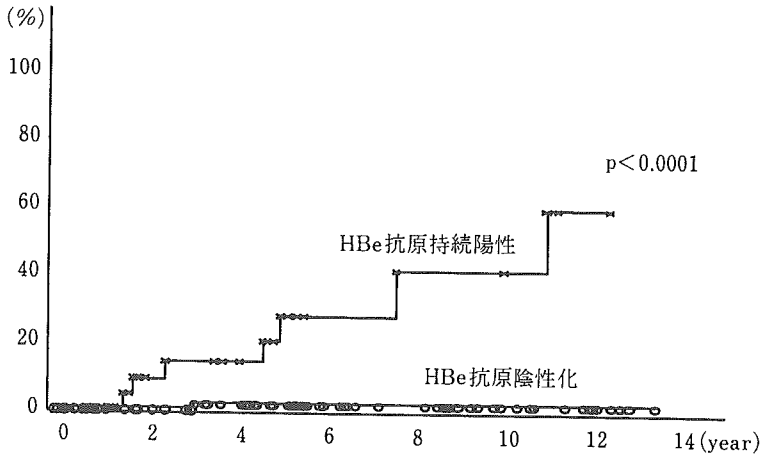

図4 累積 HCC 発癌率(カプラン・マイヤー法) HBe 抗原陰性化の有無による検討

るが，20%は ALT の異常が持続することが判った(図3)．ALT 異常が持続する半数は HBe 抗原持続陽性例で HB ステージⅠb，Ⅱb に相当し，残りの半数は HBe 抗原陰性で pre C mutant の増殖が持続するステージⅢと考えられる．肝癌発癌は8例(全例男性)に認められた(年率1.08%)．そのうち HBe 抗原持続陽性例は7例(年率4.1%)，HBe 抗原陰性化例は1例(年率0.29%)で HBe 抗原持続陽性例の発癌リスクは HBe 抗原陰性化例の約14倍であった．累積肝癌発癌率の検討でも HBe 抗原持続陽性例で有意に高率であった(図4)．HBe 抗原陰性化は，年齢が若年であるほど，壊死，炎症が高度なほど有意に高率であり，若年齢で組織学的に壊死，炎症の強い時期に IFN 治療を開始することが HBe 抗原陰性化さらに発癌抑止につながると考えられた．

おわりに

これまで IFN とラミブジンのみが保険適用の抗ウィルス剤であったが，本年12月よりアデホビルが保険適用となり，B 型慢性肝炎に対する抗ウィルス治療も新しい局面を迎えた．B 型は C 型に比し治療対象の選択がより重要で，それぞれの対象に対する適切な治療方法の選択と的確な治療の遂行が肝不全や発癌を防止し，予後の改善に寄与すると考えられる．今後，B 型慢性肝炎に対しても早期の IFN 自己注射とペグ IFN の使用が望まれるところである．

文　　献

1) 加藤道夫，伊与田賢也，結城暢一，ほか：HBVマーカーと発癌リスクよりみたHBVキャリアのステージ分類－切な抗ウィルス治療の選択に向けて－．肝臓　45：581-588，2004.
2) 加藤道夫，益沢　学，奥山卓正，ほか：B型慢性肝炎に対するヒト白血球インターフェロン少量間歇投与．肝臓　27：552-560，1986.
3) 西口修平：IFN治療．コンセンサス肝疾患2002－診断・治療と病態"B型肝炎治療"，矢野右人監修，p71-77，日本メディカルセンター，東京，2002.
4) Kato M, Yuki N, Kaneko A, et al：Changes in virus loads and precore mutations in chronic hepatitis B patients treated with 4 weeks of daily interferon alfa-2a therapy. Hepatol Res 28：73-78, 2004.
5) 林　紀夫，加藤道夫：B型慢性肝炎に対するIFN治療の長期予後とIFNの長期投与．第22回犬山シンポジウム，B型肝炎の新しい展開．犬山シンポジウム記録刊行会編，p68-69，中外医学社，東京，2001.
6) Wong DK, Cheung AM, O'Rourke K, et al：Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B：A meta-analysis. Ann Intern Med 119：312-323, 1993.
7) Cho SW, Hahn KB, Kim JH, et al：Reversion from precore/core promoter mutants to wild type hepatitis B virus during the course of lamivudine therapy. Hepatology 32：1163-1169, 2000.
8) Schalm SW, Heathcote J, Cianciara J, et al：Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection：a randomized trial. Gut 46：562-568, 2000.
9) Serfaty L, Thabut D, Zoulim F, et al：Sequential treatment with lamivudine and interferon monotherapies in patients with chronic hepatitis B not responding to interferon alone. Hepatology 34：573-577, 2001.
10) Cooksley WGE, Piratvisuth T, Lee S-D, et al：Peginterferon alfa-2a (40kDa)：an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepatitis 10：298-305, 2003.
11) Marcellin P, Lau G, Bonino F, et al：Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patientswith HBeAg-negative chronic hepatitis B.N Engl J Med 351：1206-1217, 2004.
12) Kako M, Kanai K, Aikawa T, et al：Response to interferon-alpha 2a in patients with e antigen-negative chronic hepatitis B.J Clin Gastroenterol 25：440-445, 1997.
13) Lampertico P, Ninno ED, Vigano M, et al：Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 37：756-763, 2003.
14) 加藤道夫，益澤　学：インターフェロン治療を行ったB型慢性肝炎の長期予後について．肝臓　39：679-681，1998.

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Significance of liver negative-strand HCV RNA quantitation in chronic hepatitis C

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***Background/Aims*: Liver negative-strand hepatitis C virus (HCV) RNA is the most direct indicator of active viral replication but has only been examined in a few semiquantitative studies.**

***Methods*: Positive- and negative-strand HCV RNA in the right (R) and left (L) liver lobes was quantified by rTth-based strand-specific real-time polymerase chain reaction for 48 chronic hepatitis C patients.**

***Results*: Close correlations between lobes were seen for positive- and negative-strand amounts ($r=0.950$; $P<0.001$ and $r=0.920$; $P<0.001$, respectively). The ratio of negative to positive strands (median, 0.14 for R and 0.13 for L) varied by 2 log directly in relation to HCV replication assessed by liver negative strands but had no relation to liver positive strands and circulating HCV. Only negative-strand quantitation was inversely correlated with age ($r=-0.322$; $P=0.026$ for R and $r=-0.340$; $P=0.018$ for L), while liver tissues with hepatitis B virus DNA contained larger amounts of each strand. In 27 patients treated with enhanced interferon monotherapy, the amounts of liver negative strands (<4 log copies/100 ng RNA) were the only independent predictor of a sustained virologic response.**

***Conclusions*: Negative-strand quantitation is uniform in the liver and bears distinct relevance to the disease.**

***Keywords*: Negative-strand HCV RNA; HCV replication; Chronic hepatitis C**

1. Introduction

Hepatitis C virus (HCV) replication, like that of other single-strand, positive-sense RNA viruses, is presumably preceded by the synthesis of negative-strand RNA. Thus, the amounts of negative-strand RNA-replicative intermediates in liver tissues should serve as a more reliable marker of active viral replication than positive-strand HCV RNA in the liver or in circulation. Serum HCV loads are affected by replication within the liver and extrahepatic sites and by immunologic clearance of the virus. The detection of liver positive-strand (genomic) HCV RNA can simply imply contamination by such circulating virions. Thus far, only a few semiquantitative studies have been done on the clinical relevance of liver negative-strand HCV [1–4], and controversy remains. Patients with chronic hepatitis C can show uneven distribution of liver injury, but intrahepatic variation of HCV replication also remains to be clarified. To further address these issues, we quantitatively analyzed positive- and negative-strand HCV RNA in each liver lobe by strand-specific real-time polymerase chain reaction (PCR) using rTth.

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2. Patients and methods

2.1. Patients

Forty-eight patients with chronic hepatitis C underwent laparoscopic liver biopsies. All patients were positive for serum HCV RNA (Amplicor HCV Test, Roche Diagnostics K.K., Tokyo, Japan). No confounding etiology of liver disease was found in any patient. They were negative for hepatitis B surface antigen in the serum. The group was comprised of 25 men and 23 women ranging in age from 33 to 70 years (median, 57 years). Sixteen (33%) patients had a history of blood transfusion 8–52 years (median, 36 years) earlier. Biopsies were performed using 13-gauge Tru-Cut needles (Hakko Medical Co., Ltd, Nagano, Japan), and liver tissues sufficient for histologic and virologic evaluation were obtained from the anterior segment of the right lobe and the lateral segment of the left lobe. Specimens 15 mm long and 2 mm wide were embedded in paraffin for histopathological study. The remaining portions were immediately frozen and then stored at −80 °C until PCR testing. With one patient, the specimen from the left lobe was subjected to only virologic evaluation due to its limited size. Paired serum samples were obtained from all patients at laparoscopy and stored at −80 °C without thawing until virologic tests. Of the 48 patients, 27 (Table 1) were treated with enhanced interferon (IFN) monotherapy. After laparoscopy, 3 MU of IFN-β (Feron, Toray Co., Tokyo, Japan) was administered twice a day for 2 weeks followed by 9 MU of IFN-α (Sumiferon, Sumitomo Pharm. Co., Osaka, Japan) daily for 2 weeks and thrice weekly for 20 weeks. The study was approved by the local research ethics committee in accordance with the 1975 Declaration of Helsinki, and all patients provided written informed consent.

2.2. Virologic testing

Circulating HCV genomic RNA was quantified by a PCR assay (Amplicor HCV Monitor Test version 2.0, Roche Diagnostics K.K.). HCV RNA of ≧6.4 log copies/mL was measured after serum dilution. HCV genotypes were determined by a PCR genotyping system [5].

2.3. Positive- and negative-strand HCV RNA quantitation by rTth-based strand-specific real-time reverse-transcription polymerase chain reaction (RT-PCR)

Strand-specific TaqMan RT-PCR was designed to quantify the 5′ untranslated region of the HCV genome using a thermostable enzyme, rTth (Applied Biosystems, Foster City, CA). Total hepatic RNA, 100 ng, was added to an RT reaction mixture containing 2 μL of 10× RT buffer (Applied Biosystems), 20 nmol of $MnCl_2$, 5 U of rTth, 24 U of RNasin (Promega, Madison, WI), 4 nmol of each dNTP, and 10 pmol of sense primer HCV-20F (5′-CGACACTCCACCATGAATCACT-3′) for the negative-strand assay or antisense primer HCV-114R (5′-GAGGCTG-CACGACACTCATACT-3′) for the positive-strand assay. The RT reaction was performed in a final volume of 20 μL at 70 °C for 60 min. The reaction

Table 1
Baseline patient characteristics before IFN therapy

		n
Age	<50	11 (41%)
	≥50	16 (59%)
Sex	Male	16 (59%)
	Female	11 (41%)
Transfusion history	+	9 (33%)
	−	18 (67%)
ALT	<2×ULN	17 (63%)
	≥2×ULN	10 (37%)
Liver histology		
Grading score[a]	<7	17 (63%)
	≥7	10 (37%)
Staging score[a]	<4	19 (70%)
	≥4	8 (30%)
Between-lobe grade discrepancy	+	10 (38%)
	−	16 (62%)
Between-lobe stage discrepancy	+	9 (35%)
	−	17 (65%)
HCV genotype	1	21 (78%)
	2	6 (22%)
Serum HCV RNA	<5.4 log copy/mL	6 (22%)
	≥5.4 log copy/mL	21 (78%)
+Strand[a]	<5 log copy/100 ng RNA	6 (22%)
	≥5 log copy/100 ng RNA	21 (78%)
−Strand[a]	<4 log copy/100 ng RNA	9 (33%)
	≥4 log copy/100 ng RNA	18 (67%)
−/+ Strand ratio[a]	<0.1	14 (52%)
	≥0.1	13 (48%)
Between-lobe+strand discrepancy	+	4 (15%)
	−	23 (85%)
HBV antibody (anti-HBc and/or anti-HBs)	+	7 (26%)
	−	20 (74%)
Liver HBV DNA	+	2 (7%)
	−	25 (93%)

[a] Mean values of right and left liver lobes.

was then treated with 5 μL of 10× chelating buffer (Applied Biosystems), 75 nmol of $MgCl_2$, 10 nmol of each dNTP, 10 pmol of antisense primer HCV-114R for the negative-strand assay or sense primer HCV-20F for the positive-strand assay, and 5 pmol of TaqMan probe HCV-P43 (5′FAM-CCCTGTGAGGAACTACTGTCTTCAC-GCAGATAMRA3′). The final volume was adjusted to 50 μL. The samples were promptly set in an ABI PRISM 7700 Sequence Detection System (Applied Biosystems) and incubated at 70 °C for 2 min and then at 94 °C for 2 min. Real-time PCR amplification and data analysis were subsequently performed for 45 cycles (94 °C for 20 s and 62 °C for 1 min). Copy numbers of the 95-base target sequence were determined using the standard curve based on measurements of serial 10-fold dilutions of synthetic positive- and negative-strand HCV RNA. The sensitivity was 2 log copies/reaction for the positive-strand assay and 3 log copies/reaction for the negative-strand assay. The dynamic ranges were 2–7 log copies/reaction and 3–7 log copies/reaction, respectively. In each assay, false detection of an incorrect strand occurred when the amount of incorrect strand added reached 7 log copies. The positive- and negative-strand quantitation before normalization was ≦6.4 and ≦5.9 log copies/100 ng liver RNA, respectively, in this study. Thus, the strand-specificity was unlikely to be affected by an excess of incorrect strands. Self-priming or endogenous priming was ruled out by the lack of amplification product following RT-PCR of total hepatic RNA without primers in the RT mixture. All assays were done in duplicate, and the mean values were obtained. Hepatic RNA samples from the same liver were always measured in the same run.

The HCV-specific primers and probe used are conserved among genotypes. To verify that HCV genotypes 1b, 2a and 2b could be quantified with similar efficiency, high-concentration serum samples of each genotype were obtained from eight patients and diluted to 4.4 log copies/reaction by Amplicor HCV Monitor version 2.0, which is known to equally amplify all genotypes. The positive-strand HCV quantitation by the TaqMan RT-PCR was the same for genotypes 1b (5.3±0.7), 2a (5.5±0.4) and 2b (4.9±0.5 log copies/reaction) ($P=0.141$ by one-way analysis of variance).

2.4. Normalization of hepatic HCV RNA amounts and criteria for between-lobe discrepancies

GAPDH mRNA in total hepatic RNA, 100 ng, and control total RNA (Raji cell line), 100 ng, was also quantified by real-time RT-PCR, and copy numbers were determined using the standard curve (Human GAPD Endogenous Control, Applied Biosystems). Hepatic HCV RNA and GAPDH mRNA quantitation, which were performed in separate tubes, showed a linear relationship with the amounts of target RNA (Fig. 1). The HCV RNA copy number was divided by the ratio of the sample GAPDH mRNA amounts to the TaqMan control value. Thus, normalized hepatic HCV RNA amounts were obtained and used for data analysis. In preliminary experiments, assay variance for the $\log_{10}$ transformed HCV RNA quantitation before normalization was evaluated based on five measurements of 10 liver samples (intra-assay coefficients of variation (CVs)=0.88–2.85% and inter-assay CVs=1.19–6.91% for the positive-strand assay; intra-assay CVs=2.27–9.72% and inter-assay CVs=1.52–18.11% for the negative-strand assay). Assay variance was greater for the negative-strand assay, which may be attributable to interfering factor(s) such as a large amount of positive strands in the RT reaction. The mean SDs of intra-assay variance were 0.106 and 0.081 for <5 and ≧5 log copies, respectively, in the positive-strand assay, whereas they were 0.374, 0.256 and 0.158 for <4, 4–5 and ≧5 log copies, respectively, in the negative-strand assay. The HCV RNA quantitation was assumed to vary within twice these SDs. Between-lobe HCV RNA differences were considered significant when the normalized HCV RNA amounts differed by more than the estimated variance for normalized values. All discrepancies were confirmed by repeating the assays.

2.5. Detection of liver hepatitis B virus (HBV) DNA by nested PCR

Total hepatic DNA, 100 ng, was subjected to nested PCR to amplify HBV DNA. The primers were set in the surface region (outer sense 5′-TCGTGTTACAGGCGGGGTTT-3′; outer antisense 5′-CGAACCACT-GAACAAATGGC-3′; inner sense 5′-CAAGGTATGTTGCCCGTTTG-3′; inner antisense 5′-GGCACTAGTAAACTGAGCCA-3′) and the X region (outer sense 5′-GCATGGAGACCACCGTGAA-3′; outer antisense 5′-CAGACCAATTTATGCCTACAG-3′; inner sense 5′-TACATAAGAG-GACTCTTGGACT-3′; inner antisense 5′-CAGACCAATTTATGCCTA-CAG-3′). PCR products (233 and 151 bp, respectively) were visualized by 3% agarose electrophoresis and ethidium bromide staining. All assays were done in duplicate. The sensitivity was 1 copy/100 ng liver DNA for each primer set. To avoid contamination in all PCR assays, the contamination avoidance measures [6] were strictly applied throughout the study, and positive and negative controls were used.

2.6. Histologic evaluation

After routine staining with hematoxylin–eosin, all liver biopsy specimens were examined by the same experienced pathologist without knowledge of their source. Biopsy specimens were semiquantitatively

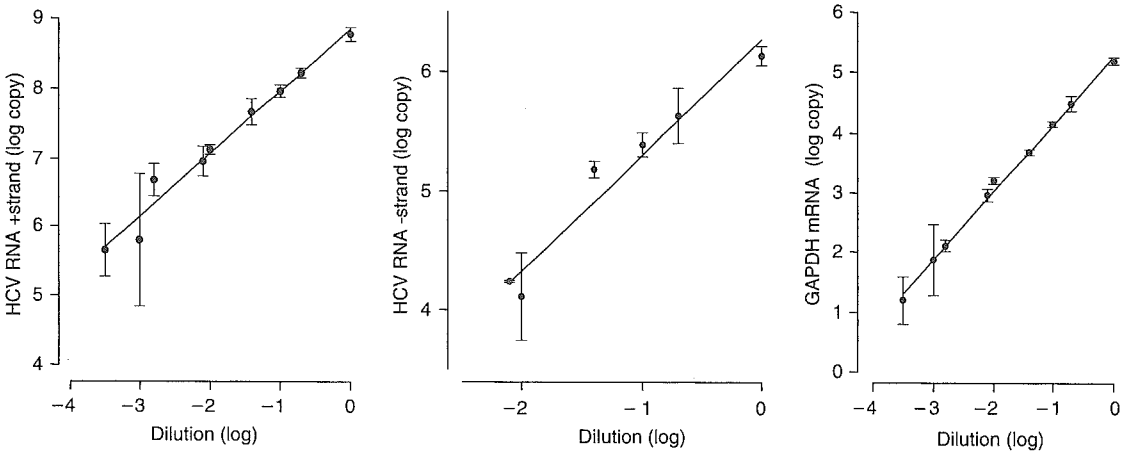

Fig. 1. Changes in hepatic HCV RNA and GAPDH mRNA quantitation in separate tubes according to the amounts of target RNA. Serial dilutions of total hepatic RNA, 100 ng, were subjected to real-time RT-PCR, and copy numbers were determined using the standard curve. The copy number (log) showed a linear relationship ($P<0.001$) with the amounts of target RNA expressed as dilution (log) ($y=8.86+0.90x$ [$n=9$, $r=0.985$] for positive-strand HCV RNA, $y=6.27+0.96x$ [$n=6$, $r=0.976$] for negative-strand HCV RNA and $y=5.27+1.13x$ [$n=9$, $r=0.998$] for GAPDH mRNA). Data are the mean±2SD of triplicate measurements. Pearson's correlation test was performed to examine the relationship.

evaluated using the modified histologic activity index described by Ishak et al. [7].

2.7. Statistical analysis

Viral load was $\log_{10}$ transformed to obtain a more symmetrical distribution without outliers. An arbitrary value of 0 log copy/100 ng liver RNA was attributed to the liver tissues negative by PCR. Data on continuous variables were presented as mean ± SD unless otherwise stated. Statistical analysis for group comparisons was performed using the Wilcoxon nonparametric test. Correlations between the variables were calculated using Spearman rank order correlations. To assess variables potentially related to virologic and histologic between-lobe discrepancies and responses to IFN, stepwise multivariate logistic regression models were used. All analyses were done with SAS (version 8.02) (SAS Institute, Inc., Cary, NC). A P value of less than 0.05 (2-tailed) was considered to indicate significance.

3. Results

3.1. Amounts of positive- and negative-strand HCV RNA in right (R) and left (L) liver lobes

Normalized positive-strand HCV loads in the right liver lobe (median, 5.9; range, 2.5–8.5 log copies/100 ng liver RNA) showed a correlation with those in the left liver lobe (median, 6.0; range, negative to 6.8 log copies/100 ng liver RNA) ($r=0.950$; $P<0.001$) (Fig. 2A). Six (13%) of the 48 patients had a between-lobe discrepancy of 0.3–2.2 log. The discrepancy was related to gender (6 [26%] of 23 women vs. none of 25 men) (odds ratio 10.9 [95% CI 1.3–90.9], $P=0.027$). Fig. 2B shows a correlation between normalized negative-strand HCV loads in the right lobe (median, 4.9; range, negative to 7.2 log copies/100 ng liver RNA) and the left lobe (median, 5.0; range, negative to 6.3 log copies/100 ng liver RNA) ($r=0.920$; $P<0.001$). A discrepancy of 2.0 log was seen in one (2%) patient (Table 2).

In 38 patients with detectable levels of positive and negative strands in each liver lobe, the ratio of negative- to positive-strand HCV (median, 0.14; range, 0.01–0.81 for R and median, 0.13; range, 0.03–0.45 for L) increased according to negative-strand liver HCV ($r=0.282$; $P=0.086$ for R and $r=0.441$; $P=0.006$ for L) (Fig. 3). The ratio showed no correlation with positive-strand liver HCV ($r=-0.192$; $P=0.248$ for R and $r=-0.097$; $P=0.564$ for L) and circulating HCV ($r=0.154$; $P=0.355$ for R and $r=0.106$; $P=0.527$ for L). Serum HCV RNA loads ranged between 3.1 and 7.6 log copies/mL (median, 6.1), and displayed a relation to the positive-strand liver HCV quantitation ($r=0.604$; $P<0.001$ for R and $r=0.634$; $P<0.001$ for L) and the negative-strand liver HCV quantitation ($r=0.632$; $P<0.001$ for R and $r=0.609$, $P<0.001$ for L).

3.2. Determinants of positive- and negative-strand HCV RNA amounts in the liver

The amounts of positive- and negative-strand HCV in each liver lobe were correlated with patient characteristics including age, gender, mode of infection, duration of infection estimated from years after blood transfusion, serum alanine aminotransferase (ALT) levels, histologic grade and stage, HCV genotypes and detection of HBV DNA in the corresponding liver lobe. An inverse correlation was found between the negative-strand liver HCV

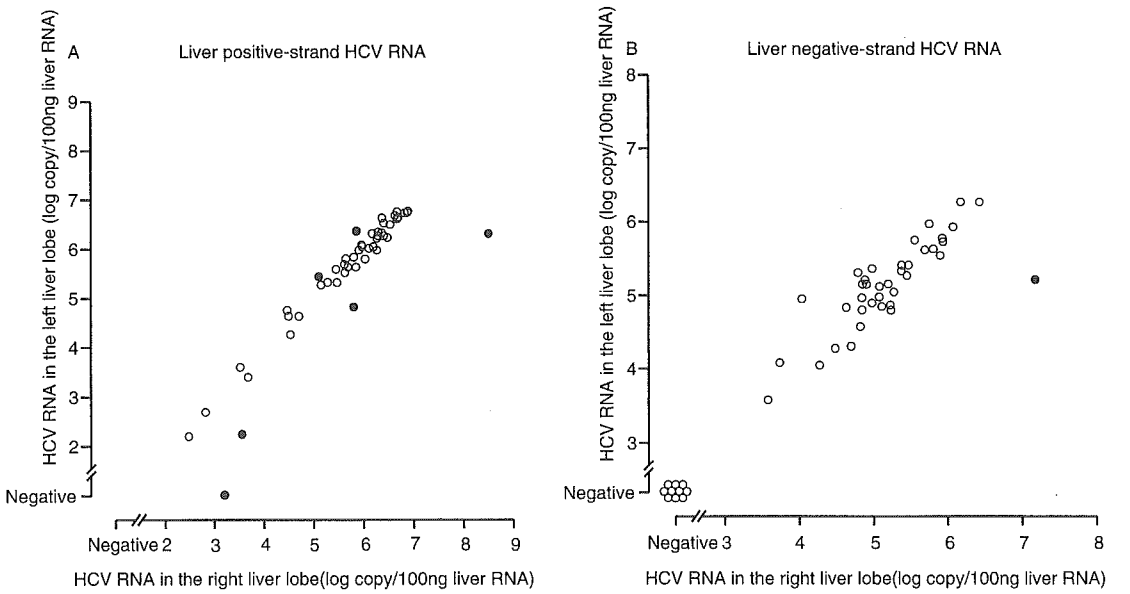

Fig. 2. Correlations between positive-strand HCV RNA levels of the right and left liver lobes ($r=0.950$; $P<0.001$) (A) and between negative-strand HCV RNA levels of each liver lobe ($r=0.920$; $P<0.001$) (B) in the 48 chronic hepatitis C patients. Between-lobe discrepancy of the viral loads was found in six (13%) patients for positive-strand HCV RNA and one (2%) patient for negative-strand HCV RNA (closed circles).

Table 2
Normalized positive- and negative-strand HCV RNA amounts in the right and left liver lobes in 48 chronic hepatitis C patients

Patient	Age (years)	Positive-strand HCV RNA[a]		Negative-strand HCV RNA[a]		HBV DNA[b]	
		Right	Left	Right	Left	Right	Left
1	62	5.96	6.08	5.23	4.80	–	–
2	47	6.88	6.73	6.18	6.26	–	–
3	66	4.48	4.74	3.59	3.58	–	–
4	52	6.68	6.60	5.90	5.53	–	–
5	68	6.38	6.62	5.28	5.04	–	–
6	49	6.04	5.79	4.98	4.89	–	–
7	64	6.11	6.00	4.99	5.36	–	–
8	49	6.54	6.48	5.94	5.72	–	–
9	54	8.51	6.30[c]	7.18	5.20[c]	–	–
10	46	6.30	6.26	5.23	4.86	–	–
11	67	4.49	4.63	4.28	4.04	–	–
12	57	5.80	5.83	5.08	4.97	–	–
13	53	4.53	4.25	–	–	–	–
14	48	5.69	5.62	4.83	4.57	–	–
15	48	5.97	6.04	4.91	5.15	–	–
16	57	5.48	5.30	4.71	4.30	–	–
17	33	6.89	6.76	6.08	5.92	–	+
18	57	5.65	5.79	4.64	4.83	–	–
19	69	3.68	3.38	–	–	–	–
20	64	6.41	6.26	5.11	4.84	–	–
21	59	6.20	6.04	5.38	5.40	–	–
22	56	5.86	6.36[c]	5.76	5.97	–	–
23	67	5.45	5.57	4.86	5.15	–	–
24	38	6.28	6.20	5.81	5.63	–	–
25	48	2.49	2.18	–	–	–	–
26	60	2.83	2.68	–	–	–	–
27	48	5.28	5.30	4.04	4.95	–	–
28	68	5.80	4.80[c]	–	–	–	–
29	44	5.62	5.68	4.85	4.96	–	–
30	43	3.20	–[c]	–	–	–	–
31	58	6.70	6.64	5.46	5.26	–	–
32	56	6.63	6.67	6.43	6.26	–	–
33	45	6.28	5.97	5.20	5.15	–	–
34	50	6.36	6.32	5.93	5.77	+	+
35	63	5.11	5.43[c]	4.49	4.28	–	–
36	70	4.71	4.62	–	–	–	–
37	52	6.83	6.72	4.80	5.30	–	–
38	41	3.56	2.23[c]	–	–	–	–
39	51	6.41	6.52	5.70	5.62	+	+
40	67	5.64	5.51	–	–	–	–
41	61	3.52	3.57	–	–	–	–
42	52	5.91	5.97	5.08	5.11	–	–
43	59	6.18	6.30	5.38	5.32	–	–
44	57	6.48	6.23	5.48	5.40	–	–
45	67	5.15	5.26	3.75	4.08	–	–
46	58	6.68	6.73	5.56	5.75	+	+
47	62	6.30	6.34	4.89	5.20	–	–
48	66	5.85	5.63	4.85	4.79	–	–

[a] Hepatic HCV RNA amounts were normalized to GAPDH mRNA amounts and expressed as log copy/100 ng liver RNA.
[b] Liver HBV DNA was detected by nested PCR using two sets of primers in the surface and X regions, respectively.
[c] Between-lobe differences in the normalized HCV RNA amounts were considered significant according to the intra-assay variance-based criteria.

quantitation and age ($r=-0.322$; $P=0.026$ for R and $r=-0.340$; $P=0.018$ for L). The positive-strand liver HCV quantitation, however, had no relation to age ($r=-0.237$; $P=0.104$ for R and $r=-0.216$; $P=0.140$ for L) (Fig. 4). The amounts of positive- and negative-strand liver HCV did not differ between 38 patients with HCV genotype 1b and 10 patients with genotype 2 (seven with genotype 2a and three with genotype 2b), but were affected by concomitant liver HBV. By using X primers, HBV DNA was detected in both liver lobes in three patients and only in the left lobe in another patient. None of the patients tested positive for liver HBV DNA using surface primers. The four

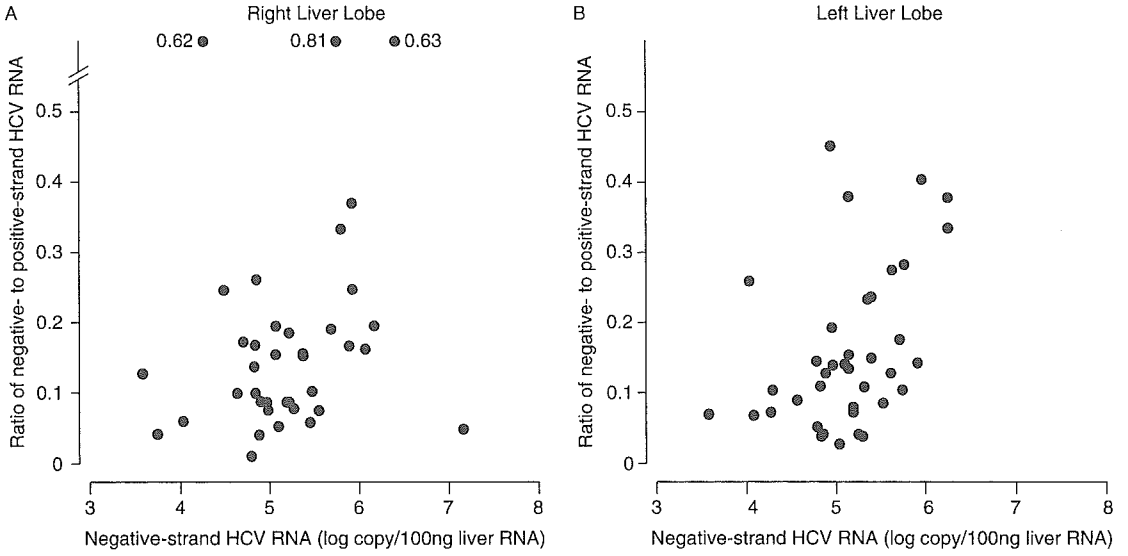

Fig. 3. Direct variation of ratios of negative- to positive-strand HCV RNA in liver tissues in relation to HCV replication, assessed by negative-strand HCV RNA in the right liver lobe ($r=0.282$; $P=0.086$) (A) and the left liver lobe ($r=0.441$; $P=0.006$) (B).

HBV DNA-positive liver tissue samples from the left lobe contained larger amounts of positive and negative strands than the 44 HBV DNA-negative tissues (6.6 ± 0.2 vs. 5.4 ± 1.4; $P=0.007$ and 5.8 ± 0.1 vs. 3.9 ± 2.2 log copies/100 ng liver RNA; $P=0.006$, respectively). For the right liver lobe, the positive- and negative-strand liver HCV quantitation also tended to be high in the three HBV DNA-positive liver tissues (6.5 ± 0.2 vs. 5.6 ± 1.2; $P=0.081$ and 5.7 ± 0.2 vs. 4.0 ± 2.3 log copies/100 ng liver RNA; $P=0.049$, respectively). None of the patient characteristics examined showed a relationship to the ratio of negative- to positive-strand HCV and serum HCV RNA load.

3.3. Histologic variation between right and left liver lobes

The total necroinflammatory grade ranged between 2 and 10 (median 7) in each liver lobe ($P=0.295$ by signed rank test). The fibrosis stage ranged from 1 to 6 (median 4) in the right lobe and from 2 to 6 (median 3) in the left lobe ($P=0.614$). Fig. 5 shows the histologic between-lobe variation among the 47 patients studied. Eleven (23%) patients showed differences of the necroinflammatory grade defined as a difference of $\geqq 2$ points, and 19 (40%) patients of the fibrosis stage defined as difference of $\geqq 1$ point. The between-lobe variation in the HCV quantitation had no impact on the histologic variation. The mean grading score of the right and left liver lobes was <7 in 10 (91%) out of the 11 patients with a grade difference compared with 16 (44%) out of the 36 patients without it (odds ratio 6.5 [95% CI 1.3–33.3], $P=0.025$). The difference in the fibrosis stage, however, had no relation to any of the patient characteristics examined.

3.4. Factors influencing the efficacy of IFN treatment

Eighteen (67%) out of the 27 patients were negative for serum HCV RNA at the end of treatment, and eight (30%) patients displayed sustained HCV clearance over 6 months posttreatment. The end-of-treatment virologic response was independently associated with an absence of between-lobe discrepancy of the necroinflammatory grade (odds ratio 0.2 [95% CI 0–0.9], $P=0.042$). However, the amounts of negative-strand HCV RNA in the liver were identified as the only independent predictor of a sustained virologic response. The mean negative-strand quantitation of the right and left liver lobes was <4 log copies/100 ng liver RNA in all sustained virologic responders (SVRs) compared with 1 (5%) of the 19 non-SVRs (odds ratio 85.4 [95% CI 5.4–999], $P=0.002$).

4. Discussion

Little has been known about the clinical significance of quantifying negative-strand RNA-replicative intermediates in the liver. The present study analyzed the ratio of liver negative- to positive-strand RNA. This ratio is the most reliable parameter since it does not depend on genotypes or normalization to the cellular GAPDH mRNA quantitation. For each liver lobe, the median ratio of 0.1 was similar to that found with cell-based HCV replicon systems [8]. Importantly, it was disclosed that the ratio was not constant but varied by 2 log values in relation to the intrahepatic HCV-replicative status. These observations suggest that the negative-strand quantitation is not merely a reflection of

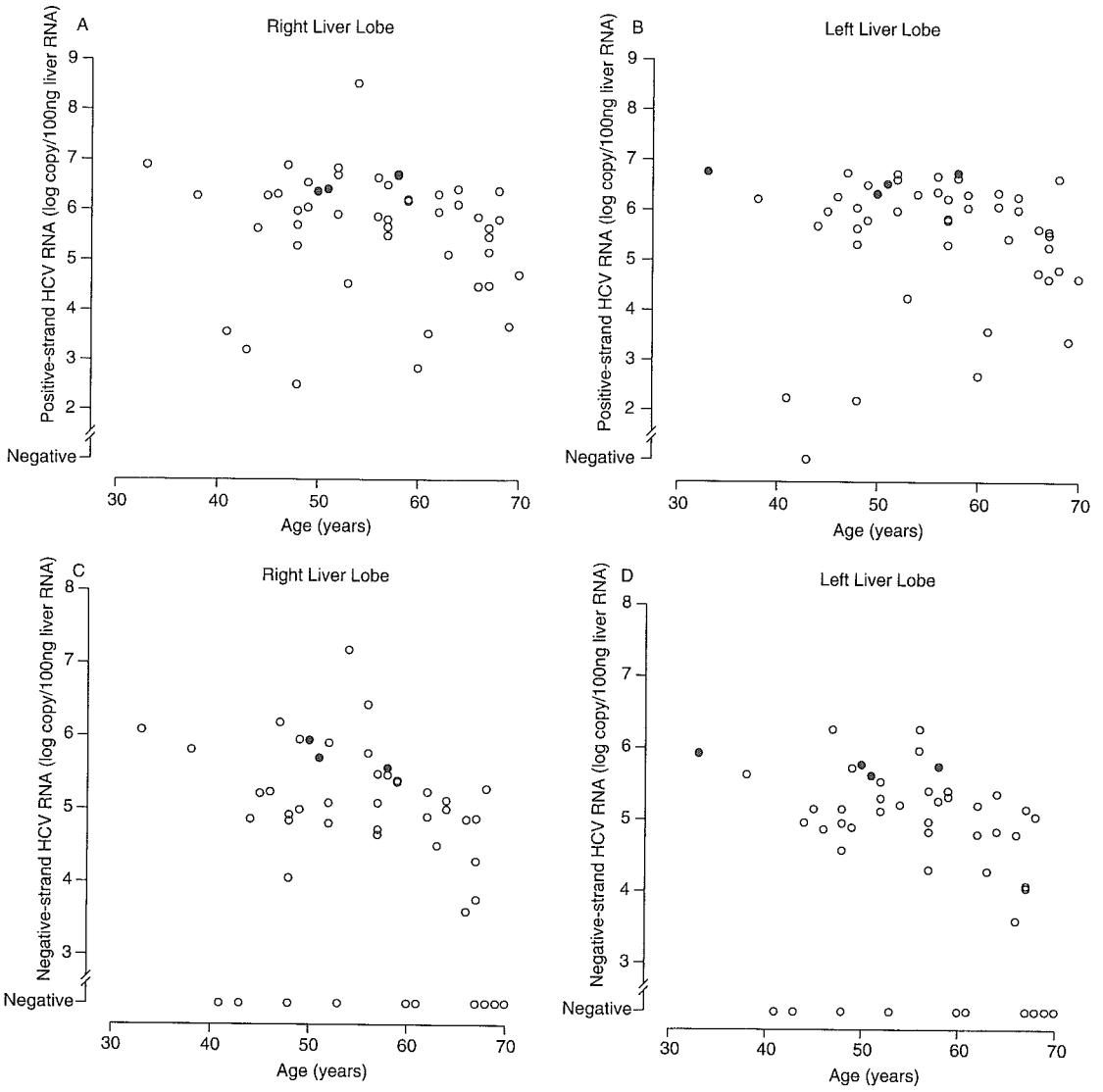

Fig. 4. Liver HCV RNA levels in relation to age and occult HBV infection in 48 chronic hepatitis C patients. No relationship was evident between age and positive-strand HCV RNA levels of the right liver lobe ($r=-0.237$; $P=0.104$) (A) and the left liver lobe ($r=-0.216$; $P=0.140$) (B), whereas inverse correlations were found between age and negative-strand HCV RNA levels of the right liver lobe ($r=-0.322$; $P=0.026$) (C) and the left liver lobe ($r=-0.340$; $P=0.018$) (D). HBV DNA-positive liver tissues (closed circles) contained higher levels of positive-strand HCV RNA ($P=0.081$ for the right liver lobe and $P=0.007$ for the left liver lobe) and negative-strand HCV RNA ($P=0.049$ for the right liver lobe and $P=0.006$ for the left liver lobe).

liver positive strands but should serve as a distinct HCV replicative marker.

Chronic hepatitis C is known as a disease with uneven distribution of lesions in the whole liver [9]. Previous studies have shown a correlation between positive-strand HCV RNA levels of the right and left liver lobes [9,10]. The present study demonstrated a close correlation between lobes not only for positive strands but also for negative strands. Thus, HCV replication within the liver was shown to be uniform, and a single biopsy seemed generally representative of the whole liver. Although the between-lobe variation of HCV RNA loads should be interpreted with caution when the difference is small, it was only found in women, raising a possibility that sex hormone(s) and sex-linked genetic factor(s) are involved in the heterogeneity of HCV replication. In the present study, the amounts of positive- and negative-strand HCV and the ratio of negative to positive strands showed no correlation with the necroinflammatory grade and the fibrosis stage. However, we must stress the possibility that the HCV replication level, especially that assessed by negative strands, may have some relevance to histologic features such as steatosis [4].

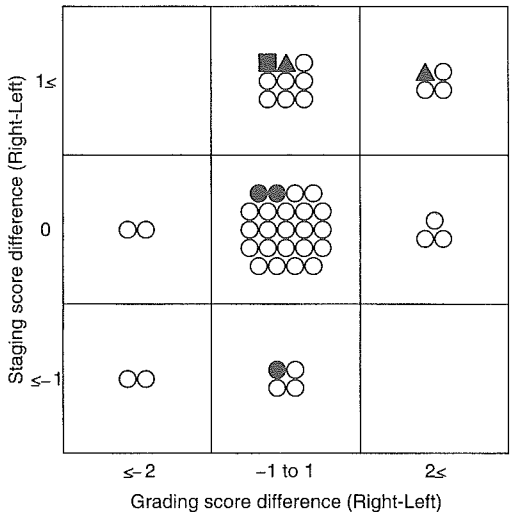

Fig. 5. Histologic and virologic discrepancies between the right and left liver lobes in chronic hepatitis C patients. ○, no between-lobe HCV RNA discrepancy; ●, larger positive-strand amounts in the right lobe; ■, larger positive- and negative-strand amounts in the right lobe; ▲, larger positive-strand amounts in the left lobe.

Factors affecting HCV replication within the liver have been the subject of controversial discussions from the standpoint of the liver and circulating positive strands. Based on the negative-strand level, HCV replication in each liver lobe was shown to be inversely correlated with age. The efficiency of negative-strand RNA synthesis can be influenced by various host factors at multiple levels [11]. The data obtained raise the possibility that some age-related factor(s) may be involved in the regulation of HCV replication within the liver. The present study further showed that liver tissues with concomitant occult HBV contained larger amounts of negative- and positive-strand HCV RNA. Among HCV patients, those carrying occult HBV can manifest severer liver disease and display a poor response to IFN [12]. Occult HBV may also have relevance for hepatocarcinogenesis [13], although the mechanism remains to be clarified. Although further studies are necessary, the data obtained raise the possibility that occult HBV exerts virulence partly by enhancing HCV replication.

As for IFN-based therapy, only limited data are available on the significance of the liver negative-strand HCV RNA quantitation. In a previous semiquantitative study, the negative-strand levels were not related to the outcomes of short-term IFN-α therapy (3 MU thrice weekly for 10 weeks) [2]. Our patients were treated with 6-month enhanced IFN monotherapy [14]. A sustained virologic response was only associated with small amounts of liver negative-strand HCV RNA (<4 log copies/100 ng liver RNA). Based on these preliminary data, further studies are warranted in populations treated with the currently standard regimen of peginterferon and ribavirin.

In conclusion, our findings combined indicate that liver negative-strand HCV RNA quantitation offers clinically relevant information distinct from that available from positive strands within the liver and in the circulation.

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References

[1] Negro F, Giostra E, Krawczynski K, Quadri R, Rubbia-Brandt L, Mentha G, et al. Detection of intrahepatic hepatitis C virus replication by strand-specific semi-quantitative RT-PCR: preliminary application to the liver transplantation model. J Hepatol 1998;29:1–11.

[2] Negro F, Krawczynski K, Quadri R, Rubbia-Brandt L, Mondelli M, Zarski J-P, et al. Detection of genomic- and minus-strand of hepatitis C virus RNA in the liver of chronic hepatitis C patients by strand-specific semiquantitative reverse-transcription polymerase chain reaction. Hepatology 1999;29:536–542.

[3] Pelletier SJ, Raymond DP, Crabtree TD, Berg CL, Iezzoni JC, Hahn YS, et al. Hepatitis C-induced hepatic allograft injury is associated with a pretransplantation elevated viral replication rate. Hepatology 2000;32:418–426.

[4] Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Malé P-J, Mentha G, et al. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. J Hepatol 2000;33:106–115.

[5] Ohno T, Mizokami M, Wu R-R, Saleh MG, Ohba K, Orito E, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 1997;35:201–207.

[6] Kwok S, Higuchi R. Avoiding false positives with PCR. Nature 1989; 339:237–238.

[7] Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22:696–699.

[8] Gu B, Gates AT, Isken O, Behrens S-E, Sarisky RT. Replication studies using genotype 1a subgenomic hepatitis C virus replicons. J Virol 2003;77:5352–5359.

[9] Fanning L, Loane J, Kenny-Walsh E, Sheehan M, Whelton M, Kirwan W, et al. Tissue viral load variability in chronic hepatitis C. Am J Gastroenterol 2001;96:3384–3389.

[10] Idrovo V, Dailey PJ, Jeffers LJ, Coelho-Little E, Bernstein D, Bartholomew M, et al. Hepatitis C virus RNA quantification in right and left lobes of the liver in patients with chronic hepatitis C. J Viral Hepatitis 1996;3:239–246.

[11] Ahlquist P, Noueiry AO, Lee W-M, Kushner DB, Dye BT. Host factors in positive-strand RNA virus genome replication. J Virol 2003; 77:8181–8186.

[12] Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. N Engl J Med 1999;341:22–26.

[13] Shibata Y, Nakata K, Tsuruta S, Hamasaki K, Hayashida Y, Kato Y, et al. Detection of hepatitis B virus X-region DNA in liver tissue from patients with hepatitis C virus-associated cirrhosis who subsequently developed hepatocellular carcinoma. Int J Oncol 1999;14:1153–1156.

[14] Asahina Y, Izumi N, Uchihara M, Noguchi O, Tsuchiya K, Hamano K, et al. A potent antiviral effect on hepatitis C viral dynamics in serum and peripheral blood mononuclear cells during combination therapy with high-dose daily interferon alfa plus ribavirin and intravenous twice-daily treatment with interferon beta. Hepatology 2001;34:377–384.

B型慢性肝炎の病態をどう把握し，治療方針を立てるか？

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はじめに●

B型肝炎ウイルス(HBV)キャリアはHBe抗原陽性無症候性キャリアから慢性肝炎，肝硬変，肝細胞癌あるいは臨床的治癒とされているHBe抗体陽性無症候性キャリアまでさまざまな病態が存在する．そして，その経過もさまざまであるが，大別すると肝硬変，肝細胞癌に進行する群と臨床的治癒の状態に落ち着く群に2分される．これらHBVキャリアのそれぞれが現在どの病期にいるのか，発癌リスクはどの程度であるのか，積極的な治療の必要性はあるのか，そしてあるならどのような治療を選択すべきかという問いに対処するため，われわれはHBVキャリアのステージ分類を提唱した[1]．

HBVキャリアのステージ分類●

1995年11月以降に当院を初診したHBVキャリア207例を対象にHBVキャリアを8ステージに分類した(**表1**)．対象の性別は男性138例，女性69例で，平均年齢はそれぞれ44.3±13.4歳，42.8±15.6歳であった．

HBステージ0：HBs抗原陽性，HBe抗原陽性，ALT正常値持続のいわゆる無症候性キャリアの状態．

HBステージI：HBs抗原陽性，HBe抗原陽性，ALT異常値(持続正常以外)でHBV-DNA量が$10^{7.6}$copies/mL以上の高ウイルス群．若年例(男性：30歳未満，女性：35歳未満)をステージIa，高年例(男性：30歳以上，女性：35歳以上)をステージIbとする．

HBステージII：HBs抗原陽性，HBe抗原陽性，ALT異常値(持続正常以外)でHBV-DNA量が$10^{7.6}$copies/mL未満の低ウイルス群．若年例をステージIIa，高年例をステージIIbとする．

HBステージIII：HBs抗原陽性，HBe抗原陰性，HBV-DNA10^5copies/mL以上のプレコア変異株の増殖が持続していると考えられる群である．

HBステージIV：HBs抗原陽性，HBe抗原陰性，HBV-DNA10^5copies/mL未満のいわゆる臨床的治癒の状態である．

HBステージV：HBキャリア(HBs抗原陽性の時期が確認されている例)でHBs抗原が消失した状態である．

各ステージの例数，性別，平均年齢，ALT値，血小板数および発癌率は**表2**に示す．HBe抗原

表1　HBVキャリアのステージ分類

HBステージ	0	I	II	III	IV	V
HBsAg	＋	＋	＋	＋	＋	−**
HBeAg	＋	＋	＋	−	−	−
HBV-DNA (copies/mL)	不問	$10^{7.6}\leqq$	$10^{7.6}>$	$10^5\leqq$	$10^5>$	不問
ALT	持続正常	持続正常以外	持続正常以外	不問	不問	不問
年齢	不問	若年/高年* (Ia/Ib)	若年/高年* (IIa/IIb)	不問	不問	不問
発癌リスク	きわめて小	小/大	小/きわめて大	きわめて大	きわめて小	きわめて小

*若年：男性30歳未満，女性35歳未満
高年：男性30歳以上，女性35歳以上
**HBsAg(＋)の時期が確認されていること

1345-1551/06/￥100/頁/JCLS

●臨床的治癒コースはステージ Ia から IIa となり，速やかにステージ IV に移行する．
●病態進展コースはステージ Ia から Ib，IIb と進行し，
III までは到達するが IV には至らない．
●ステージ III とステージ IV は時間的経過の差ではなくて，
病態の異なる集団である．

表2　各 HB ステージの背景因子と発癌率

HB ステージ	0	Ia	Ib	IIa	IIb	III	IV
例数(%)	9 (4.3)	23 (11.1)	44 (21.3)	10 (4.8)	31 (15.0)	49 (23.7)	41 (19.8)
性別 (男性/女性)	3/6	16/7	32/12	4/6	24/7	38/11 **	21/20 **
年齢(歳)	34.4±9.1	25.5±3.4	44.8±11.0	24.0±2.5	48.5±9.8	53.1±9.7 **	45.6±15.7 **
ALT (IU/L)	17.7±4.4	129.0±101.4	193.6±204.2	105.6±80.3	130.5±194.2	117.2±112.3 ***	41.0±39.7 ***
血小板数 ($\times 10^4$)	20.4±4.2	20.1±3.6	16.5±6.2	18.1±4.3	15.4±7.9	14.4±5.9 ***	19.3±7.5 ***
初診時発癌 (−/+)	9/0	23/0	44/0	9/1	24/6	39/10	35/6
初診後発癌例	0	0	3	0	4	9	1
発癌率(%)	0	0	6.8	0	16.7	23.1 *	2.9 *

* p<0.05, ** p<0.01, *** p<0.001

陰性期のステージ III とステージ IV を比較すると，平均年齢はステージ IV が有意($p<0.01$)に若年齢であり，性別は女性は有意($p<0.01$)にステージ III 例で少数であった．また，ALT 値はステージ IV が有意($p<0.001$)に低値であった．ステージ III とステージ IV はステージ III からステージ IV へと移行するという時間的経過の差ではなくて，病態の異なる集団と考えられる．HBV キャリアの大多数が歩む臨床的治癒の状態へのコースはステージ Ia からステージ IIa となり，その後短期間ステージ III を経由した後速やかにステージ IV に移行するものと考えられる．そしてステージ IV が長期間続いた後 HBs 抗原が消失し，ステージ V となる．一方，肝硬変進展・肝癌発癌ハイリスク群はステージ Ia からステージ Ib，ステージ IIb と進行し，HBe 抗原が陰性化してステージ III までは到達するが HBV の増殖は持続し，ステージ IV に至ることはない(図1)．臨床的治癒コースの各ステージにおける初診時の血小板数と発癌リスクは，ステージ 0，Ia，

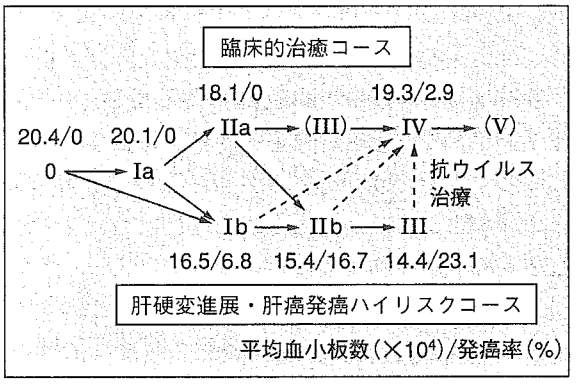

図1　HBV キャリアの経過(臨床的治癒コースと肝硬変進展・肝癌発癌ハイリスクコース)

IIa および IV でそれぞれ 20.4 万，0%，20.1 万，0%，18.1 万，0% および 19.3 万，2.9% とほとんど変化を認めないが，肝硬変進展・肝癌発癌ハイリスクコースにあたるステージ Ib，IIb および III ではそれぞれ 16.5 万，6.8%，15.4 万，16.7% および 14.4 万，23.1% とステージの移行に従っての血小板数の低下と発癌率の増加が認められ，ステージ Ib，IIb および III のキャリアに対する抗ウイルス治療の必要性が強く示唆される．

●ステージ Ib，IIb および III は肝硬変進展・肝癌発癌のハイリスクコースである．
●ステージ III では CP，Pre C 両領域ともに変異型が有意に高率である．
●ステージ IV では両領域で野生型の残存率が高く，
ウイルス量の減少による HBe 抗原消失と考えられる．

肝癌発癌例，肝予備能低下例と HB ステージ分類●

各ステージ別の発癌率はステージ 0 0%，ステージ Ia 0%，ステージ Ib 6.8% (3/44)，ステージ IIa 0%，ステージ IIb 16.7% (4/24)，ステージ III 23.1% (9/39)，ステージ IV 2.9% (1/35) であった．ステージ Ib，ステージ IIb およびステージ III は B 型肝癌発癌のハイリスク群で積極的に抗ウイルス治療を行う必要がある．また全発癌例 (初診時有発癌例を含む) における性別および発癌確認時の年齢，ALT 値についてみると，性差は，男性 24.6% (138 例中 34 例)，女性 10.1% (69 例中 7 例) と男性で有意に発癌率が高率 ($p < 0.02$) であった．発癌例の年齢分布は 50 歳代が 55.0% と最も多く，60 歳代，40 歳代がそれぞれ 17.5%，15.0% で 40 歳未満は 25 歳と 35 歳の 2 例のみであった．また，発癌確認時の ALT 値は 30IU/L 未満が 6 例 (15.0%)，40IU/L 未満 12 例 (30.0%) および 50IU/L 未満 19 例 (47.5%) と ALT 低値例が約半数を占めた．

また，初診時血小板数 10 万未満例を肝予備能低下例とすると，各ステージ別の肝予備能低下例の割合はステージ 0 0%，ステージ Ia 4.3% (1/23)，ステージ Ib 13.6% (6/44)，ステージ IIa 0%，ステージ IIb 25.8% (8/31)，ステージ III 26.5% (13/49)，ステージ IV 7.3% (3/41) であった．発癌例と同様にステージ Ib，ステージ IIb およびステージ III において肝予備能低下例が高率に認められた．

プレコア，コアプロモーター変異と HB ステージ分類●

対象 207 例中 111 例においてプレコア (PreC) およびコアプロモーター (CP) 変異について検討した．各ステージにおける野生型の割合は PreC 領域，CP 領域でそれぞれ，ステージ 0 100%，75.0%，ステージ Ia 66.7%，33.3%，ステージ Ib 65.2%，34.8%，ステージ IIa 42.9%，14.3%，ステージ IIb 53.3%，13.3%，ステージ III 3.7%，7.4%，ステージ IV 37.5%，31.8% であった．HBe 抗原陰性期でもステージ III とステージ IV では様相が異なり，PreC 領域では野生型と変異型がステージ III ではそれぞれ 1 例，15 例であるが，ステージ IV では 9 例，12 例とステージ III で変異型が有意 ($p < 0.05$) に高率であった．また，CP 領域でも野生型と変異型を比較すると，ステージ III ではそれぞれ 2 例，25 例であるが，ステージ IV では 7 例，13 例とステージ III で PreC 領域と同様，変異型が有意 ($p < 0.05$) に高率であった．PreC 領域と CP 領域のいずれかが野生株である率はステージ III ではわずか 11.1% (3/27) であったが，ステージ IV では 52.0% (13/25) と過半数を占めた．臨床的治癒期と考えられるステージ IV では両領域で野生型の残存率が高く，ウイルス量の減少によって HBe 抗原が消失した例が多いことを示す成績と考えられる．

HBV genotype と病態との関連●

HBV は分子進化学の発展により A 型から H 型までの 8 種の genotype に分類されている．Orito らのわが国における genotype 分布の解析[2] によると，沖縄と東北地方には genotype B が多く，それ以外の地域では genotype C が大半を占めており，わが国全体の比率としては genotype B が 12.2%，genotype C が 84.7% であった．genotype B は genotype C に比し予後良好と考えられており，PreC 領域と CP 領域の変異の有無についての検討でも，変異型は genotype B の 16% に比し genotype C では 58% と genotype C で有意に高率と報告されている[3]．当院で無作為に抽

●ステージ Ib では若年齢を過ぎても HBV-DNA 量高値が持続し，抗ウイルス薬治療が必要である．
●ステージ IIb 全例とステージ III の ALT 異常男性例は抗ウイルス治療の絶対適応である．
●ステージ III の発癌数は全ステージ中最大で，ALT の正異に関係なく発癌例がみられる．

出したB型慢性肝疾患60例中56例(93.3%)はgenotype Cであり，その他はgenotype A，B，FおよびB+Cが1例ずつであった．大阪でもB型慢性肝疾患の大半はgenotype Cであり，前述のPreC，CP変異とステージ分類との関係もgenotype Cのキャリアにおいての成績と考えられるが，genotype Bのキャリアでは変異型が有意に低値とのことで，HBe抗原陰性期でのステージIIIの比率がきわめて低率ではないかと推察される．

HBステージ分類と抗ウイルス治療の必要性●

ステージIaはステージ0の無症候性キャリアが肝炎期に移行した状態のすべてのHBキャリアが通過する高ウイルスのステージであり，発癌リスクがきわめてまれで通常は抗ウイルス治療の必要はない．しかし，組織学的に線維化ステージがF2以上に進行している例は早期に肝硬変に進展する可能性があり，抗ウイルス治療の適応と考えられる．ALT値が高値を持続する例は通常HBV-DNA量が減少しステージIIaとなるが，ステージIIaからは若年発症のB型肝癌例があり，ALT値持続高値例は抗ウイルス治療の適応となる．Ia，IIaとも薬剤としては若年で免疫応答が良好であるのでインターフェロン(IFN)が第一選択となると考える．ステージIbは若年齢を過ぎてもHBV-DNA量の高値が持続する群で，発癌リスクはステージIIbよりは低頻度であるがリスク大で抗ウイルス治療の必要がある．Suzukiら[4]は多変量解析によって，高ウイルス群であることがYMDD変異株出現に最も寄与する因子であることを報告しており，ラミブジン(ゼフィックス®)単独での治療効果の持続は困難で，エンテカビルなどの抗ウイルス効果の強い薬剤あるいは併用治療が適応になると考えられる．ステージIIbは発癌リスクがきわめて大で抗ウイルス治療の絶対適応である．薬剤はラミブジンなどの核酸アナログ単独あるいはIFN，HBワクチンとの併用の選択が考えられる．ステージIIIの発癌数は全ステージ中最大でALT値の正異に関係なく発癌例がみられる．受診キャリア中の頻度も最大で，全例に対して治療が必要かどうかは今後の検討課題と考えられるが，少なくともALT値異常の特に男性例は絶対適応であろう．薬剤は高年例が大半を占め，ラミブジンの治療効果が良好でYMDD変異株の出現も低率であるため，現在のところラミブジンが第一選択であり，YMDD変異株出現例にはアデホビル(ヘプセラ®)などの他の核酸アナログの併用あるいは切り替えで対応できると考えられる．ステージIVはいわゆる臨床的治癒といわれる病態で，抗ウイルス治療の最終目標である．まれに発癌例を認めるが，治療の対象にはならない．ステージVも非B非C肝癌におけるオカルトB型肝癌の問題も残るが抗ウイルス治療の対象にはならないと考えられる．

おわりに●

B型肝癌発癌抑止のためには，HBVキャリアがどの病期にいるかを診断することが肝要である．われわれが提唱したこのHBステージ分類はその診断に有用と考える．治療適応例には早期に適切な抗ウイルス治療を開始し，発癌例を1名でも減少させたいと考えている．

文　献

1) 加藤道夫，伊与田賢也，結城暢一ほか：HBマーカーと発癌リスクよりみたHBVキャリアのステージ分類—適切な抗ウイルス治療の選択に向けて—．肝臓 45：581-588, 2004
2) Orito, E., Ichida, T., Sakugawa, H. et al.: Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV

infection in Japan. Hepatology **34** : 590-594, 2001
3) Orito, E., Mizokami, M., Sakugawa, H. et al. : A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. Hepatology **33** : 218-223, 2001
4) Suzuki, F., Tsubota, A., Arase, Y. et al. : Efficasy of lamivudine therapy and factors associated with emergence of resistance in chronic hepatitis B virus infection in Japan. Intervirology **46** : 182-189, 2003

Late Liver-Related Mortality From Complications of Transfusion-Acquired Hepatitis C

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Although several cohort studies have been reported in individuals with chronic hepatitis C virus (HCV) infection, little is known about liver-related mortality among the elderly. We conducted a cohort study in 302 patients with tuberculosis sequelae who had received a blood transfusion at a young age and had subsequently been treated at a chest clinic. The cohort consisted of 147 patients with antibody to HCV (anti-HCV), of whom 81% were positive for HCV RNA, and 155 without anti-HCV. The cohort was followed for a mean duration of 5.7 years. There were no differences between the two groups in the mean age of the patients at the time of transfusion (31 vs. 34 years) or at the time of entry into the study (65 vs. 66 years). The outcome of 143 patients with, and 145 without, anti-HCV could be traced; 92 (64%) and 82 (57%) had died, respectively. The main cause of death was tuberculosis sequelae in 61 (42%) and 66 (46%) patients, respectively. Eight (6%) of the 143 patients with anti-HCV died of liver disease (hepatocellular carcinoma: seven; rupture of varices: one). The average annual mortality from liver disease from study entry in the patients with anti-HCV was 9.8 per 1,000 person-years. The patients with anti-HCV had a significantly lower cause-specific survival probability for liver disease (92% vs. 100% at 10 years, $P < .005$). In conclusion, in our study, liver-related mortality appeared to be high among elderly HCV-infected individuals. (HEPATOLOGY 2005;41:819-825.)

The clinical features and prognosis of individuals with chronic hepatitis C virus (HCV) infection vary widely. Some persons suffer from chronic progressive liver disease, which may eventually develop into cirrhosis and hepatocellular carcinoma (HCC).[1-5] Others show persistently normal levels of serum alanine aminotransferase (ALT) and remain without clinical symptoms.[6,7] It is difficult to determine the prognosis of persons with chronic HCV infection.. Although a limited number of cohort studies have been reported,[8-14] most have been conducted in subjects whose average age was 45 years or younger[8-13]; the prognosis for individuals in the older population remains unclear.

To evaluate the impact of HCV infection on the liver-related mortality in the older population, we conducted a study in a cohort of patients aged 52 years and older, diagnosed with tuberculosis sequelae and positive for antibody to HCV (anti-HCV), who had received a blood transfusion at a young age. As a control, we used a group of tuberculosis sequelae patients who were negative for anti-HCV. The condition *tuberculosis sequelae* refers to any complication related to pulmonary tuberculosis that has appeared after the infection has been cured, such as pulmonary dysfunction, cor pulmonale, or pulmonary mycosis. In general, these conditions are much more serious in patients who have undergone chest surgery than in those who have not. In Japan, most blood for transfusion was obtained commercially between 1951 and 1967, which is known to have frequently caused hepatitis.[15,16]

Patients and Methods

Patients and HCV Infection. A total of 328 tuberculosis sequelae patients, receiving care at the Department of Respiratory Disease in the Tokyo National Hospital (previously called the National Tokyo Sanatorium) between July 1989 and June 1995 had received surgery for pulmonary tuberculosis between 1946 and 1990. Of them, 306 patients who had received a blood transfusion at that time were enrolled in the study; four of the enrolled

Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; ALT, alanine aminotransaminase; EIA, enzyme-linked immunosorbent assay; RIBA, recombinant immunoblot assay.

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Table 1. Characteristics of Patients With Tuberculosis Sequelae With or Without Anti-HCV

Features	Anti-HCV(+) (n = 147)	Anti-HCV(−) (n = 155)	Differences
Male	98 (67%)	88 (57%)	
Age (years) (range)	65.2 ± 6.2 (52-83)	65.7 ± 7.4 (44-85)	
40-49	0	4 (3%)	
50-59	23 (16%)	20 (13%)	
60-69	88 (60%)	86 (54%)	
70-79	34 (23%)	38 (25%)	
80-89	2 (1%)	7 (5%)	
Age at transfusion (range)	31.4 ± 8.1 (15-62)	33.5 ± 11.5 (13-64)	
Period after transfusion until study entry (years) (range)	33.7 ± 6.5 (8-47)	32.1 ± 10.2 (2-48)	
Year of transfusion (range)	1959 ± 6.3 (1946-1982)	1961 ± 9.9 (1946-1990)	
<1950*	9 (6%)	17 (11%)	
1951-1967†§	128 (87%)	107 (69%)	
>1968‡§	10 (7%)	31 (20%)	
Alcohol consumption (>50 g/day)	6 (4%)	5 (3%)	
ALT (IU/L)	38.8 ± 35.3	16.3 ± 15.1	$P<.0001$
≤34	85 (58%)‖	146 (94%)	
35-68	41 (28%)	7 (5%)	
≥69	21 (14%)	2 (1%)	
Albumin (g/100 mL) (range)	4.04 ± 0.40 (2.8-4.9)	4.10 ± 0.43 (2.4-4.9)	
Platelets ($\times 10^4/mm^3$) (range)	18.6 ± 5.9 (7.2-35.1)	22.7 ± 5.9 (11.2-43.2)	$P<.0001$

NOTE: Anti-HCV(+): One hundred nineteen HCV RNA-positive patients and 28 anti-HCV by recombinant immunoblot assay (RIBA)-positive patients. Anti-HCV(−): Patients who were negative for anti-HCV by ELISA or RIBA in serum. Mean ± SD is shown for continuous variables.

* Both commercial and donated blood was used for transfusion.

† Most blood was obtained commercially.

‡ Almost all blood was provided by volunteer donor.

§ The prevalence of anti-HCV was significantly higher in patients who received transfusion between 1951 and 1967 than in those receiving it after 1967 (54% (128/235) vs. 24% (10/41), $P < .0005$).

‖ ALT values were within normal limits (≤34 IU/L) in 59 (49%) of 119 HCV RNA-positive patients.

patients had serum positive for hepatitis B surface antigen and were excluded from the study.

All 302 patients were tested for anti-HCV by enzyme-linked immunosorbent assay (EIA), and HCV RNA was determined in those patients who tested positive for anti-HCV. Then, in patients who tested negative for HCV RNA, anti-HCV was measured by recombinant immunoblot assay (RIBA). We regarded the patients as truly possessing anti-HCV if they were positive for HCV RNA or anti-HCV in serum was detected by RIBA. These patients were considered to be currently infected with HCV at high or low level, or to have been infected in the past; they were named HCV-infected patients. Mortality was compared between 147 patients with anti-HCV and 155 patients without anti-HCV (they were named HCV non-infected patients). Demographic characteristics of these two patient groups are shown in Table 1.

Entry. To be eligible for the study, patients had to have tuberculosis sequelae and have received a blood transfusion at the time of surgery for pulmonary tuberculosis. Clinical records retained at the clinic were used to make the initial selection of tuberculosis sequelae patients who had had an operation for pulmonary tuberculosis. Subsequent clinical interviews ascertained whether the selected patients had received a blood transfusion at the time of their operation, later, or not at all. The amount of alcohol a patient consumed was also noted. Patients with a history of 10 years or more of drinking more than 50 g of alcohol per day were defined as heavy drinkers. The entry into the study was defined as the time when the assessments of these covariates had been completed.

Of the 147 patients with anti-HCV, 12 had already been receiving care at the liver clinic in our hospital at the time of their entry into the study. All 12 had been referred to the chest clinic prior totheir care at the liver clinic. An additional 44 patients were referred to the liver clinic at least once after enrollment.

Outcome. Patients were followed-up until October 2002, when mortality was compared between the patients with (N = 147) and without (N = 155) anti-HCV. Prognosis and cause of death could be confirmed for 288 patients by clinical records (261 patients), by telephone questionnaires to them or their family members (19 patients, of whom 10 had died), or by telephone questionnaires to the last physicians in charge of these patients, who were asked to confirm clinical records (8 patients, all of whom had died). Information could not be obtained for the remaining 14 patients; the end points for the observation of them were the last days they saw the doctors in our hospital, and at that time they were censored. We

questioned family members of the 10 patients on cause of death recorded on a death certificate. Regarding the 27 patients, or their closest kin, on whom information was obtained through telephone questionnaires, they all understood the purpose of the study and gave their informed consent. The cause of death was classified into three categories: (a) tuberculosis sequelae, (b) liver disease, and (c) other diseases. Tuberculosis sequelae that led to death consisted mainly of respiratory failure, and to a lesser extent suffocation by hemoptysis. Liver disease that caused death was HCC and severe complications of cirrhosis. Two patients with anti-HCV received interferon therapy 2 or 4 years after the entry: one was a nonresponder, and another was a sustained virological responder. No other patients received antiviral treatments.

Both the overall and the cause-specific survival curves were calculated. In calculating the cause-specific survival curve, for which the end point was liver-related death, deaths of tuberculosis sequelae or other diseases were censored.

Transfusion History. Fifty patients had received an additional transfusion 1 year or more after their first, mostly because of repeated surgery. The mean period between the first and the last transfusion was 8.5 ± 6.1 years (1-26 years), and for these patients the period after transfusion was measured from the year in which they received their first transfusion. Of these 50 patients, 40 (80%) received their first transfusion between 1951 and 1967.

Markers of HCV Infection. Anti-HCV was tested by EIA(Abbott HCV EIA 2nd Generation, Abbott Japan, Tokyo, Japan) and RIBA (Chiron HCV RIBA test 3rd Generation, Ortho-Clinical Diagnostics, Tokyo, Japan), and hepatitis B surface antigen by passive hemagglutination (Mycell, Institute of Immunology Co., Ltd., Tokyo, Japan). Serum samples, which were kept frozen at −20°C, were tested for HCV RNA within 3 years after collecting the blood. Nucleic acids were extracted from 100 μL of serum by the guanidine-thiocyanate-phenol-method[17] and reverse-transcribed to complementary DNA, which was then amplified by a two-stage polymerase chain reaction with nested primers deduced from the well-conserved 5′-noncoding region of the HCV genome.[18]

Statistical Analysis. Differences in baseline characteristics between the groups were evaluated by the chi-square test for dichotomous variables and by the Wilcoxon rank-sum test for continuous variables. Kaplan–Meier survival curves were calculated and compared using the log-rank test. *P* values less than .05 were considered to be significant.

Results

HCV Infection in Patients With Tuberculosis Sequelae. Anti-HCV by EIAwas detected in 162 (54%) of the 302 patients, of which 119 were positive for HCV RNA. Of the 43 anti-HCV–positive patients who were positive by EIA but HCV RNA negative, 28 were positive for anti-HCV by RIBA. Therefore, 147 (49%) of the 302 patients were positive for HCV RNA or anti-HCV by RIBA, and considered to truly possess anti-HCV antibody. Of the 147 patients, 119 (81%) were positive for HCV RNA; this corresponded to 39% of all patients. The remaining 155 patients were negative for anti-HCV by EIA or RIBA. No differences were observed between the anti-HCV–positive (n = 147) and –negative patients (n = 155) with respect to age, sex, age at first transfusion, year of transfusion, time after transfusion until study entry, or in the percentage of heavy drinkers (Table 1). In the anti-HCV–positive patients, the mean ALT value was significantly higher than in the anti-HCV–negative patients (38.8 IU/L ± 35.3 vs. 16.3 IU/L ± 15.1, $P <$ .0001); nevertheless, values were within normal limits (≤34 IU/L) in 58% of the anti-HCV–positive patients and 49% of the HCV RNA–positive patients. There were no differences between groups in mean albumin value; the platelet count was significantly lower in the patients with anti-HCV than in those without ($P <$ .0001).

Seventy-eight percent of 302 patients (87% of anti-HCV–positive patients and 69% of anti-HCV–negative patients) received transfusions between 1951 and 1967. The prevalence of anti-HCV was significantly higher in patients who had received transfusions before 1967 than in those who had received it after 1967 (54% (128/235) vs. 24% (10/41), $P <$ .0005).

Outcome of the Patients at the End of Follow Up. Outcome at the end of follow-up was compared between the patients with and without anti-HCV; 143 (97%) patients with and 145 (94%) without anti-HCV completed the follow up. The duration of follow-up (measured from date of entry until death or lost to follow-up, or October 2002, whichever came first) did not differ significantly between the two groups (Table 2). Of the 288 patients who completed follow-up, 92 patients (62%) with anti-HCV and 82 (53%) without had died. Among the anti-HCV–positive patients, the cause of death was tuberculosis sequelae in 61 (42%), whereas 23 (16%) died of other diseases. Among the anti-HCV–negative patients, 66 (46%) died of tuberculosis sequelae and 16 (11%) of other diseases. Eight (6%) patients with anti-HCV died of liver disease, which accounted for 26% of the deaths not related to tuberculosis sequelae; none of the anti-HCV–negative patients died of liver disease. The av-