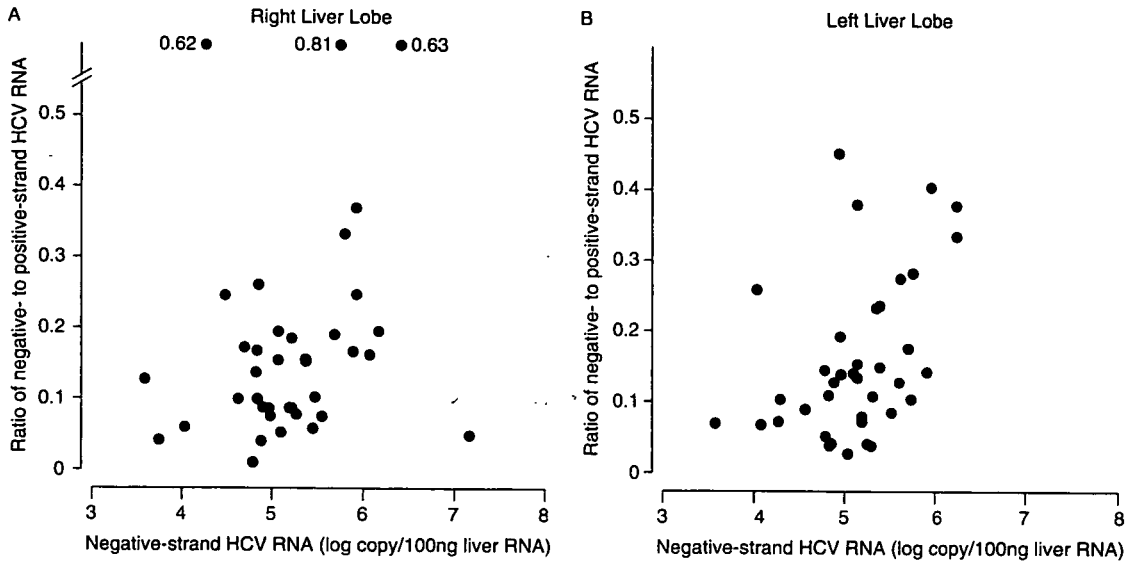

Fig. 3. Direct variation of ratios of negative- to positive-strand HCV RNA in liver tissues in relation to HCV replication, assessed by negative-strand HCV RNA in the right liver lobe ($r=0.282$; $P=0.086$) (A) and the left liver lobe ($r=0.441$; $P=0.006$) (B).

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

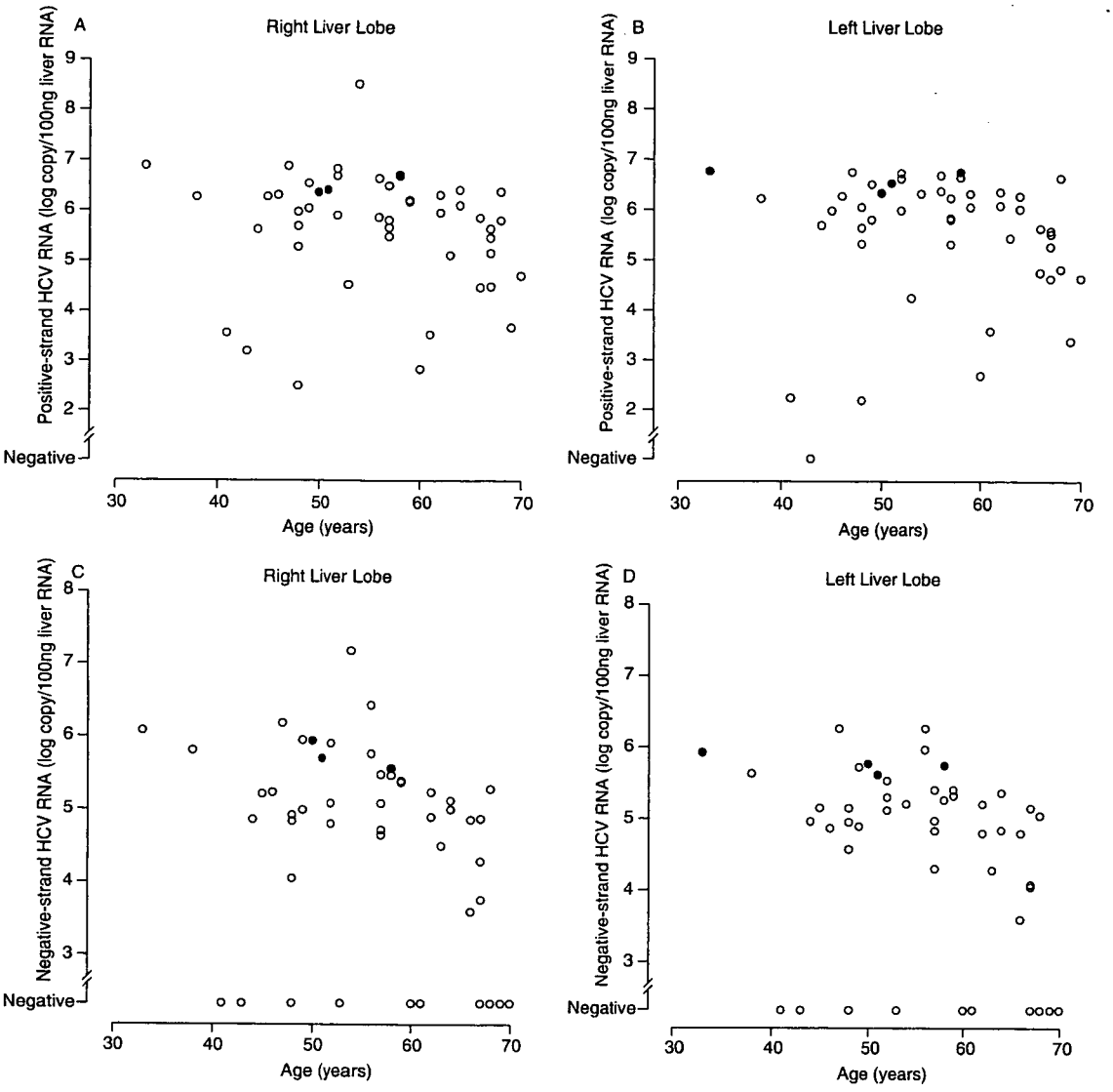

Fig. 4. Liver HCV RNA levels in relation to age and occult HBV infection in 48 chronic hepatitis C patients. No relationship was evident between age and positive-strand HCV RNA levels of the right liver lobe ($r=-0.237$; $P=0.104$) (A) and the left liver lobe ($r=-0.216$; $P=0.140$) (B), whereas inverse 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].

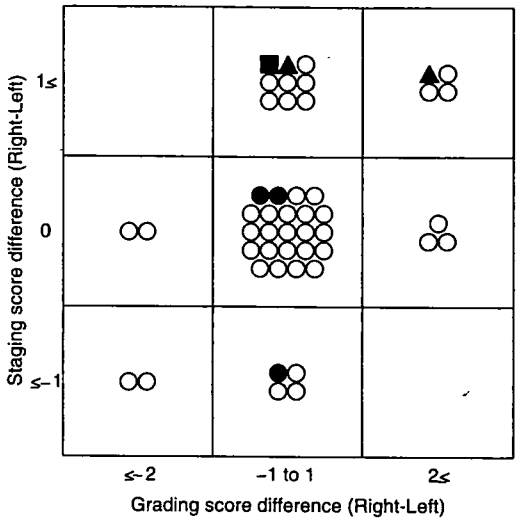

Fig. 5. Histologic and virologic discrepancies between the right and left liver lobes in chronic hepatitis C patients. ○, no between-lobe HCV RNA discrepancy; ●, larger positive-strand amounts in the right lobe; ■, larger positive- and negative-strand amounts in the right lobe; ▲, larger positive-strand 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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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	$10^{7.6}$ ≦	$10^{7.6}$ >	10^5 ≦	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　　**HBsAg(＋)の時期が確認されていること
高年：男性30歳以上，女性35歳以上

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●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，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．

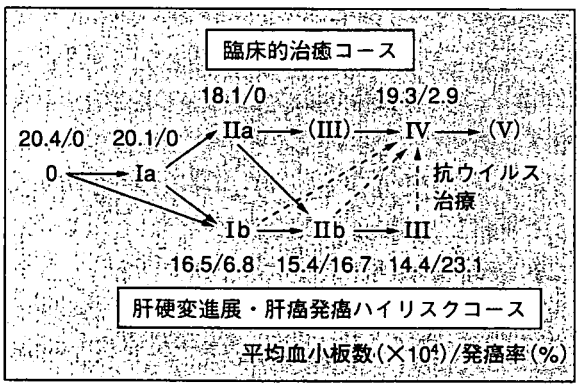

図1　HBV キャリアの経過(臨床的治癒コースと肝硬変進展・肝癌発癌ハイリスクコース)

●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．

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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-

Table 2. Outcomes of Patients With Tuberculosis Sequelae With or Without Anti-HCV

	Anti-HCV(+) (n = 147)	Anti-HCV(−) (n = 155)
Duration of follow-up (years)		
Mean ± SD	5.5 ± 3.3	5.9 ± 3.4
(range)	(0.2-13.3)	(0.1-13.3)
Alive	51 (36%)	63 (43%)
Dead	92 (64%)	82 (57%)
Due to tuberculosis sequelae	61 (42%)	66 (46%)
Due to liver disease	8 (6%)	0
Other causes	23 (16%)	16 (11%)
Unknown	4 (3%)	10 (6%)

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.

erage annual mortality from liver disease from study entry in anti-HCV–positive patients was 9.8 per 1,000 person-years.

There was no significant difference between the 2-, 5-, and 10-year overall survival probabilities from study entry of the patients with anti-HCV (84%, 60% and 35%, respectively), compared with those of the patients without anti-HCV (85%, 66% and 44%, respectively) ($P = .12$) (Fig. 1). The patients with anti-HCV, however, had significantly lower cause-specific survival probabilities for liver disease from study entry than did those without it: 99%, 96%, and 92%, at 2, 5, and 10 years, respectively ($P < .005$) (Fig. 2).

Of the liver-related deaths in the eight patients with anti-HCV (six men, two women), seven were caused by HCC and one by massive bleeding from esophageal varices (Table 3). All of the patients were positive for HCV RNA and showed abnormal ALT values at entry. None had a history of heavy alcohol use. They had received their transfusions at the ages of 25 to 41 years, had been diagnosed with HCC at 65 to 75 years, and died at 66 to 76 years. The period between transfusion and diagnosis of HCC was 33 to 45 years. Of the seven patients with HCC, two died within 1 year of diagnosis, two within 2 years, and two within 3 years. The remaining patient with HCC, who had been treated successfully with percutaneous ethanol injection therapy, died of recurrent HCC after 7 years. Seven of the eight patients who died of liver disease had been diagnosed with cirrhosis by histological and/or clinical examinations.

Details of the causes of death of patients from "other diseases" are shown in Table 4.

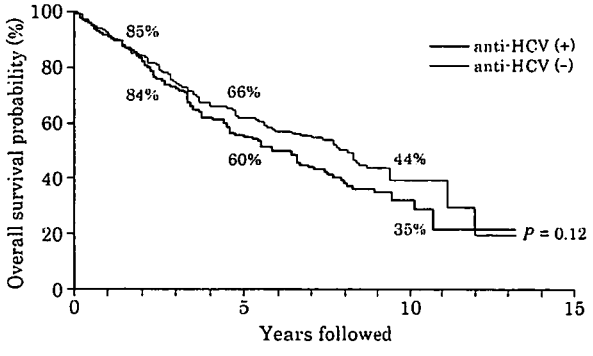

Fig. 1. The cumulative overall survival curves from study entry in anti-HCV-positive patients (**solid line**) and the anti-HCV-negative controls (**dotted line**). The 2-, 5-, and 10-year overall survival probabilities for the subjects were 84%, 60%, and 35%, respectively, and those for the controls were 85%, 66%, and 44%, respectively. The differences between groups was not significant ($P = .12$). Abbreviation: anti-HCV, antibody to HCV; NOTE: Anti-HCV(+): HCV RNA positive or anti-HCV by recombinant immunoblot assay (RIBA) positive patients. Anti-HCV(−): Patients who were negative for anti-HCV by EIA or RIBA in serum.

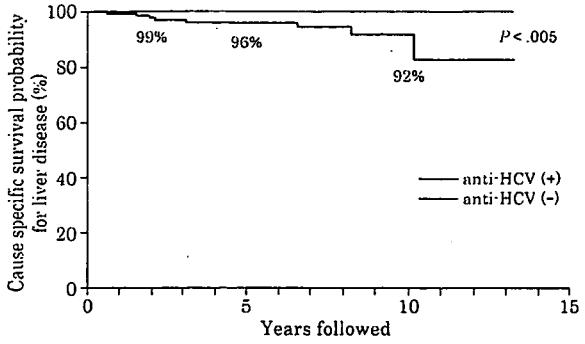

Fig. 2. The cause-specific survival curves for liver disease from study entry in the anti-HCV-positive patients (**solid line**) and the anti-HCV-negative controls (**dotted line**). The anti-HCV-positive patients showed significantly lower cause-specific survival probabilities for liver disease ($P < .005$), 99%, 96%, and 92% at 2, 5, and 10 years, respectively, than the controls. Abbreviation: anti-HCV, antibody to HCV; NOTE: Anti-HCV(+): HCV RNA positive or anti-HCV by recombinant immunoblot assay (RIBA) positive patients. Anti-HCV(−): Patients who were negative for anti-HCV by EIA or RIBA in serum.

Discussion

In Japan, the surgical treatment of pulmonary tuberculosis, such as thoracoplasty or lobectomy, was common in the 1950s until the early 1960s; more than 20,000 patients per year underwent such surgeries during this time.[19] These operations required a large volume of blood by transfusion.[15] Between 1951 and 1967, such blood was obtained commercially. It has been documented that hepatitis was acquired posttransfusion in up to 50% to 80% of patients who had undergone a major operation that involved the use of commercial blood, including those undergoing surgery for pulmonary tuberculosis.[15,16] We could not examine other risk factors for HCV infection than transfusion, so we are unable to completely discount

Table 3. Patients With Anti-HCV Who Died of Liver Disease

Case No.	Sex	Age at Transfusion	Age at Diagnosis of HCC	Age at Death	HCV RNA	ALT (IU/L)	Esophageal Varices	Liver Histology	Causes of Death
1	F	25	65	66	(+)	72	(+)	Unknown	HCC
2	F	26	71	73	(+)	59	(+)	Cirrhosis	HCC
3	M	27	66	66	(+)	37	(+)	Unknown	HCC
4	M	31	64	71	(+)	71	(+)	Unknown	HCC
5	M	33	68	70	(+)	68	(−)	Cirrhosis	HCC
6	M	35	75	76	(+)	139	(−)	Cirrhosis	HCC
7	M	36	–	70	(+)	95	(+)	Cirrhosis	Varices rupture
8	M	41	75	75	(+)	41	Unknown	Unknown	HCC

NOTE: ALT at the entry is shown.
Abbreviations: F, Female; M, Male; HCV RNA, hepatitis C virus ribonucleic acid; HCC, hepatocellular carcinoma.

potential routes such as sexual exposure, medical procedures, reused needles, or intravenous drug use. However, judging from the results that 78% of the patients received transfusion between 1951 and 1967, and the prevalence of anti-HCV was significantly higher in patients who received transfusion at that time than in those receiving it after 1967, it is reasonable to assume that, most of the patients were infected with HCV via a blood transfusion. Furthermore, it is not surprising that 49% of our patients with tuberculosis sequelae were positive for anti-HCV antibody and 39% for HCV RNA; the prevalence of the HCV-RNA–positive patients might be underestimated, because their samples were stored only at −20°C.

Table 4. Details of the Reasons for Death in Patients Who Died of "Other Causes"

Cause of Death	Anti-HCV(+)	Anti-HCV(−)
Cerebral vascular diseases	4	3
Myocardial infarction	1	1
Rupture of aortic aneurysm	1	1
Renal failure	2	1
Myelodysplastic syndrome	1	0
Multiple organ failure	0	1
Sudden death of unknown etiology	2	0
Senile decay	0	3
Accident	1	0
Brain tumor	1	0
Malignancy		
Thyroid cancer	0	1
Lung cancer	2	0
Esophageal cancer	1	0
Gastric cancer	2	1
Colon cancer	2	2
Gallbladder cancer	0	1
Pancreatic cancer	1	0
Multiple myeloma	1	0
Malignant lymphoma	1	1

NOTE: Anti-HCV(+): HCV RNA positive or anti-HCV by recombinant immunoblot assay (RIBA) positive patients. Anti-HCV(−): Patients who were negative for anti-HCV by ELISA, or RIBA in serum.
Abbreviation: anti-HCV, antibody to HCV.

In the United States, patients with chronic HCV infection die more frequently of decompensated cirrhosis than of HCC.[20] By contrast, in Japan, HCC has been the major cause of death in this patient group for a long time. This trend is growing increasingly stronger, and the proportion of death by HCC among all causes of death in patients with cirrhosis and chronic HCV infection has reached 81%.[21] Hamada et al.[22] reported that the age of the patient and duration of infection were independent risk factors affecting the development of HCC, and of them, age was the more significant. On average, approximately 30 years pass between the time of transfusion and the time of diagnosis of HCC.[1,3,22] According to a nationwide survey in Japan,[23] the mean age at such diagnosis was 63.0 years for males and 66.5 years for females (75% of all patients were anti-HCV–positive). Ninety-two percent of HCC patients were 60 years or older at the time they were diagnosed with HCC.[22] In the west of Scotland, age-specific incidence of HCC in men aged over 55 years increased dramatically between 1975 and 1985, particularly among those aged 75 to 84 years, but not in those younger than 55 years; the major etiology was considered to be HCV infection.[24] The patients in our study survived an average of 34 years after having received blood transfusion in early adulthood (at a mean age of 31 years); at the time of entry into the study they had become relatively elderly (average age, 65 years). Accordingly, they were considered to be at high risk for carcinogenesis in view of age and duration of infection.

When investigating the prognosis of liver disease in HCV-infected persons, it would be preferable to start studying patients at the time of infection and continue until they reach old age several decades later, when the risk becomes high for developing HCC. However, it is difficult to carry out such a prospective study. A retrospective cohort study on older HCV-infected persons, for whom a considerable period has elapsed between the presumed

time of their infection and their enrollment into the study, and who were selected with as little bias as possible, could serve as a good alternative to clarify, relatively accurately, the eventual outcome of their liver disease, although the study would miss liver-related events that occurred before entry. Previous retrospective studies identified patients referred for liver disease.[1-5] The patients in our study were recognized at a chest clinic while receiving treatment of sequelae to pulmonary tuberculosis, which had placed them at risk for HCV infection through transfusion. Thus, the advantage of this study was that selection bias would be less likely. Moreover, only two patients received antiviral treatment.

In our study, eight of the anti-HCV–positive patients and none of the anti-HCV–negative patients died of liver disease. The average annual mortality from liver disease from study entry of HCV-infected patients was 9.8 per 1000 person-years, and the cause-specific survival probabilities for liver disease from study entry were 99%, 96% and 92% at 2, 5 and 10 years, respectively. The mortality from liver disease might be underestimated, because chronic illnesses, including tuberculosis sequelae, might confound recognition of liver disease complication, and in some patients the cause of death was obtained from death certificates.

We showed by univariate analysis that the cause-specific survival probability for liver disease was significantly lower in patients with anti-HCV than in those without anti-HCV antibody. Subsequently, we should examine survival by using a Cox proportional hazards regression analysis. Nevertheless, in our study, this analysis was inappropriate, because none of the patients without anti-HCV died of liver disease. However, the patients in both groups were selected on the same conditions, and the background was not different between the two groups, as shown in Table 1. We could therefore conclude that HCV infection was probably an independent risk factor for the death of liver disease. Then, we showed that the overall mortality from study entry was not significantly different between the two patient groups. Because the overall mortality was extremely high owing to a large number of deaths from tuberculosis sequelae in both groups, the impact on mortality of liver-related deaths resulting from HCV infection would have been underestimated. In fact, of the deaths unrelated to tuberculosis sequelae, death from liver disease accounted for 26%.

Many of the patients in our study who died of liver disease died due to HCC, as the previous report from Japan had shown.[21] All the patients were positive for HCV RNA. None of the patients positive for anti-HCV and negative for HCV RNA died of liver disease, and there was no excess liver-related mortality in those patients. Although ALT values at entry were within normal limits in 49% of the HCV RNA–positive patients, no patient who died of liver disease showed normal ALT values.

Several studies reporting on the prognosis of liver disease in cases of chronic HCV infection have looked at patients infected in childhood (average age, 0-8 years)[8,9] or early adulthood (average age, 19-28 years).[10-13] At a median or average of 14 to 35 years after infection, clinical or histological cirrhosis was found in 0% to 8% of patients,[8-13] and no or mild histological fibrosis in 81% to 87%.[8,9,11,12] End-stage liver disease developed with an incidence of 3.1 per 1,000 person-years,[13] and liver-related mortality was 0 to 0.4%,[8,10] with a much better prognosis than was seen in our study. The difference is likely to be attributable to the difference in age of the patients at the time of the investigations. The patients in these studies were young, with an average age of 20 to 45 years. Our study showed that the eight patients who died of liver disease had apparently been infected in early adulthood (25-41 years) and died after they had reached old age (66-76 years). These results suggest that for patients who were infected in early adulthood, the long-term prognosis of liver disease, once they reach old age, is not good. It has been reported that fibrosis begins to accelerate at 50 years of age[25] and that the evolution from chronic hepatitis to cirrhosis occurs more frequently and rapidly in patients aged 50 years or older than in those younger than 50 years.[26] Accordingly, young individuals infected with HCV, and having a favorable course, may undergo rapid progression of the fibrosis once they reach middle age and then develop severe liver disease, including HCC, when they reach old age.

Seeff et al.[14] reported the long-term mortality over approximately 25 years in 222 patients with posttransfusion hepatitis C, with an average age of 49 years, of whom approximately 77% were considered to have been positive for HCV RNA in serum. Their report is the only study that includes a control group and also deals with individuals who had reached old age at the end point of observation. Liver-related mortality was significantly higher among the cases than among the control group of matched, transfused, and nonhepatitis patients (4.1% vs. 1.3%, respectively). However, the all-cause mortality was not different between the two groups (67.1% vs. 65.0%, respectively). Furthermore, the mortality attributed to chronic hepatitis C infection was only about 3%, and liver disease was considered to be a relatively minor cause of patient death. Our study showed distinctly high mortality from liver disease compared with that observed in their study, although both studies dealt with older patients. The reason for the differences in the prognosis between

their study and ours is unclear. It might be related to the difference in duration of infection, which was apparently longer in our patients than in theirs. In our patients, past tuberculosis infection, past treatment for tuberculosis, or tuberculosis sequelae might contribute to more rapid progression of liver disease. Another influencing factor might be differences in race, and this aspect warrants further study.

In conclusion, for the 147 HCV-infected patients (average age, 65 years), of whom 81% were positive for HCV RNA, with tuberculosis sequelae who had received blood transfusion at a younger age, liver-related mortality from study entry was high at 9.8 per 1,000 person-years. Among the deaths unrelated to tuberculosis sequelae, death of liver disease was the most frequently reported cause.

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HEPATOLOGY

Risk factors for the development of hepatocellular carcinoma among patients with chronic hepatitis C who achieved a sustained virological response to interferon therapy

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Abstract

Background and Aim: Hepatitis C virus (HCV)-infected patients who responded to interferon (IFN) treatment with clearance of serum HCV RNA may rarely develop hepatocellular carcinoma (HCC). The aim of the present study was to elucidate the risk factors for liver carcinogenesis among such patients.

Methods: In total, 126 patients with chronic hepatitis C (CHC) who achieved a sustained virological response (SVR) to IFN monotherapy, which was defined as the absence of detectable HCV RNA in the serum at 6 months after completion of treatment, were enrolled and possible risk factors for HCC were analyzed.

Results: During the observation period of 66 ± 36 months after cessation of IFN treatment, five (4.0%) of the 126 patients developed HCC. The cumulative incidence of HCC at 3, 5 and 10 years was estimated to be 0.9, 4.7 and 7.5%, respectively. The cumulative incidence of HCC was significantly higher among patients with severe fibrosis (F3 or F4) than among patients with no or mild fibrosis (F0 to F2) in the liver before treatment ($P = 0.007$); among patients with alcohol intake of ≥ 27 g/day than among patients with that of < 27 g/day ($P = 0.015$); and among patients who were ≥ 65 years old than among patients who were < 65 years old at the start of treatment ($P = 0.026$).

Conclusions: Patients with CHC who had severe fibrosis, who had regularly taken moderate amounts of alcohol, or who were ≥ 65 years at the start of IFN treatment should be carefully followed to detect small and controllable HCC, even after eradication of HCV.

Key words: hepatocellular carcinoma, interferon, retrospective cohort study, risk factor, sustained virological response.

INTRODUCTION

In Japan, hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths. Approximately 30 000 patients died of HCC in 2002, and 70–80% of these cases were associated with hepatitis C virus (HCV) infection. It has been demonstrated that HCC frequently develops during the advanced stages of chronic hepatitis C (CHC). Thus, it is considered that preventing the progression of CHC would reduce the risk for developing HCC. Interferon (IFN), administered with or without ribavirin, has been widely used for the treatment of CHC patients. Many investigators have reported that IFN treatment is effective for reducing the serum alanine aminotransferase (ALT) level, reducing and eliminating HCV RNA from the circulation, and improving liver histology in CHC patients.[1–5] There is accumulating evidence that a sustained virological response (SVR) to IFN therapy, defined as the absence of serum HCV RNA at follow-up 6 months after the end of treatment, is highly predictive of long-term remission of the disease.[1–3] Furthermore, the long-term outcome of HCV-infected patients who achieved a SVR to IFN treatment has been shown to be excellent with

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improvement of liver fibrosis.[1-5] Therefore, it would seem unlikely that patients who have responded to IFN therapy with loss of HCV RNA subsequently develop liver cirrhosis or HCC. However, the development of HCC among CHC patients with a SVR to IFN therapy has been reported.[4-10] Although risk factors for the development of HCC among CHC patients who underwent IFN therapy have been described in previous studies,[4,5,11-14] it remains unclear as to whether particular subsets of patients with a SVR to IFN therapy should be carefully followed for the early diagnosis of HCC. In the present study, we investigated the risk factors for the development of HCC among CHC patients who had undergone IFN monotherapy and had a SVR.

METHODS

Patients

In total, 126 histologically proven CHC patients who received IFN treatment at the Department of Gastroenterology, National Tokyo Hospital, between January 1992 and December 2001 and achieved a SVR, were enrolled in this retrospective study. In the present study, a SVR was defined as negativity for detectable HCV RNA in the circulation at 6 months after the end of IFN treatment, using a polymerase chain reaction (PCR) assay with a sensitivity of at least 100 copies (50 IU) per ml.[15,16] The exclusion criteria were as follows: patients with hepatitis B surface antigen, indicating ongoing infection of hepatitis B virus (HBV); patients who were complicated with autoimmune hepatitis; patients who had been diagnosed as having HCC and were cured; and patients with late relapse of HCV infection. No patient had concurrent infection of human immunodeficiency virus type 1. The diagnosis of chronic HCV infection was based on continuous positivity for second-generation antibodies to HCV (Abbott Japan, Tokyo, Japan) and positivity for serum HCV RNA[17] for more than 6 months before IFN treatment was started. All patients underwent liver biopsy just before IFN treatment was started. Histological staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] in which staging is defined as follows: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis). The 126 patients underwent IFN-α monotherapy for 24 ± 3 weeks (range: 9–30 weeks). The total dose of IFN was 722 ± 188 million units (range: 430–980 million units). They received 6–10 million units of IFN-α daily for 2–4 weeks, followed by 6–10 million units of IFN-α three times a week.

The following parameters were assayed in each patient just before IFN therapy was started: serum levels of aspartate aminotransferase (AST) (normal range: 9–31 IU/L) and ALT (normal range: 4–34 IU/L), platelet count (normal range: 15–30 × 10^4/μL), antibody against hepatitis B core antigen (anti-HBc, enzyme immunoassay, Abbott Japan), and HCV genotype. When a serum sample was positive for anti-HBc (inhibition percentage ≥ 70%), the serum diluted at 1:200 was also assayed for anti-HBc. The HCV genotype was determined by the method described previously.[19] This study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of National Tokyo Hospital. Informed written consent was obtained from each patient.

Follow-up and diagnosis of hepatocellular carcinoma

Follow-up of patients was performed by blood examinations including ALT, AST, qualitative detection of HCV RNA, α-fetoprotein (AFP: normal < 10 ng/mL), lectin-reactive AFP (AFP-L3: normal < 15%) and vitamin K absence or antagonist II (PIVKA II: normal range: 0–39 mAU/mL) at regular intervals of within 6 months. Imaging diagnosis was performed at least twice a year by ultrasonography (US) or computed tomography (CT). In all patients studied, serum samples were continuously negative for HCV RNA after the end of IFN treatment using Amplicor HCV v2.0 (Nippon Roche, Tokyo, Japan). The diagnosis of HCC was made using liver imaging (US, CT or magnetic resonance imaging) and/or angiography. In patients whose angiogram did not demonstrate a typical hypervascular image of HCC, microscopic examination of liver specimens obtained by echo-guided fine needle biopsy was performed. Consequently, in all patients with a SVR who developed HCC, a histological diagnosis of HCC was made using surgically resected specimens and/or biopsied specimens.

Detection of hepatitis B virus DNA

The presence of HBV DNA was determined by the method described previously.[20] Briefly, nucleic acids were extracted from 100 μL of serum using a commercially available kit (SMITEST EX-R&D; Genome Science, Tokyo, Japan), and were tested for HBV DNA by nested PCR using primers derived from the well-conserved areas in the S gene region of the HBV genomes of all eight genotypes (A to H) and Perkin-Elmer AmpliTaq DNA polymerase (Roche Molecular Systems, Branchburg, NJ, USA). The first-round PCR (94°C for 2 min before the start of cycling; 94°C for 30 s; 55°C for 30 s; 72°C for 90 s, with an additional 7 min in the last cycle) was performed for 35 cycles with primers HB095 (sense: 5′-GAG TCT AGA CTC GTG GTG GAC-3′) and HB184 (antisense: mixture of two sequences, 5′-CGA ACC ACT GAA CAA ATG GCA CCG C-3′ and 5′-CGC ACC ACT GAA CAA ATT GCA C-3′). The second-round PCR for 25 cycles was carried out under the same conditions as the first-round PCR except for extension for 60 s with primers HB097 (sense: 5′-GAC TCG TGG TGG ACT TCT CTC-3′) and S2-2 (antisense: 5′-GGC ACT AGT AAA CTG AGC CA-3′). The amplification product of the first-round PCR was 461 base pairs, and that of the second-round PCR was 437 base pairs.

Statistical analyses

The Kaplan-Meier method was used to calculate the cumulative incidence of HCC and the log-rank test was used to compare the cumulative incidence of HCC between two groups. Differences were considered to be statistically significant at $P < 0.05$. Data are presented as mean ± standard deviation (SD).

RESULTS

The baseline characteristics of the 126 patients at the start of IFN treatment (baseline) who subsequently achieved a SVR to IFN treatment are summarized in Table 1. All patients showed virological clearance and biochemical normalization 6 months after the end of treatment. During the observation period of 66 ± 36 months (range: 7–139 months) after the end of IFN treatment, the sera continued to be negative for HCV RNA in all 126 patients. However, five patients (4.0%) developed HCC. The cumulative incidence of HCC at 3, 5 and 10 years was estimated to be 0.9, 4.7 and 7.5%, respectively. The baseline characteristics of the five patients who developed HCC are presented in Table 2. All five patients who developed HCC were males, whose age ranged from 51 to 70 years at the start of IFN treatment. Four patients were assumed to have contracted HCV infection from a blood transfusion, and one patient was assumed to have contracted HCV infection from home medical therapy. The age at which the five patients were assumed to have contracted HCV infection ranged from 21 to 49 years, and the duration of persistent HCV infection was estimated to be 13–48 years. None of the five patients were heavy drinkers, which was defined as alcohol intake of 80 g or more per day, but four patients (80%) were moderate drinkers who took 27–54 g alcohol per day. The HCV genotype was 1b in one patient, 2a in three patients, and 2b in the remaining patient. Four patients (80%) were positive for anti-HBc but negative for the antibody in the serum diluted at 1:200, indicating that the titer of anti-HBc was too low to support ongoing HBV infection.

Table 1 Characteristics of the 126 patients who achieved a sustained virological response to interferon (IFN) monotherapy

Age at the start of IFN treatment (years)	53 ± 14 (range: 19–75)
Sex (male/female)	78/48
Alcohol intake	
Drinkers (≥ 27 g/day)	42 (33%)
Heavy drinkers (≥ 80 g/day)	15 (12%)
Observation period after IFN treatment (months)	66 ± 36 (range: 7–139)
Development of HCC	5 (4%)
Laboratory data before IFN treatment	
AST (IU/L)	86 ± 74 (range: 12–498)
ALT (IU/L)	119 ± 105 (range: 11–612)
Platelet count ($\times 10^4/\mu L$)	17.3 ± 6.3 (range: 5.6–36.4)
HCV genotype (genotype 1/genotype 2)	44/82
Positive for anti-HBc	49 (39%)
Positive for anti-HBc (diluted at 1:200)	5 (4%)
Liver histology before IFN treatment	
F0	2 (2%)
F1	45 (36%)
F2	47 (37%)
F3	29 (23%)
F4	3 (2%)

The normal range of platelet count is 15–30 × $10^4/\mu L$. Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] ALT, alanine aminotransferase (normal range: 4–34 IU/L); anti-HBc, antibody against hepatitis B core antigen (positive inhibition ≥ 70%); AST, aspartate aminotransferase (normal range: 9–31 IU/L); HCC, hepatocellular carcinoma.

Table 2 Baseline characteristics of five patients with a sustained virological response who developed hepatocellular carcinoma (HCC) after interferon (IFN) treatment

				Before IFN treatment					
Case	Sex	Age at the start of IFN treatment (years)	Assumed cause of HCV infection (duration of infection, years)	Alcohol intake	HCV genotype	Anti-HBc	AST (IU/L)	ALT (IU/L)	Liver histology
1	Male	51	Blood transfusion (13)	27 g/day	2a	Positive	58	76	F3
2	Male	63	Home medical therapy (14)	54 g/day	2a	Positive	57	99	F3
3	Male	69	Blood transfusion (48)	None	1b	Positive	105	141	F3
4	Male	66	Blood transfusion (38)	27 g/day	2a	Positive	66	74	F2
5	Male	70	Blood transfusion (36)	54 g/day	2b	Negative	61	82	F3

Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] ALT, alanine aminotransferase (normal range: 4–34 IU/L); anti-HBc, antibody against hepatitis B core antigen (positive inhibition ≥ 70%); AST, aspartate aminotransferase (normal range: 9–31 IU/L); HCV, hepatitis C virus.

Furthermore, none had detectable HBV DNA in the circulation. The stage of liver fibrosis at baseline was F3 in four patients (80%) and F2 in the remaining one patient.

The laboratory data at the time of diagnosis of HCC and pathological characteristics of the HCCs in the five patients are presented in Table 3. HCC was detected 54 ± 27 months (range: 25–99 months) after the end of IFN treatment. Four patients (80%) had a single HCC tumor and one patient (case 3) had three definable tumors. The size of a HCC tumor ranged from 8 to 30 mm in diameter, and the tumor was pathologically diagnosed as 'moderately differentiated' in four patients (80%) and 'well differentiated' in the patient with three tumor nodules (case 3). At the time of diagnosis of HCC, one patient (case 5) had a slightly elevated serum AFP level; however, no other HCC-related markers (AFP-L3 and PIVKA II) were elevated in any of the patients, including case 5. The histological findings of non-tumor liver tissues obtained at the time of diagnosis of HCC had remarkably improved in each patient compared with the histological findings at baseline. The stage of liver fibrosis had improved from F3 to F2 in case 1; from F3 to F1 in cases 2, 3 and 5; and from F2 to F1 in case 4.

Univariate analyses with the Kaplan-Meier method and the log-rank test were performed to compare the cumulative incidence of HCC with regard to various possible risk factors including age at baseline, sex, alcohol intake, laboratory data at baseline (AST, ALT, platelet count, HCV genotype, and anti-HBc) and the degree of liver fibrosis at baseline (Table 4). These factors were stratified into two groups, and the cumulative incidences of HCC between the two groups were compared. The cumulative incidence of HCC was significantly higher among the 32 patients with severe fibrosis (F3 or F4) than among the 94 patients with no fibrosis (F0) or mild fibrosis (F1 or F2) in the liver tissues before IFN treatment ($P = 0.007$, Fig. 1); among the 42 patients with alcohol intake of ≥ 27 g per day than among the 84 patients with alcohol intake of < 27 g per day ($P = 0.015$); and among the 28 patients who were

Table 3 Characteristics of hepatocellular carcinoma (HCC) in five patients who achieved a sustained virological response to interferon (IFN) therapy

Case	Months between the end of IFN therapy and the detection of HCC	HCC			Laboratory and histological data at the time of diagnosis of HCC				
		Number	Size (mm in diameter)	Differentiation	AFP (ng/mL)	AFP-L3 (%)	PIVKA II (mAU/mL)	Anti-HBc antibody	Liver histology
1	25	One	15	Moderate	2.1	0	25	Positive	F2
2	99	One	16	Moderate	1.8	0	28	Positive	F1
3	53	Three	8, 15, 30	Well	2.3	0	18	Positive	F1
4	42	One	23	Moderate	2.6	0	19	Positive	F1
5	52	One	20	Moderate	14.9	0	25	Negative	F1

Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] AFP, α-fetoprotein (normal < 10 ng/mL); AFP-L3, lectin-reactive AFP (normal < 15%); anti-HBc, antibody against hepatitis B core antigen (positive inhibition ≥70%); PIVKA II, vitamin K absence or antagonist II (normal range: 0–39 mAU/mL).

Table 4 Risk factors associated with the development of hepatocellular carcinoma (HCC) in patients who achieved a sustained virological response to interferon (IFN) therapy

Factor	Comparison	*P*-value (log-rank test)
Age at the start of IFN treatment (years)	≥ 65 *vs* < 65	0.026
Sex	Male *vs* female	0.059
Alcohol intake	≥ 27 g/day *vs* < 27 g/day	0.015
	≥ 80 g/day *vs* < 80 g/day	0.447
Laboratory data before IFN treatment		
AST (IU/L)	≥ 80 *vs* < 80	0.446
ALT (IU/L)	≥ 80 *vs* < 80	0.890
Platelet count (× 10^4/μL)	≥ 15.0 *vs* < 15.0	0.326
HCV genotype	Genotype 1 *vs* genotype 2	0.428
Positive for anti-HBc	Positive *vs* negative	0.097
Positive for anti-HBc (diluted at 1:200)	Positive *vs* negative	0.646
Liver histology before IFN treatment	F0, F1 and F2 *vs* F3 and F4	0.007

The normal range of platelet count is 15–30 × 10^4/μL. Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] ALT, alanine aminotransferase (normal range: 4–34 IU/L); anti-HBc, antibody against hepatitis B core antigen (positive inhibition ≥ 70%); AST, aspartate aminotransferase (normal range: 9–31 IU/L); HCV, hepatitis C virus.

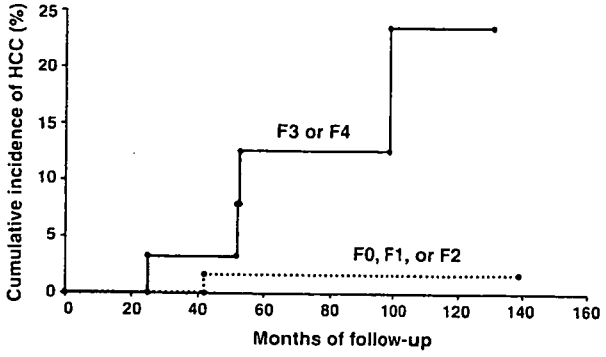

Figure 1 Cumulative incidence of hepatocellular carcinoma (HCC) among 32 patients with severe fibrosis (F3 or F4) and among 94 patients with no or mild fibrosis (F0 to F2) of the liver before interferon treatment who subsequently achieved a sustained virological response using the Kaplan-Meier method and the log-rank test. Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] in which staging is defined as follows: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis). $P = 0.007$ (log-rank test).

≥ 65 years old than among the 98 patients who were < 65 years old at the start of IFN treatment ($P = 0.026$). In addition, the cumulative incidence of HCC tended to be higher among the 78 males than among the 48 females ($P = 0.059$), and among the 49 patients with anti-HBc than among the 77 patients without anti-HBc in their sera before IFN treatment ($P = 0.097$).

DISCUSSION

Patients with CHC who have achieved a SVR to IFN treatment are likely to be considered cured. However, the development of HCC in patients who had a SVR to IFN therapy for a long period of time has recently been reported. In these case reports, HCC developed in the CHC patients with a SVR at 72 months,[6] 77 months,[9] 80 months,[7] and 90 months[10] after the end of IFN therapy. In the present study, five (4.0%) of the 126 patients who had achieved a SVR to IFN therapy developed HCC at 25 months, 42 months, 52 months, 53 months, and 99 months after the end of IFN therapy. Although the growth pattern varies among tumors, the tumor volume doubling time (growth rate) has been estimated to range from 1 to 20 months (median: 6 months),[21] and it has been estimated that the length of time between the occurrence of HCC and the time point at which the HCC tumor has grown to a diameter approximately 1 cm, when it is detectable by conventional US or CT, is more than 72 months.[13] Thus, it seems likely that four of five patients who developed HCC in the present study had had undetectable HCC before IFN therapy. However, it is difficult to distinguish between de novo HCC and HCC that developed before or during IFN therapy. It has been reported that poorer differentiation of a HCC tumor is associated with a shorter doubling time of HCC.[22] Considering the differentiation of the tumor in our five HCC patients, four of the five were moderately differentiated and one was well differentiated. One patient with a SVR in the present study developed moderately differentiated HCC 99 months after the end of IFN therapy. Thus, it is important to investigate the risk factors for the development of HCC among patients with a SVR, separate from those without a SVR.

Many previous studies have revealed various risk factors for the development of HCC among patients with CHC. The risk factors thus far reported are: no history of IFN therapy;[4,11,23] no response to IFN therapy;[4,5,11–14] older age;[4,5,11–14,23,24] male sex;[4,5,12–14,23,24] past history of blood transfusion;[25] heavy alcohol intake;[21,25,26] severe fibrosis of the liver;[4,5,11,13,14,25] high histological activity score;[11] portal inflammation;[12] HCV genotype 1b;[24] high HCV RNA level;[12] lower platelet count;[13,14,23] high serum AFP level;[23] high serum γ-glutamyl transpeptidase level;[5] low serum albumin level;[25] and high serum ALT level.[23] However, it seems unlikely that all of these risk factors are applicable to patients who have achieved a SVR, for early diagnosis and treatment of HCC, because the incidence of HCC among patients with a SVR is very low compared with that among patients who did not achieve a SVR.[4,5]

In the present study, the risk factors for HCC were analyzed among patients with CHC, focusing on those who achieved a SVR to IFN monotherapy, and the following three factors were found to be statistically significant: severe fibrosis (F3 or F4) of the liver before IFN treatment; alcohol intake of 27 g or more per day; and age of 65 years or above at the start of IFN treatment. Of note, a moderate amount of alcohol intake (≥27 g/day) was significantly associated with the development of HCC in patients with a SVR in this study. Although it is well documented that excessive alcohol intake is one of the important risk factors for the development of HCC in patients with CHC,[16,21,25] the effect of lower levels of alcohol consumption is still unclear.[16] Some investigators have pointed out the effect of light drinking on HCV-associated liver disease.[26–29] In the present study, 15 patients with excessive alcohol intake of ≥80 g/day had not developed HCC within the observation period of 26–127 months. This might indicate the existence of a synergistic effect between excessive alcohol intake and other risk factors for HCC. Multivariate analysis (e.g. Cox proportional hazards model) was not performed in this study, because the number of patients who developed HCC were too few ($n = 5$) to draw a plausible conclusion. Therefore, extended studies are required to determine whether or not these three risk factors are independent risk factors for HCC among CHC patients with sustained loss of serum HCV RNA after completion of IFN treatment.

In the current study, we did not examine HCV RNA in the liver tissues at 6 months after completion of IFN treatment when HCV RNA was not detectable in the circulation. Therefore, we cannot rule out the possibility of HCV persistence in the liver tissues of the five patients who developed HCC. However, the histological findings of non-tumor liver tissues obtained at the time of diagnosis of HCC had improved markedly in each patient as compared with those at baseline.