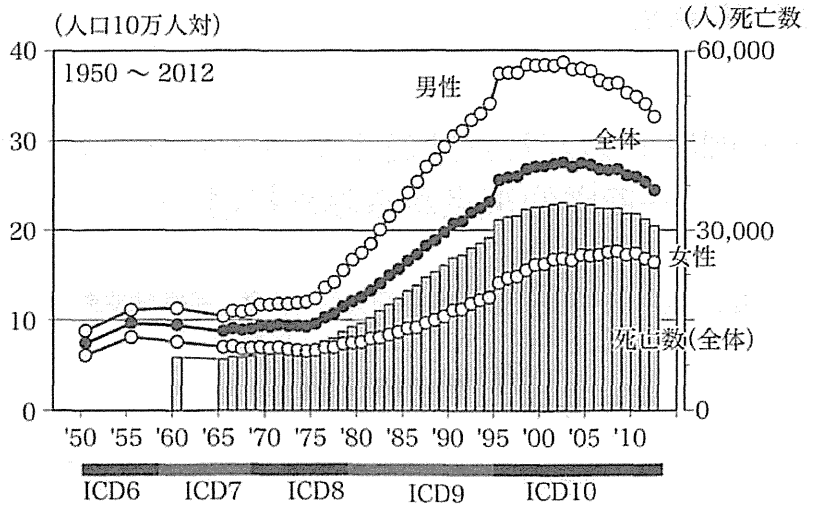
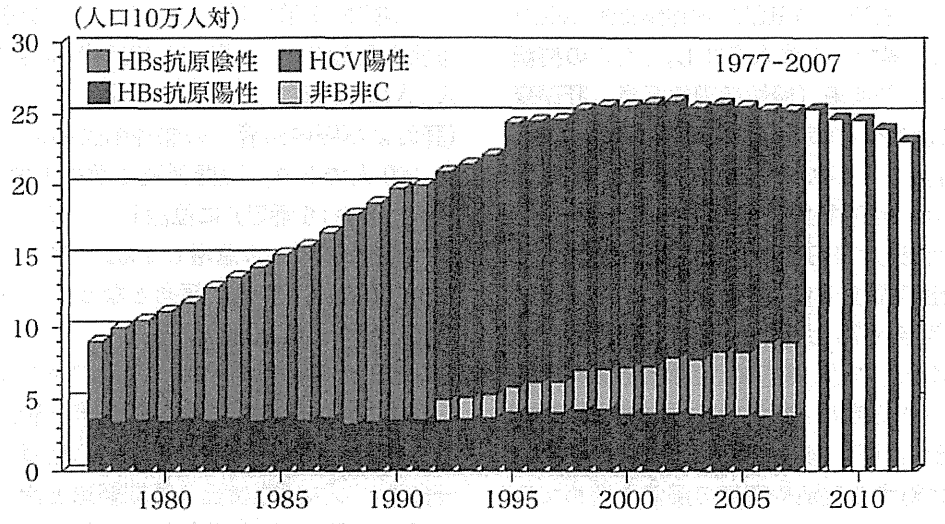


人口動態統計より作成：2014.4

図 1　わが国における肝がんによる死亡の推移



厚労省 肝炎等克服政策研究事業
「急性感染も含めた肝炎ウイルス感染状況・長期経過と治療導入対策に関する研究」班

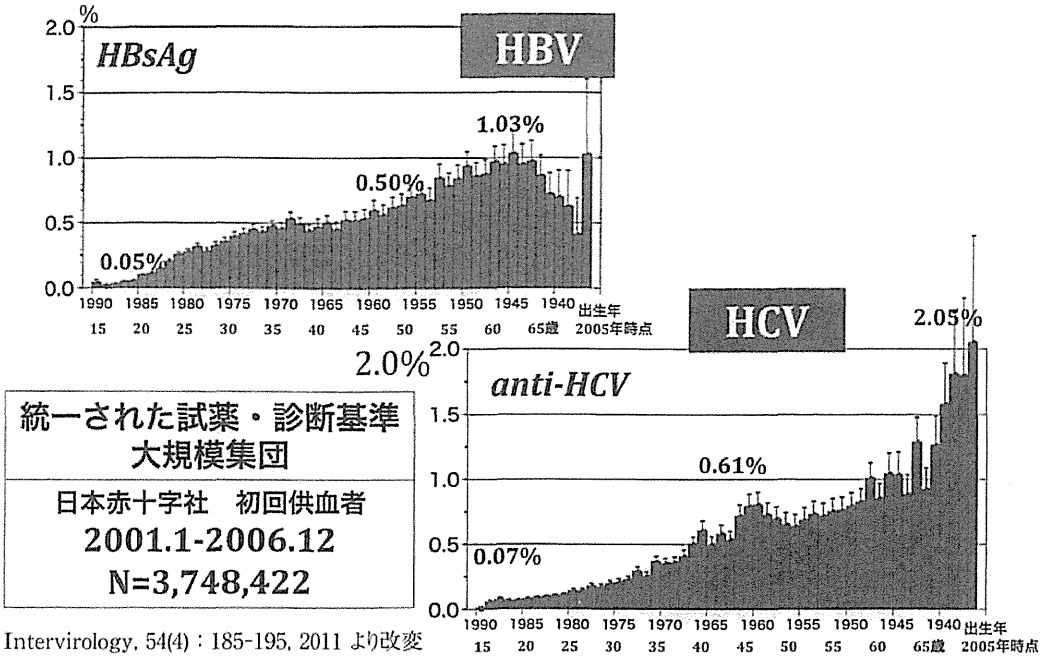
図 2　病因別にみた肝細胞がんによる死亡の経年的推移

ある.

依然としてわが国の肝細胞がん死亡の約8割はHCVあるいはHBVの持続感染に起因し，その多くはHCVによる持続感染であることから，肝がん撲滅には，肝炎ウイルス感染予防と肝炎ウイルスの持続感染者（キャリア）対策が重要であり，そのためにキャリア率と数を把握することが治療の推進とともに肝炎対策の柱となっている.

3）市町村別肝がん死亡の状況とその推移

全国市町村別肝がん死亡の状況とその推移を把握することを目的として，同研究班で1971年以後2005年までの肝がん標準化死亡比（Standard Mortality Ratio：SMR）の算出を試みた[5]. 第1期（1971～1975年）と第7期（2001年～2005年）を比較すると，第1期には顕著な地域差は認められないが，第7期には，西日本地域を中心とした地域差が顕著となっている.



Intervirology, 54(4) : 185-195, 2011 より改変

図 3　初回供血者集団における年齢階級別にみた HBs 抗原陽性率と HCV 抗体陽性率（2000 年以後）

都道府県別の肝がん死亡（人口 10 万人対）の順位を集計すると，2012 年には，佐賀県（人口 10 万人対 39.4），和歌山県（36.8），愛媛県（36.4）が上位 3 県である．1993 年から 2012 年までの上位 10 県の約 4 分の 3 は中国・四国・九州地域に位置する県が占め，肝がん死亡率には地域に特徴があることがわかる．

2．肝炎ウイルス持続感染者（キャリア数）の全体数の把握

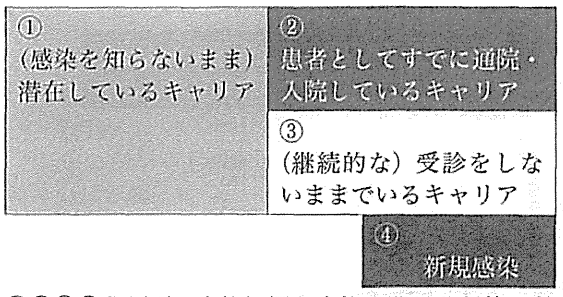
1）肝炎ウイルスキャリア率

わが国では全国の血液センターで輸血用血液の安全性を確保するため，統一された試薬と診断基準により高い精度で検査が行われている．わが国の一般集団における HCV および HBV の感染状況については，初回供血者 3,748,422 人（2001～2006 年）の資料から日赤の協力により肝炎疫学研究班で算出した成績[6]を基に検討を行ってきた（図 3）．HCV 抗体陽性率は全体平均では 0.26% であるが，高年齢層では 2% を超え，一方若年層ではきわめて低い値を示している．また，HBs 抗原陽性率は全体平均では 0.31% であったが，団塊の世代では 1% を超える高い値を示している．献血を契機に肝炎ウイルス感染が判明した集団は，それまで感染を知らないまま社会に潜在していたと考えられ，これらの成績は，2014 年時点換算で 70 歳以上の高年齢集団では，感染を知らないまま社会に潜在している HCV キャリア，HBV キャリアが依然として多いと推測される．

2）肝炎ウイルスキャリア数

前項に示した初回供血者集団の成績と節目検診受診者集団の成績を用いて，2005 年時点のキャリア数の推定を行ったところ，HCV キャリア 80.8 万人（68.0～97.4 万人），HBV キャリア約 90.3 万人（83.7～97.0 万人）と推計され，これは「①感染を知らないまま社会に潜在しているキャリア」数に相当すると考えられる．

一方，この推計値は，1990 年代に同様の方法で推定したキャリア数と比較すると減少しており，1990 年代後半から行政や医師会などが中心となって行ってきた種々の施策や広報による知識の普及による効果と考えられる．しかし，2005 年時点にはいまだ 100 万人以上が検査を受けることなく感染を知らないままでいると推定されることか



①②③④分類別の実態把握，実態に即した対策が効果的

肝炎等克服政策研究事業「急性感染も含めた肝炎ウイルス感染状況・長期経過と治療導入対策に関する研究」班
a．肝炎ウイルス感染状況に関する疫学基盤研究

図4　肝炎ウイルスキャリアの社会における存在状態4分類

ら，自覚症状がなくても一度は肝炎ウイルス検査を受検することを国民に勧めることが大事といえる．

社会における存在状態別キャリアには「①感染を知らないまま社会に潜在しているキャリア」「②患者としてすでに通院・入院しているキャリア」「③受診にいたっていない，あるいは継続受診にいたっていないキャリア」「④新規感染によるキャリア」が考えられ，この4分類によるキャリアの実態の把握がさまざまなアプローチで行われているところである（図4）．

3．肝炎ウイルス検査後の動向について：検査の通知と受診勧奨

肝炎・肝がん対策の取り組みの一つとして，肝炎ウイルス検査の推進が重要であるが，検査を受けて陽性と判定された以後の動向については興味深い．

肝炎ウイルス検査後のキャリアの動向を把握する目的で行われた無記名自記式調査の結果[7]，肝炎ウイルス検査で陽性と判定された2,177人のうち，「検査を受けたことを忘れていた者」は14.3%，受検したことは覚えているが結果通知が「陰性」であると間違って認識していたのは9.3%にのぼった．したがって，検査で「陽性」と判定されたキャリアの医療機関受診率は66.2%と低率であり，3分の1は医療機関を受診していないことになる．これらのことから，「陽性」判定を通知する際には，医療機関受診の必要性と受診勧奨のための具体的な情報提供をすることが重要であることが明らかとなった．

そこで，肝炎ウイルス検査を受けた人全員に，検査結果を伝える際に説明用下敷きを用いて説明を行い，カード（「肝炎ウイルス検査の記録」：検査日を記録）を配布する取り組み[8]を開始している（図5）．また，国は，肝炎対策基本法に基づいて告示された「肝炎対策基本指針」において「手術前等に行われる肝炎ウイルス検査の結果の通知について，受検者に適切に説明を行うよう」医療機関に要請している．さまざまなツール等を用いて，検査で「陽性」と判明した受検者が，医療費助成制度を活用し，適切な治療を受けられるしくみの構築が急務である．

4．公費治療助成制度について

現在，特にHCVキャリアに対して，次々に治療効果の高い抗ウイルス薬が開発・市販導入されているが，抗ウイルス薬を用いた治療には高額の医療費負担がかかる．

わが国では2008年よりB型肝炎・C型肝炎のインターフェロン（IFN）治療に対する医療費助成が開始され，以後，助成期間の延長などの運用変更（2009年），自己負担限度額の引き下げとB型肝炎治療の核酸アナログ製剤が助成対象へ追加（2010年），さらに，テラプレビルを含む三剤併用療法等の新たな治療法が助成対象に追加（2011年）されてきた．

助成されるのは，月ごとのIFN治療等にかかる保険診療の負担額から患者自己負担額の上限額を除いた全額である．患者自己負担額は世帯全員の市町民税の合算によって区分され，2万円あるいは1万円のいずれかとなっている．指定医療機関に受診したうえで，住民票がある自治体に交付申請書等必要書類を提出・申請し，自治体ごとに設置された認定審査会で審査後，受給者証の発行を受けることができる．

2012年度治療受給者証交付実績[9]は，核酸アナログ製剤治療の新規は10,971件，更新分は43,461件，C型・B型肝炎のIFN治療（単独あるいはリバビリン併用）の初回治療は11,196件

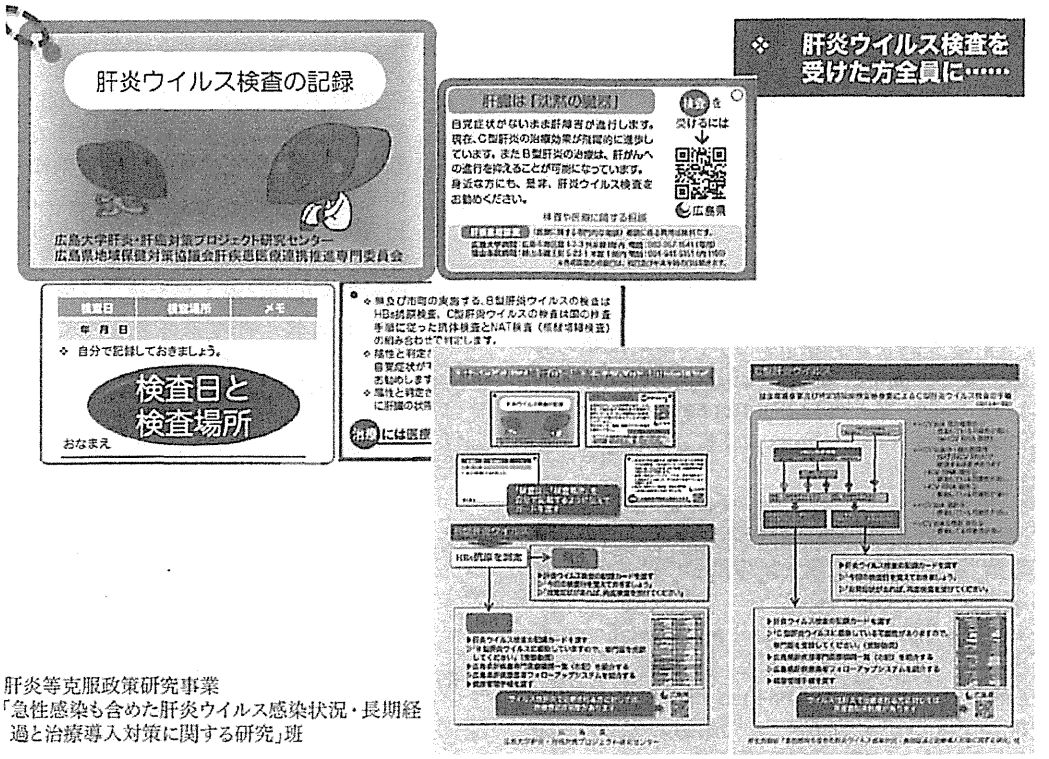


図 5 「肝炎ウイルス検査の記録」カードと検査結果補助説明用下敷き

(HCV), 1,244 件 (HBV), 2 回目治療は 1,070 件 (HCV), 143 件 (HBV) であった. また, テラプレビルを含む三剤併用療法には 6,889 件が交付された. なお, IFN 治療は 2008 年から 2012 年に合計 130,486 件が交付されている.

おわりに

わが国では, 輸血用血液への HCV 抗体スクリーニング導入や肝炎ウイルス検査の住民健診への導入など, 世界に先駆けた感染防止対策を講じてきた. また, 肝炎対策基本法や医療費助成制度の制定や無料検査を取り入れ積極的に肝がん対策を行ってきている.

その結果, わが国の一般集団における新規 HCV 感染率は低率にとどまっているものの (10 万人年当たり 1.86 人[10]), 観血的治療を頻回に実施する血液透析患者集団等ハイリスク集団における感染防止対策は引き続き重要であることも忘れてはならない (1,000 人年当たり 3.3 人[11]). 一方, HBV 母子感染防止事業が効果的に運用されたことから, 1986 年以後に出生した若年世代の HBV キャリア率はきわめて低く, 次世代の HBV 母子感染はほぼ消滅することが期待されている.

現在, 次々開発される治療効果の高い抗ウイルス薬の導入を見据えると, 肝炎ウイルス検査の推進と同時に治療にいたっていないキャリアへの対策が重要である. また, 抗ウイルス治療に対する医療費助成制度が整っている状況からみると, 手術前検査等さまざまな機会に行われている肝炎ウイルス検査の結果を受検者に適切に通知し, 「陽性」と判定されている場合には, 一度は肝臓専門医を紹介することが必要である. 新薬の導入と併せて, かかりつけ医と肝臓専門医, 自治体との連携により効果的な肝炎・肝がん対策を推し進めることにより, 国民の健康増進につながることから, 医師会や行政, 大学, 病院のそれぞれの役割が期待されている.

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Journal of Medical Virology 86:169–175 (2014)

Impact of Virus Clearance for the Development of Hemorrhagic Stroke in Chronic Hepatitis C

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The aim of this retrospective cohort study was to assess the cumulative incidence and predictive factors for intracerebral hemorrhagic stroke after the termination of interferon (IFN) therapy in Japanese patients with hepatitis C virus (HCV). A total of 4,649 HCV-positive patients treated with IFN were enrolled. The primary goal is the first onset of intracerebral hemorrhagic stroke. The mean observation period was 8.0 years. Evaluation was performed using the Kaplan–Meier method and the Cox proportional hazard model. A *P*-value of less than 0.05 was considered statistically significant. A total of 28 developed intracerebral hemorrhagic stroke. The cumulative incidence of intracerebral hemorrhagic stroke was 0.3% at 5 years, 0.8% at 10 years, and 1.7% at 15 years. Intracerebral hemorrhagic stroke occurred when patients had age increments of 10 years (hazard ratio: 2.77; 95% confidence interval (CI) 1.48–5.18; $P=0.001$), hypertension (hazard ratio: 2.30; 95% CI 1.09–4.83; $P=0.021$), liver cirrhosis (hazard ratio: 4.50; 95% CI 2.07–9.78; $P<0.001$), and HCV non-clearance (hazard ratio: 3.22; 95% CI 1.22–8.53; $P=0.018$). On the intracerebral hemorrhagic stroke based on the difference of liver fibrosis and efficacy of IFN therapy, HCV clearance reduced to 24.3% (1/4.11) compared to HCV non-clearance in cirrhotic patients ($P=0.040$). In conclusion, HCV clearance reduced the development of intracerebral hemorrhagic stroke. In particular, HCV clearance reduced intracerebral hemorrhagic stroke to about one-fourth in cirrhotic patients. ***J. Med. Virol. 86:169–175, 2014.***

KEY WORDS: hepatitis C virus; interferon therapy; hemorrhagic stroke

INTRODUCTION

There are 170 million people affected with chronic hepatitis C virus (HCV) infection worldwide, which may cause an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20–50% of cases over a period of 10–30 years [Kiyosawa and Furuta, 1991; Alter et al., 1992]. In addition, HCV is a major risk for hepatocellular carcinoma (HCC) [Hasan et al., 1990; Kew et al., 1990; Ikeda et al., 1993; Tsukuma et al., 1993; Arase et al., 2012]. In addition, several authors have reported that HCV clearance decreases the rate of fibrosis progression and the development of HCC in patients with chronic HCV infection [Kasahara et al., 1998; Yoshida et al., 2002; Arase et al., 2013].

On the other hand, hemorrhagic stroke is a medical emergency and can cause permanent neurological damage and death [Truelsen et al., 2003; Iso et al., 2007; Donnan et al., 2008]. It is becoming a great health burden in most countries. However, there is a little information on the incidence and risk factors on the incidence of hemorrhagic stroke in HCV patients treated with interferon (IFN). Furthermore, it is not clear whether the HCV clearance is useful for

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CT, computed tomography; GGT, gamma-glutamyltransferase; HbA_{1C}, hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon; LDL, low density lipoprotein

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reducing the development of hemorrhagic stroke in HCV patients.

With this background in mind, the present retrospective cohort study was initiated to investigate the cumulative incidence and risk factors of cerebral stroke after prolonged follow-up in HCV patients treated with IFN. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

PATIENTS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection and treated for the first time with IFN monotherapy or combination therapy between September 1990 and May 2010 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 7,635. Of these, 4,649 patients satisfied with the following enrolled criteria: (1) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy within 1 year before the initiation of IFN therapy; (2) positivity for serum HCV-RNA before the initiation of IFN therapy; (3) period of $\geq$1 month to $\leq$1 year of IFN therapy; (4) negativity for hepatitis B surface antigens (HBsAg), antibody to hepatitis B core, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence assay; (5) age of $\geq$30 to $\leq$80 years; and (6) no autoimmune systemic disease, such as systemic lupus erythematosus or rheumatic arthritis. Patients with either of the following criteria were excluded from the study: (1) they had illnesses that could seriously reduce their life expectancy; (2) they had a history of coronary and/or cerebrovascular disease; (3) they had a history of carcinogenesis; and (4) they had been given anticoagulant and antiplatelet drugs.

The primary outcome is the first development of hemorrhagic stroke. Hemorrhagic stroke was regarded as intracerebral hemorrhagic stroke in the present study. Thus, patients with subarachnoid hemorrhagic stroke or subdural hematoma were excluded from analyses. The development of hemorrhagic stroke was diagnosed by clinical symptoms and imaging (computed tomography and/or magnetic resonance imaging) based on the World Health Organization definition [Truelsen et al., 2003; Iso et al., 2007; Donnan et al., 2008]. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The physicians in charge explained the purpose, method, and side effect of IFN therapy to each patient and/or patients' family. In addition, the physicians in charge got permission of serum stores and future uses of stored serum. Informed consent for IFN therapy and future uses of stored serum was obtained from all patients. This study had been approved by Institutional Review Board of our hospital.

Medical Evaluation

Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline, and the body mass index (BMI) was calculated as kg/m^2. All patients were interviewed by physicians or nurse staff in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and health-related habits including questions on alcohol intake and smoking history.

Hemoglobin A_{1C} (HbA_{1C}) was estimated as National Glycohemoglobin Standardization Program equivalent value (%) and fasting plasma glucose [American Diabetes Association, 2010]. Patients were defined as having type 2 diabetes mellitus when HbA_{1C} level was $\geq$6.5% and/or fasting plasma glucose level was $\geq$126 mg/dl. Patients were defined as hypertensive when blood pressure was $\geq$140/90 mmHg or pharmacological treatment for high blood pressure was given. Smoking index (package per day $\times$ year) and total alcohol intake were evaluated by the sum of before, during, and after the IFN therapy.

Laboratory Investigation

Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Anti-HCV was detected using an enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-genotype was examined via polymerase chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported [Dusheiko et al., 1994]. HCV-RNA was determined by the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland). The serum samples stored at −80°C before IFN therapy were used. The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative. A HCV clearance was defined as clearance of HCV RNA using the COBAS TaqMan HCV test 6 months after the cessation of IFN therapy.

Evaluation of Liver Cirrhosis

Status of liver was mainly determined on the basis of peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas [Desmet et al., 1994].

Follow-Up

The observation starting point was 6 months after the termination of IFN therapy. After that, patients were followed up at least twice a year in our hospital.

Biochemical tests were conducted at each examination together with regular check-up. Four hundred fifty patients were lost to follow-up. The final date of follow-up in 452 patients with loss of follow-up was regarded as last consulting day.

Patients with either of the following criteria during follow-up were regarded as censored data in statistical analysis [Fleming et al., 1984]: (1) they were retreated with IFN (N = 949); (2) they had new onset of carcinogenesis (N = 645); and (3) they had been given anticoagulant and antiplatelet drugs (N = 28). The final date of follow-up in these patients with censored data was regarded as the time of the initiation of criteria described above. The mean follow-up period was 6.7 [standard deviation (SD) 4.3] years in 452 patients with loss of follow-up and 7.4 (SD 4.7) years in 1,722 patients who had censored data. Patients with loss of follow-up and censored data were counted in the analysis.

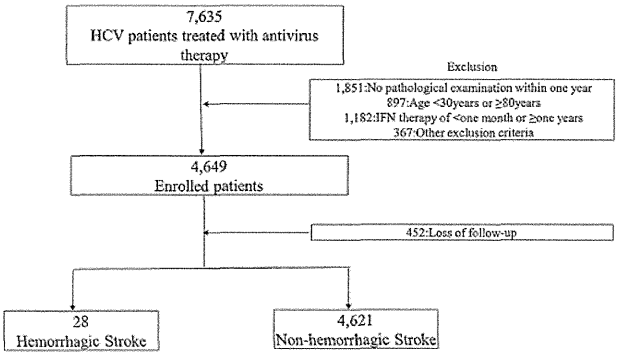


Fig. 1. An algorithm of the study population.

Statistical Analysis

Clinical differences between patients with hemorrhagic stroke and those without events were evaluated using Mann–Whitney test. The cumulative incidence of hemorrhagic stroke were calculated by using the Kaplan–Meier technique, and differences in the curves were tested using the log-rank test [Kaplan and Meier, 1958; Harrington and Fleming, 1983]. Independent risk factors associated with hemorrhagic stroke were studied using the stepwise Cox regression analysis [Cox, 1972]. The following

TABLE I. Clinical Backgrounds at the Initiation of Follow-Up in Enrolled Patients

	Total	Hemorrhagic stroke group	Without events group	*P*-value
N	4,649	28	4,621	
Age (years)	51.9 ± 11.8	60.4 ± 6.7	51.8 ± 11.9	<0.001
Gender (M/F)	2,966/1,883	16/12	2,950/1,871	0.781
Height (cm)	163.1 ± 9.2	159.5 ± 9.4	163.2 ± 9.2	0.171
Weight (kg)	61.4 ± 12.8	57.9 ± 8.0	61.4 ± 12.7	0.113
BMI	22.7 ± 3.1	23.4 ± 2.8	22.7 ± 3.1	0.582
BP (systolic, mmHg)	128 ± 18	140 ± 20	127 ± 18	0.007
BP (diastolic, mmHg)	77 ± 13	86 ± 15	77 ± 13	0.001
Total alcohol intake (kg)[a]	95 ± 92	148 ± 105	94 ± 92	0.002
Smoking index[a]	6.5 ± 9.5	11.8 ± 12.4	6.4 ± 9.4	<0.001
AST (IU/L)	41 ± 43	48 ± 28	41 ± 43	<0.001
ALT (IU/L)	44 ± 53	53 ± 38	43 ± 52	0.004
GGT (IU/L)	53 ± 60	59 ± 47	52 ± 61	0.078
Albumin (g/dl)	4.0 ± 0.3	3.5 ± 0.4	4.0 ± 0.3	0.110
Triglyceride (mg/dl)	101 ± 52	108 ± 46	100 ± 52	0.097
Cholesterol (mg/dl)	170 ± 31	171 ± 27	170 ± 31	0.893
HDL-C (mg/dl)	48 ± 14	45 ± 12	48 ± 14	0.002
LDL-C (mg/dl)	104 ± 29	108 ± 37	103 ± 29	0.049
Fasting plasma glucose (mg/dl)	99 ± 22	103 ± 23	100 ± 22	0.093
HbA_{1C} (%)	5.7 ± 1.1	5.9 ± 1.2	5.7 ± 1.1	0.024
Platelet ($\times 10^4/mm^3$)	17.2 ± 5.2	14.1 ± 6.2	17.3 ± 5.4	0.001
Staging (cirrhosis/non-cirrhosis)[b]	485/4,164	12/16	473/4,148	<0.001
HCV genotype (1b/2a/2b/other)[b]	2,859/1,109/497/184	22/5/1/0	2,837/1,104/496/184	0.104
HCV RNA (log IU/ml)[b]	6.07 ± 1.05	6.03 ± 1.03	6.08 ± 1.05	0.387
IFN monotherapy/combination therapy[c]	3,000/1,649	24/4	2,976/1,645	<0.001
Efficacy (HCV; clearance/non-clearance)	2,103/2,546	5/23	2,098/2,523	0.006

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA_{1C}; hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon.
Data are number of patients or mean ± standard deviation.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.
[b]Value before IFN treatment.
[c]Outbreak of IFN monotherapy: recombinant IFN alpha 2a, 238 cases; recombinant IFN alpha 2b, 183 cases; natural IFN alpha, 1,750 cases; natural IFN beta, 750 cases; total dose of IFN = 554 ± 164 MU. Outbreak of peg IFN monotherapy: peg IFN alpha 2a, 93 cases, total dose of peg IFN = 7.54 ± 2.20 mg.
Outbreak of combination therapy: recombinant IFN alpha 2b + ribavirin, 335 cases, total dose of IFN = 508 ± 184 MU, total dose of ribavirin = 160 ± 68 g; natural IFN beta + ribavirin, 127 cases, total dose of IFN = 502 ± 177 MU, total dose of ribavirin = 155 ± 67 g; peg IFN alpha 2b + ribavirin, 1,173 cases, total dose of peg IFN = 4.12 ± 1.10 mg, total dose of ribavirin = 205 ± 58 g.

variables were analyzed for potential covariates for incidence of primary outcome: (1) age, gender, type 2 diabetes mellitus, hypertension, BMI at the initiation time of follow-up, (2) HCV genotype, HCV load, and hepatic fibrosis before IFN therapy, (3) average value of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and platelet during follow-up, (4) sum value of smoking and alcohol before, during, and after the IFN therapy, (5) efficacy of IFN therapy, combination of ribavirin, type of IFN, and total dose of IFN. A P-value of less than 0.05 was considered statistically significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

RESULTS

Patients Characteristics

Figure 1 shows the algorithm of the study population. For the mean observation period of 8.0 years, 28 of 4,649 patients developed hemorrhagic stroke. Table I shows the baseline characteristics of the enrolled 4,649 patients at the initiation of follow-up. The patients are divided into two groups of patients with hemorrhagic stroke and without event. There are significant differences in several baseline characteristics between the two groups. The HCV clearance rate was 34.7% (1,042/3,000) in IFN monotherapy and 64.3% (1,061/1,649) in combination therapy of IFN and ribavirin. Thus, the number of patients with HCV clearance was 2,103. The mean follow-up was 8.0 (SD 5.0) years. The 28-day vascular disease-related mortality rate was 33% (10/28) in hemorrhagic stroke.

Predictive Factors for the Development of Intracerebral Hemorrhagic Stroke

The cumulative incidence of intracerebral hemorrhagic stroke was 0.3% at 5 years, 0.8% at 10 years, and 1.7% at 15 years (Fig. 2A). The factors associated with the development of intracerebral hemorrhagic stroke are shown in Table II. Intracerebral hemorrhagic stroke occurred when patients had age increments of 10 years [hazard ratio: 2.77; 95% confidence interval (CI) 1.48–5.18; $P = 0.001$], hypertension

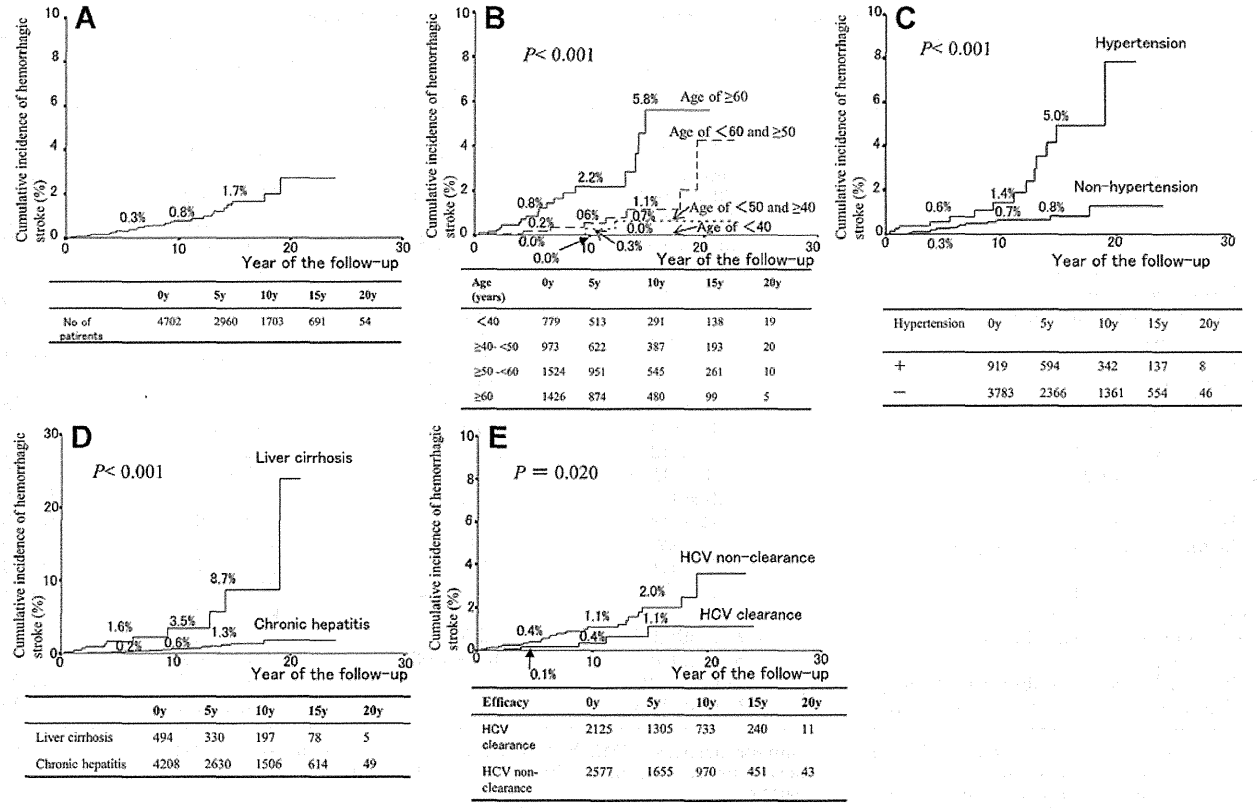


Fig. 2. **Panel A**: Cumulative development rate of intracerebral hemorrhagic stroke in total HCV patients treated with IFN therapy. **Panel B**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of age. **Panel C**: Cumulative development rate of ischemic stroke based on the difference of blood pressure. **Panel D**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of liver fibrosis. **Panel E**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of interferon efficacy.

TABLE II. Predictive Factors for the Development of Intracerebral Hemorrhagic Stroke

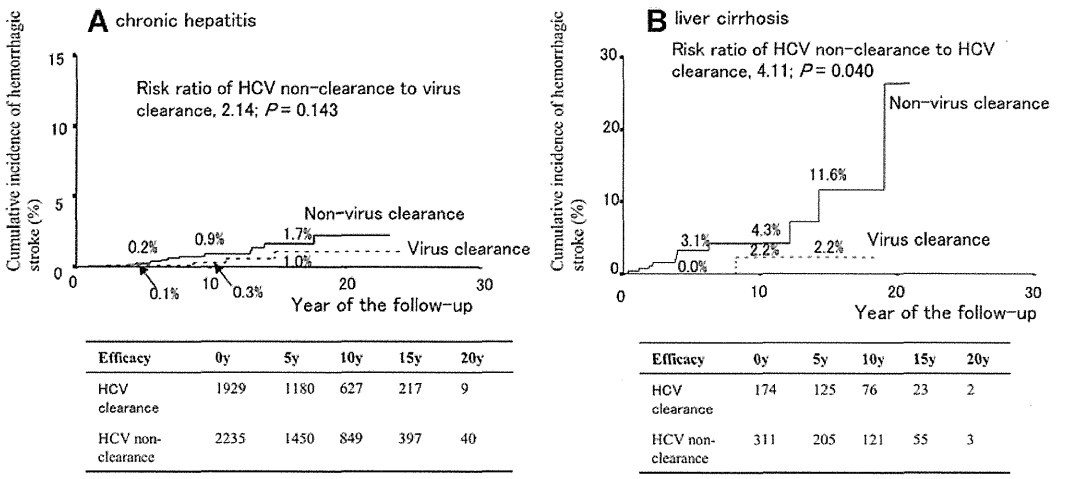
	Univariate analysis		Cox regression	
Variables	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years, per 10)	3.55 (1.96–6.43)	<0.001	2.77 (1.48–5.18)	0.001
Gender (M/F)	1.26 (0.65–2.44)	0.334		
BMI (≥22/<22)	0.97 (0.75–1.24)	0.767		
Diabetes (+/−)	3.40 (1.26–9.15)	0.015		
Hypertension (+/−)	4.07 (1.94–8.54)	<0.001	2.30 (1.09–4.83)	0.021
Smoking index (≥20/<20)[a]	2.12 (0.95–4.76)	0.068		
Total alcohol intake (kg, ≥200/<200)[a]	1.10 (0.53–4.37)	0.138		
AST (IU/L, ≥34/<34)	2.79 (1.17–6.66)	0.020		
ALT (IU/L, ≥36/<36)	2.68 (1.14–6.29)	0.023		
GGT (IU/L, ≥109/<109)	1.28 (0.610–1.89)	0.655		
Albumin (g/dl, <3.9/≥3.9)	2.96 (1.24–7.09)	0.015		
Triglyceride (mg/dl, ≥100/<100)	1.19 (0.83–1.49)	0.283		
Total cholesterol (mg/dl, <150/≥150)	1.06 (0.48–1.91)	0.936		
HDL-C (mg/dl, ≥40/<40)	0.96 (0.38–2.50)	0.960		
LDL-C (mg/dl, ≥120/<120)	0.81 (0.50–2.51)	0.572		
Platelet ($\times 10^4$/mm^3, <15/≥15)	3.22 (1.41–7.35)	0.005		
Histological diagnosis (cirrhosis/non-cirrhosis)	7.40 (3.30–16.77)	<0.001	4.50 (2.07–9.78)	<0.001
Combination of ribavirin (+/−)	0.80 (0.25–2.54)	0.701		
Type of IFN (α/β)	1.29 (0.65–2.33)	0.116		
Total dose of IFN (MU, ≥500/<500)	0.87 (0.39–1.99)	0.744		
HCV genotype (1/2)	1.53 (0.62–3.80)	0.360		
HCV RNA (log IU/ml, ≥5/<5)	1.35 (1.02–1.79)	0.035		
Efficacy (HCV: non-clearance/clearance)	2.98 (1.13–6.59)	0.020	3.22 (1.22–8.53)	0.018

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; IFN, interferon.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.

(hazard ratio: 2.30; 95% CI 1.09–4.83; $P = 0.021$), liver cirrhosis (hazard ratio: 4.50; 95% 2.07–9.78; $P < 0.001$), and HCV non-clearance (hazard ratio: 3.22; 95% CI 1.22–8.53; $P = 0.018$). Figure 2B–E shows the cumulative incidence of hemorrhagic stroke based on difference of age, blood pressure, liver fibrosis, and efficacy of IFN therapy.

Hemorrhagic Stroke Based on the Difference of Liver Fibrosis and Efficacy

Figure 3A,B shows the cumulative incidence of intracerebral hemorrhagic stroke based on the difference of liver fibrosis and efficacy of IFN therapy. As shown in Figure 3B, HCV clearance reduced



Efficacy	0y	5y	10y	15y	20y
HCV clearance	1929	1180	627	217	9
HCV non-clearance	2235	1450	849	397	40

Efficacy	0y	5y	10y	15y	20y
HCV clearance	174	125	76	23	2
HCV non-clearance	311	205	121	55	3

Fig. 3. **Panel A**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of efficacy after interferon treatment in HCV patients with chronic hepatitis. **Panel B**: Cumulative development rate of intracerebral hemorrhagic stroke based on the difference of efficacy after interferon treatment in HCV patients with liver cirrhosis.

TABLE III. Comparison in Clinical Backgrounds Between HCV Clearance and HCV Non-Clearance in Patients With Liver Cirrhosis

	HCV clearance group	HCV non-clearance group	*P*-value
N	174	311	
Age (years)	56.7 ± 9.6	57.0 ± 9.9	0.721
Gender (M/F)	108/66	184/127	0.562
BMI	23.8 ± 3.7	23.6 ± 3.5	0.479
BP (systolic, mmHg)	132 ± 18	131 ± 17	0.791
BP (diastolic, mmHg)	80 ± 11	79 ± 12	0.775
Total alcohol intake (kg)[a]	112 ± 97	128 ± 101	0.057
Smoking index[a]	6.2 ± 10.7	5.9 ± 10.2	0.129
AST (IU/L)	33 ± 20	73 ± 47	<0.001
ALT (IU/L)	34 ± 28	79 ± 61	<0.001
GGT (IU/L)	24 ± 26	61 ± 65	<0.001
Albumin (g/dl)	3.7 ± 0.4	3.5 ± 0.4	0.149
Triglyceride (mg/dl)	110 ± 47	104 ± 45	0.243
Cholesterol (mg/dl)	157 ± 29	161 ± 31	0.373
HDL-C (mg/dl)	42 ± 12	45 ± 12	0.257
LDL-C (mg/dl)	96 ± 26	95 ± 30	0.748
Fasting plasma glucose (mg/dl)	104 ± 22	109 ± 26	0.085
HbA_{1C} (%)	5.7 ± 1.2	6.0 ± 1.3	0.024
Platelet ($\times 10^4/mm^3$)	14.1 ± 6.2	17.3 ± 5.4	0.097
HCV genotype (1b/2a/2b/other)[b]	75/72/24/3	209/54/15/33	<0.001
HCV RNA (log IU/ml)[b]	5.32 ± 1.12	6.38 ± 1.00	<0.001
IFN monotherapy/combination therapy[c]	110/64	232/79	0.012

Data are number of patients or mean ± standard deviation, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA_{1C}, hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.
[b]Value before IFN treatment.
[c]Outbreak of IFN monotherapy: natural IFN alpha, 252 cases; natural IFN beta, 90 cases; total dose of IFN = 518 ± 156 MU.
Outbreak of combination therapy: natural IFN beta + ribavirin, 41 cases, total dose of IFN = 490 ± 171 MU, total dose of ribavirin = 151 ± 64 g; peg IFN alpha 2b + ribavirin, 102 cases, total dose of peg IFN = 3.96 ± 1.03 mg, total dose of ribavirin = 188 ± 51 g.

hemorrhagic stroke to one-fourth in cirrhotic patients. Table III shows the clinical backgrounds between HCV clearance and HCV non-clearance in patients with liver cirrhosis. There are significant differences in AST, ALT, GGT, HCV genotype, HCV RNA, and HbA_{1C} between HCV clearance group and HCV non-clearance group. However, there are no significant differences in age and hypertension between HCV clearance group and HCV non-clearance group.

DISCUSSION

The incidence of hemorrhagic stroke after the termination of IFN therapy in HCV patients has been described in the present study. The strengths of the present study are a prolonged follow-up in the large numbers of patients included.

The present study shows several findings with regard to the cumulative incidence and predictive factors for hemorrhagic stroke after IFN therapy for HCV patients. First, intracranial hemorrhagic stroke occurred significantly when patients had advanced age of ≥60 years, hypertension, liver cirrhosis, and HCV non-clearance. Several authors have reported that the most common risk factor for hemorrhagic stroke is aging, high levels of blood pressure [Turin et al., 2010; O'Donnell et al., 2010; Naidech, 2011; Cervera et al., 2012]. In addition, antiplatelet and anticoagulant medications also increase the risk of hemorrhagic stroke [Cervera et al., 2012]. Our results evaluated hemorrhagic stroke in HCV patients agreed with these reports concerning aging and hypertension.

Second, HCV clearance reduced hemorrhagic stroke to about one-fourth in cirrhotic patients. In general, patients with advanced liver fibrosis have often the hemorrhagic tendency due to prothrombin deficit and platelets diminution. Thus, our result suggests that the HCV clearance prevent the aggravation of prothrombin deficit and platelets diminution. Our previous reports have indicated that HCV clearance reduces type 2 diabetes mellitus [Arase et al., 2009], bone fracture [Arase et al., 2010], and chronic kidney disease [Arase et al., 2011]. In the present study, HCV clearance reduced the incidence of intracerebral hemorrhagic stroke. In particular, HCV clearance reduced intracerebral hemorrhagic stroke to about one-fourth in cirrhotic patients.

A hemorrhagic stroke is the rapid loss of brain function due to hemorrhage. As a result, a hemorrhagic stroke is a medical emergency and can cause permanent neurological damage and death. Recently, the life span has been long in Japan. Thus, in near the future, a large number of patients with HCV will be >60 years of age. A hemorrhagic stroke might be increasing in HCV positive patients in aging society. Our results show that physicians in charge of HCV

patients with hypertension, liver cirrhosis, and HCV non-clearance should be noted the development of hemorrhagic stroke.

The present study was limited by a retrospective cohort trial. Another limitation of the study was that patients were treated with different types of antivirus therapy for different duration. In addition, these patients were treated with different types of drugs for diabetes, hypertension, and dyslipidemia during follow-up. Finally, our cohort contains Japanese subjects only. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

In conclusion, HCV clearance reduced hemorrhagic stroke to about one-fourth in cirrhotic patients.

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The Japanese Society of Gastroenterology

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Seroclearance rate of hepatitis B surface antigen in 2,112 patients with chronic hepatitis in Japan during long-term follow-up

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Abstract
Background Rate of hepatitis B surface antigen (HBsAg) seroclearance was determined in 2,112 Japanese patients with chronic hepatitis B who were followed up for at least 15 years.
Methods Patients had a median age of 37 years and included 1,431 (67.8 %) men. Median values were AST/ALT, 43/62 IU/L; platelet counts, 182 × 10^3/mm^3; HBsAg, 3,400 IU/mL; and hepatitis B virus (HBV) DNA, 6.2 log copies/mL. Factors influencing HBsAg seroclearance were evaluated by the Cox proportional model and annual rate of HBsAg seroclearance by the Kaplan–Meier life table method.
Results The overall annual rate of HBsAg seroclearance was 1.75 % in 2,112 patients; it was 1.65 % in 1,130 untreated and 2.05 % in 982 treated patients ($p = 0.289$). In untreated patients, seroclearance was influenced by age, no HBV infections in third-degree or closer relatives, and HBsAg levels in univariate analysis. Seroclearance was influenced by a median age ≥50 years [relative risk (RR) 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] in multivariate analysis. In treated patients, age, male gender, no HBV infections in third-degree or closer relatives, interferon therapy, chronic hepatitis, high AST and γ-GTP levels, low platelet counts, hepatitis B e antigen (HBeAg)-negative status, low HBsAg levels and the wild-type precore sequence significantly influenced HBsAg seroclearance. In multivariate analysis, no family history [RR 2.22 ($p = 0.006$)], interferon treatment [RR 3.15 ($p < 0.001$)], and HBeAg-negative status [RR 3.75 ($p < 0.001$)] significantly influenced HBsAg seroclearance.
Conclusions In this retrospective cohort study, the annual rate of HBsAg seroclearance was 1.65 % in untreated patients and 2.05 % in treated patients.

Keywords Seroclearance · Hepatitis B surface antigen · Hepatitis B virus · Chronic hepatitis B

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ETV	Entecavir
HBeAg	Hepatitis B e antigen
HBcrAg	Hepatitis B core-related antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B virus DNA
HBsAg	Hepatitis B surface antigen
IFN	Interferon
LAM	Lamivudine

Introduction

Worldwide, an estimated 400 million people are infected with hepatitis B virus (HBV) persistently. HBV infection is a common disease that can induce a chronic carrier state

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and is associated with the risk of developing progressive disease and hepatocellular carcinoma (HCC) [1–5]. In regions highly endemic for HBV, such as Asia and Africa, the persistent carrier state is established by perinatal transmission or early in infancy. Carriers serve as the reservoir of HBV in the community and can spread the infection to susceptible individuals. The incidence of HCC is decreased extremely by eradicating HBV from the circulation that is responsible for liver damage [6–9]. In Japan, interferon (IFN) was introduced for the treatment of persistent HBV infections, and long-term IFN increased seroclearance of hepatitis B surface antigen (HBsAg) [10]. Since 2000, the effect of long-term nucleot(s)ide analogues, such as lamivudine [11, 12] and entecavir [13], on HBsAg seroclearance has been monitored in Japan.

In the current study, we followed untreated or treated patients for at least 15 years. We evaluated the seroclearance of HBsAg, achieved in both groups of patients, by using highly sensitive assays. Our aim was to determine factors that can lead to HBsAg seroclearance and to elucidate the factors associated with its success.

Patients and methods

Patients

During at least 15 years from 1968, 2,112 consecutive patients, chronically mono-infected with HBV (confirmed by HBsAg-positivity for at least 6 months) were followed at the Department of Hepatology, Toranomon Hospital, in Metropolitan Tokyo. Patients met the following inclusion and exclusion criteria: (1) negativity for hepatitis C antibody and/or hepatitis C virus RNA by polymerase chain reaction (PCR) in the serum; (2) no history of HCC; and (3) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis B. Thus, the 2,112 patients were enrolled in this cohort study. A written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved a priori by the institution's human research committee.

Treatment

Nine hundred and eighty-two patients received antiviral treatments. Of them, 156 patients received prednisolone (PSL) 40 mg daily for 1 week, 30 mg daily for 1 week, 20 mg daily for 1 week, and then 10 mg daily for 1 week until it was abruptly withdrawn (total 700 mg). A total of 428 patients received 100 mg lamivudine (LAM) daily as an initial therapy. In total, 333 patients received 3–12 MU of IFN-α or IFN-β. The durations and regimens of treatment were as follows: daily for 2 or 4 weeks and then 2 or 3 times per week for 26–104 weeks. The median duration of treatment was 26 weeks (range 4–981). There were 190 (57 %) patients who received multiple treatments of IFN.

LAM treatment was continued as a rule; median duration of LAM treatment was 75 months (55–102). LAM-resistant rtM204I/V mutants developed in 151 (35 %) of the 428 patients, and they were provided with adefovir dipivoxil (10 mg) added on LAM, as a rescue therapy. The remaining patients continued to receive LAM monotherapy. In addition, 65 patients received 0.5 mg entecavir (ETV) daily as an initial therapy. ETV treatment was continued as a rule, and median duration of ETV treatment was 45 months (1.0–104).

Markers of HBV infection

Serum HBsAg titers were determined annually using ARCHITECT HBsAg QT assay kits (Abbott Laboratories, Tokyo, Japan), which have a lower limit of detection of 0.05 IU/mL and an upper limit of detection of 250 IU/mL. To expand the upper limit from 250 to 125,000 IU/mL, serum samples going off the scale were diluted stepwise to 1:20 and 1:500 with ARCHITECT diluents following instructions from the manufacturer.

Hepatitis B e antigen (HBeAg) was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). HBV DNA was quantified using the Amplicor monitor assay (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.6–7.6 log copies/mL, or COBAS TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.1–9.0 log copies/mL. Hepatitis B core-related antigen (HBcrAg) was determined by chemiluminescence enzyme immunoassay (CLEIA) with the HBcrAg assay kit (Fujirebio Inc., Tokyo, Japan). A commercial kit (HBV Genotype EIA; Institute of Immunology, Tokyo, Japan) was used to serologically determine HBV genotypes by the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the 7 major genotypes (A–G).

Statistical analysis

Baseline data were obtained on the day of the first visit in untreated patients. In patients who received antivirals, baseline data were obtained at the start of the first day of treatment. Categorical data were compared between groups by chi-squared or Fisher's exact tests. Continuous variables with a nonparametric distribution were analyzed by Mann–Whitney *U* tests, whereas those with a parametric distribution were analyzed by the Student's *t* test. Cox

regression analyses were used to assess variables that were significantly associated with HBsAg seroclearance. All baseline factors that were found to be significantly associated with HBsAg seroclearance by univariate analysis were entered into a multivariate analysis. Independent baseline factors associated with the seroclearance of HBsAg were evaluated using a stepwise Cox regression analysis. We then performed a time-dependent Cox regression to analyze independent factors associated with HBsAg seroclearance while on-treatment factors and independent baseline factors had been adjusted.

Cumulative HBsAg seroclearance rates were analyzed using the Kaplan–Meier method; differences in the resulting curves were evaluated using log-rank tests. Significance was defined as $p < 0.05$ for all two-tailed tests. Data analysis was performed with the SPSS software package version 11.0.1 J (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics in the 2,112 patients

The baseline characteristics of studied patients are shown in Table 1. They had a median age of 37 years (range 1–81), included 1,431 (67.8 %) men, and 2,031 (96.2 %) of them had chronic hepatitis. Their baseline values were AST/ALT, 43 (3–2,192)/62 (2–3,020 IU/L); γ-GTP, 27 (4–1,494) IU/L; platelet counts, 182 (40–483) × 10^3/mm^3; and HBV markers were HBsAg, 3,400 (0.06–27,700) IU/mL; and HBV DNA, 6.2 (<2.1 to >9.1) log copies/mL. HBeAg was not detectable in 5.4 % of studied patients, and the distribution of genotypes A/B/C/others was 4.5:15.6:79.6:0.3 %.

The HBsAg seroclearance rate analyzed by the Kaplan–Meier method was 9 % in 5 years, 17 % in 10 years, 27 % in 15 years, 35 % in 20 years, 44 % in 25 years, and 54 % in 30 years. The annual rate of HBsAg seroclearance was 1.75 % during 20 years (Fig. 1).

In the 2,112 patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were cirrhosis [relative risk (RR) 2.40 ($p = 0.014$)]; HBeAg negative [RR 3.01 ($p = 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.13 ($p = 0.004$)]. In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: HBeAg negative [RR 1.81 ($p < 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.60 ($p < 0.001$)] (Table 2).

Untreated patients and treated patients

Differences in the baseline characteristics between 1,130 untreated and 982 treated patients are shown in Table 3: age [31 years vs. 36 ($p < 0.001$)]; male gender [62.4 vs. 71.9 % ($p < 0.001$)]; AST [median 27 vs. 56 IU/L ($p < 0.001$)]; ALT [median 28 vs. 96 IU/L ($p < 0.001$)]; γ-GTP [median 20 vs. 45 IU/L ($p < 0.001$)]; total bilirubin

Table 1 Baseline characteristics 2,112 patients infected with HBV followed for longer than 15 years

Features at the baseline	Patients (n = 2,112)
Demographic data	
Age (years)	37 (1–81)
Men	1,431 (67.8 %)
Liver disease	
Chronic hepatitis	2,031 (96.2 %)
Cirrhosis	81 (3.8 %)
Laboratory data	
AST (IU/L)	43 (3–2,192)
ALT (IU/L)	62 (2–3,020)
γ-GTP (IU/L)	27 (4–1,494)
Total bilirubin (mg/dL)	0.7 (0.1–21.2)
Albumin (g/dL)	4.3 (1.1–5.8)
Platelets (×10^3/mm^3)	182 (40–483)
α-Fetoprotein (μg/L)	4 (1–2,060)
HBV markers	
HBeAg-negative status	1,169 (55.4 %)
HBsAg (IU/mL)	3,400 (0.06–277,000)
HBcrAg (log U/mL)	5.4 (<3.0 to >6.8)
Genotypes (A/B/C/others)	4.5 %/15.6 %/79.6 %/0.3 %
HBV DNA (log copies/mL)	6.2 (<2.1 to >9.1)

Median values with the range in parentheses or numbers with the percentage in parentheses are given

HBV hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

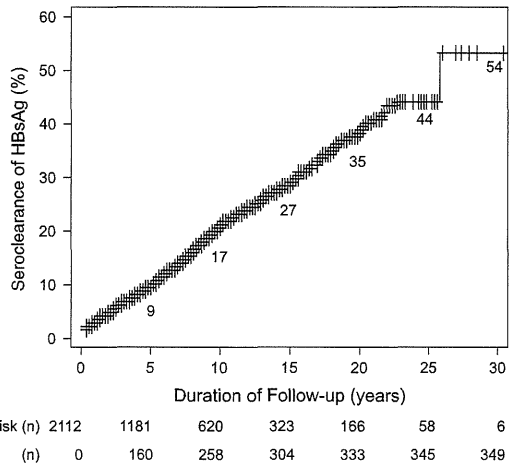

Fig. 1 Seroclearance of HBsAg in the 2,112 patients studied. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

Table 2 Factors influencing the seroclearance of HBsAg in 2,112 patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value
Age ≥50 years	1.06 (0.64–1.76)	0.824		
Male gender	1.15 (0.69–1.90)	0.594		
No HBV infection in family	1.55 (0.93–2.57)	0.092		
Treatment	1.26 (0.72–2.19)	0.413		
Cirrhosis	2.40 (1.20–4.83)	0.014		
AST ≥50 IU/L	1.30 (0.66–2.57)	0.454		
ALT ≥50 IU/L	1.81 (0.89–3.70)	0.104		
γ-GTP ≥20 IU/L	1.26 (0.72–2.23)	0.418		
Total bilirubin ≥1 mg/dL	1.39 (0.69–2.79)	0.358		
Albumin ≥4 g/dL	1.03 (0.58–1.81)	0.927		
Platelets >150 × 10^3/mm^3	1.22 (0.68–2.18)	0.501		
α-Fetoprotein ≤10 μg/L	1.06 (0.59–1.89)	0.845		
Genotype A or B, C	1.55 (0.86–2.76)	0.142		
HBeAg-negative status	3.01 (0.79–2.07)	0.001	1.81 (1.30–2.77)	<0.001
HBV DNA ≥5 log copies/mL	1.17 (0.64–2.15)	0.612		
HBsAg ≤2,000 IU/mL	2.13 (1.27–3.56)	0.004	2.60 (1.94–3.50)	<0.001
HBcrAg ≥4 log U/mL	1.11 (0.61–2.03)	0.731		
Wild-type precore sequence	0.98 (0.59–1.53)	0.964		
Wild-type core promoter sequence	2.74 (0.80–9.30)	0.104		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

[median 0.5 vs. 0.7 mg/dL ($p < 0.001$)]; albumin [median 4.4 vs. 4.3 g/dL ($p < 0.001$)]; platelets [median 202 vs. 181 × 10^3/mm^3 ($p < 0.001$)]; α-fetoprotein [median 4 vs. 4 μg/L ($p < 0.001$)]; HBeAg-negative status [75.8 vs. 31.8 % ($p < 0.001$)]; HBsAg levels [median 2,240 vs. 5,270 IU/mL ($p < 0.001$)]; HBcrAg [median 3.6 vs. >6.8 log U/mL ($p < 0.001$)]; distribution of genotypes A/B/C/others (5.7/20.0/72.6/1.7 vs. 3.4/11.1/84.9/0.5 %, $p < 0.001$); and HBV DNA [median 4.7 vs. 8.0 log copies/mL ($p < 0.001$)].

The rate of HBsAg seroclearance in treated patients was 8 % in 5 years, 20 % in 10 years, 28 % in 15 years, 41 % in 20 years, 49 % in 25 years, and 49 % in 30 years, with an annual HBsAg seroclearance rate of 2.05 % (Fig. 2). The rate in untreated patients was 9 % in 5 years, 18 % in 10 years, 26 % in 15 years, 33 % in 20 years, 42 % in 25 years, and 56 % in 30 years, with an annual HBsAg seroclearance rate of 1.65 %. No differences in the annual HBsAg seroclearance rate were noted between treated and untreated patients ($p = 0.289$).

HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, HBsAg persisted in 930 (82.3 %), whereas HBsAg seroclearance occurred in 200 (17.7 %). In the baseline characteristics, significant differences were found for age ($p < 0.001$), male gender ($p = 0.003$), chronic hepatitis ($p = 0.020$), γ-GTP ($p < 0.001$), albumin ($p = 0.004$), HBV genotypes ($p < 0.001$), HBeAg-negative status ($p < 0.001$), HBV DNA ($p < 0.001$), HBsAg level ($p < 0.001$), HBcrAg ($p < 0.001$), precore wild-type ($p < 0.001$), and core promoter wild-type ($p = 0.001$) (Table 4).

Factors contributing to HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.63 ($p = 0.002$)]; no family history in third-degree or closer relatives [RR 1.38 ($p = 0.037$)]; and HBsAg ≤2,000 IU/mL [RR 1.87 ($p < 0.006$)].

In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: age ≥50 [RR 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] (Table 5).

HBsAg seroclearance in treated patients

In the 982 treated patients, HBsAg persisted in 833 (84.8 %). HBsAg seroclearance occurred in 149 (15.2 %). In the baseline characteristics, significant difference were found for male gender ($p = 0.004$), no family history in third-degree or closer relatives ($p = 0.010$), chronic hepatitis ($p = 0.001$), AST ($p = 0.010$), γ-GTP ($p = 0.023$), platelet counts ($p < 0.001$), HBeAg-negative status

Table 3 Baseline characteristics in untreated and treated patients

Features at the baseline	Untreated (n = 1,130)	Treated (n = 982)	Differences p value
Age (years)	31 (1–81)	36 (6–75)	<0.001
Men	705 (62.4 %)	726 (71.9 %)	<0.001
Chronic hepatitis	1,094 (96.8 %)	937 (96.4 %)	0.079
Cirrhosis	36 (3.2 %)	45 (3.6 %)	
AST (IU/L)	27 (3–1,776)	56 (6–2,192)	<0.001
ALT (IU/L)	28 (2–3,020)	96 (8–2,740)	<0.001
γ-GTP (IU/L)	20 (4–1,494)	45 (4–1,278)	<0.001
Total bilirubin (mg/dL)	0.5 (0.1–20.1)	0.7 (0.2–21.2)	<0.001
Albumin (g/dL)	4.4 (2.2–5.8)	4.3 (1.1–5.4)	<0.001
Platelets ($\times 10^3$/mm^3)	202 (40–443)	181 (40–483)	<0.001
α-Fetoprotein (μg/L)	4 (1–2,060)	4 (1–1,610)	<0.001
HBeAg-negative status	857 (75.8 %)	312 (31.8 %)	<0.001
HBsAg (IU/mL)	2,240 (0.06–141,000)	5,270 (0.09–277,000)	<0.001
HBcrAg (log U/mL)	3.6 (<3.0 to >6.8)	> 6.8 (<3.0 to >6.8)	<0.001
Genotypes [A/B/C/others (%)]	5.7/20.0/72.6/1.7	3.4/11.1/84.9/0.5	<0.001
HBV DNA (log copies/mL)	4.7 (<2.1 to >9.1)	8.0 (<2.1 to >9.1)	<0.001

Median values with the range in parentheses or numbers with the percentage in parentheses are given

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

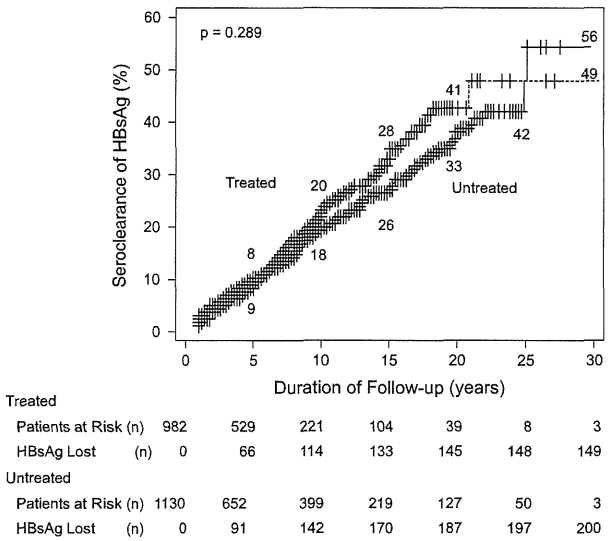

Fig. 2 Comparison of HBsAg seroclearance rates between 982 treated and 1,130 untreated patients. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

($p < 0.001$), HBV DNA ($p = 0.002$), HBsAg ($p < 0.001$), HBcrAg ($p = 0.003$), and precore wild-type ($p = 0.013$) (Table 6).

Factors contributing to HBsAg seroclearance in treated patients

In the 982 treated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.91 ($p = 0.001$)]; male gender [RR 2.14 ($p = 0.001$)], no family history in third-degree or closer relatives [RR 1.58 ($p = 0.005$)]; previous treatment with interferon [RR 2.13 ($p < 0.001$)]; chronic hepatitis [RR 3.12 ($p < 0.001$)]; AST ≥50 IU/L [RR 1.47 ($p = 0.031$)]; γ-GTP ≥20 IU/L [RR 1.87 ($p = 0.001$)]; platelets ≤150 × 10^3/mm^3 [RR 2.10 ($p < 0.001$)]; HBeAg-negative status [RR 2.53 ($p < 0.00$)]; HBV DNA ≤5 log copies/mL [RR 2.07 ($p = 0.001$)]; HBsAg ≤2,000 IU/mL [RR 2.29 ($p < 0.001$)]; HBcrAg ≤4 log U/mL [RR 2.28 ($p = 0.003$)]; and the wild-type precore sequence [RR 2.04 ($p = 0.011$)].

In multivariate analysis, only 3 factors contributed to HBsAg seroclearance: no family history in third-degree or closer relatives [RR 2.22 ($p = 0.006$)]; previous treatments with interferon [RR 3.15 ($p < 0.001$)]; and HBeAg-negative status [RR 3.75 ($p < 0.001$)] (Table 7).

Discussion

In Japan, perinatal materno-fetal transmission was the main route of HBV infection, but this transmission has been prevented since 1986 by the national campaign to prevent it by immunoprophylaxis with combined passive-active immunization of babies born to HBeAg-positive carrier mothers. However, HCC develops in about 10 % of the patients who have established chronic HBV infection by materno-fetal infection or through child-to-child transmission. Hence, HBsAg seroclearance is crucially required for preventing the development of cirrhosis followed by HCC.

In the present study, we analyzed 2,112 patients with persistent HBV infection to establish the factors

Table 4 Differences between the baseline characteristics of 917 untreated patients in whom HBsAg persisted and 213 those who lost HBsAg

Features at the baseline	HBsAg persisted ($n = 917$)	HBsAg lost ($n = 213$)	Differences p value
Age (years)	37 (1–81)	44 (0–80)	<0.001
Men	553 (60.3 %)	152 (71.4 %)	0.003
HBV in family members	349 (38.1 %)	76 (35.7 %)	0.509
Chronic hepatitis	893 (97.4 %)	201 (94.4 %)	0.020
AST (IU/L)	27 (3–1,144)	25 (6–1,776)	0.283
ALT (IU/L)	28 (6–1,960)	27 (6–3,020)	0.389
γ-GTP (IU/L)	22 (1–1,494)	29 (4–1,092)	<0.001
Total bilirubin (mg/dL)	0.6 (0.2–20.1)	0.7 (0.1–4.0)	0.257
Albumin (g/dL)	4.3 (2.0–5.3)	4.4 (1.6–5.7)	0.004
Platelets ($\times 10^3/mm^3$)	203 (40–443)	203 (33–417)	0.473
α-Fetoprotein (μg/L)	3 (1–2,060)	1 (1–478)	0.373
Genotypes [A/B/C/others (%)]	5.7/19.0/73.3/1.9	5.5/24.7/69.2/0.7	<0.001
HBeAg-negative status	663 (72.3 %)	194 (91.1 %)	<0.001
HBV DNA (log copies/mL)	4.9 (<2.1 to >9.1)	3.8 (<2.1 to >9.1)	<0.001
HBsAg (IU/mL)	3,100 (1.94–141,000)	149 (0.06–88,800)	<0.001
HBcrAg (log U/mL)	3.9 (<3.0 to >6.8)	2.9 (<3.0 to >6.8)	<0.001
Wild-type precore sequence	441 (48.1 %)	160 (75.0 %)	<0.001
Wild-type core promoter sequence	320 (34.9 %)	47 (22.0 %)	0.001

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 5 Factors influencing the seroclearance of HBsAg in untreated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.63 (1.19–2.23)	0.002	1.61 (1.09–2.37)	0.018
Male gender	1.08 (0.79–1.48)	0.618		
No HBV infection in family	1.38 (1.02–1.86)	0.037		
Cirrhosis	1.19 (0.73–1.93)	0.484		
AST ≥50 IU/L	1.01 (0.70–1.45)	0.979		
ALT ≥50 IU/L	0.93 (0.68–1.27)	0.633		
γ-GTP ≥20 IU/L	1.17 (0.85–1.61)	0.330		
Total bilirubin ≥1 mg/dL	1.41 (0.80–2.49)	0.239		
Albumin ≥4 g/dL	0.78 (0.51–1.18)	0.239		
Platelets >150 × $10^3/mm^3$	0.99 (0.67–1.46)	0.946		
α-Fetoprotein ≤10 μg/L	0.84 (0.48–1.47)	0.543		
Genotype A or B	1.17 (0.81–1.69)	0.410		
HBeAg-negative status	0.78 (0.79–2.07)	0.314		
HBV DNA ≥5 log copies/mL	0.84 (0.58–1.24)	0.383		
HBsAg ≤2,000 IU/mL	1.87 (1.19–2.91)	0.006	1.77 (1.12–2.77)	0.014
HBcrAg ≥4 log U/mL	0.85 (0.50–1.45)	0.555		
Wild-type precore sequence	0.99 (0.60–1.52)	0.967		
Wild-type core promoter sequence	0.78 (0.35–1.73)	0.538		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

contributing to HBsAg seroclearance. The overall rate of HBsAg seroclearance was 1.75 % annually. The annual seroclearance rates of HBsAg are reported to be 1.7 % in Korea [14] and 1.6 % in Taiwan [15–17], as well as 2.5 % in Goto Islands of Japan, where HBV infections are very prevalent [18]. In 1,271 natives in Alaska, the rate of HBsAg seroclearance was 0.7 % annually [19]. These differences could be ascribed, in part, to HBV genotypes distinct among Asian countries and Alaska. Since treatment with IFN and/or nucleot(s)ide analogues has suppressive effects on the development of HCC [6, 20], they may influence HBsAg seroclearance.

Table 6 Differences in baseline characteristics between the 833 treated patients in whom HBsAg persisted and 149 those who lost HBsAg

Features at the baseline	HBsAg persisted (n = 833)	HBsAg lost (n = 149)	Differences p value
Age (years)	41 (13–88)	43 (17–71)	0.285
Men	601 (72.2 %)	124 (83.2 %)	0.004
HBV in family members	496 (59.6 %)	72 (48.3 %)	0.010
Chronic hepatitis	802 (96.3 %)	134 (89.9 %)	0.001
AST (IU/L)	54 (6–2,192)	78 (7–888)	0.010
ALT (IU/L)	93 (8–2,740)	118 (8–1,700)	0.117
γ-GTP (IU/L)	44 (4–1,278)	46 (4–1,278)	0.023
Total bilirubin (mg/dL)	0.7 (0.2–21.2)	0.7 (0.3–8.4)	0.273
Albumin (g/dL)	4.3 (1.1–5.4)	4.5 (1.4–5.3)	0.281
Platelets ($\times 10^3$/mm^3)	182 (40–483)	171 (50–391)	<0.001
α-Fetoprotein (μg/L)	4 (1–1,610)	4 (1–765)	0.682
Genotypes [A/B/C/others (%)]	3.2/10.7/85.1/1.0	5.1/12.4/81.6/0.9	0.565
HBeAg-negative status	230 (27.6 %)	79 (53.0 %)	<0.001
HBV DNA (log copies/mL)	7.8 (<2.1 to >9.1)	8.3 (<2.1 to >9.1)	0.002
HBsAg (IU/mL)	7,880 (0.04–277,000)	1,380 (0.04–188,000)	<0.001
HBcrAg (log U/mL)	6.9 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	0.003
Wild-type precore sequence	554 (66.6 %)	61 (41.2 %)	0.013
Wild-type core promoter sequence	274 (32.9 %)	67 (45.0 %)	0.836

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 7 Factors influencing the seroclearance of HBsAg in treated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.91 (1.32–2.77)	0.001		
Male gender	2.14 (1.37–3.33)	0.001		
No HBV infection in family	1.58 (1.15–2.19)	0.005	2.22 (2.32–3.94)	0.006
Treatments (interferon vs. others)	2.13 (1.53–2.98)	<0.001	3.15 (1.69–5.87)	<0.001
Chronic hepatitis	3.12 (2.05–4.74)	<0.001		
AST ≥50 IU/L	1.47 (1.04–2.09)	0.031		
ALT ≥50 IU/L	1.29 (0.82–1.92)	0.201		
γ-GTP ≥20 IU/L	1.87 (1.30–2.70)	0.001		
Total bilirubin ≥1 mg/dL	1.35 (0.87–2.08)	0.179		
Albumin ≥4 g/dL	1.11 (0.66–1.86)	0.688		
Platelets ≤150 × 10^3/mm^3	2.10 (1.49–2.96)	<0.001		
α-Fetoprotein ≤10 μg/L	1.33 (0.92–1.92)	0.136		
Genotype A or B vs. others	1.16 (0.74–1.82)	0.529		
HBeAg-negative status	2.53 (1.83–3.50)	<0.001	3.75 (2.09–6.74)	<0.001
HBV DNA ≤5 log copies/mL	2.07 (1.37–3.13)	0.001		
HBsAg ≤2,000 IU/mL	2.29 (1.52–3.47)	<0.001		
HBcrAg ≤4 log U/mL	2.28 (1.31–3.97)	0.003		
Wild-type precore sequence	2.04 (1.18–3.55)	0.011		
Wild-type core promoter sequence	1.18 (0.63–2.21)	0.608		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A176.2/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Therefore, we went on to extend our analysis to untreated patients and those treated with IFN or nucleotide analogues separately. Criteria for upper or lower levels of each parameter were set, taking into consideration the median value or a cutoff value with the lowest p value of the entire 2,112-patient cohort (Table 1), and unified for untreated and treated patients (Tables 5, 7).

Firstly, in the univariate analysis, age, no family history of HBV infection in third-degree or closer relatives, and decreased HBsAg levels lowered the annual rate of HBsAg seroclearance significantly. In multivariate analysis, age $\geq$50 years (RR 1.61, $p = 0.018$) and HBsAg $\leq$2,000 IU/mL (RR 1.77, $p = 0.014$) decreased the annual rate of HBsAg seroclearance significantly. Kato et al. [18] reported high HBsAg seroclearance rates in patients over 40 or over 50 years; in our patients, also, age $\geq$50 years increased RR to 1.61 ($p = 0.018$). As for HBsAg and HBV DNA, low HBsAg and HBV DNA levels increased the HBsAg seroclearance rate to 37.7 %, and therefore, low HBsAg levels are an important factor. In actuality, HBsAg levels $\leq$2,000 IU/mL increased the rate of HBsAg seroclearance with RR 1.77 ($p = 0.014$).

In treated patients, by contrast, age, the male gender, no HBV infections in third-degree or closer relatives, treatment with IFN, chronic hepatitis, high AST levels, high γ-GTP levels, low platelet counts, HBeAg-negative status, low HBsAg levels, low HBcrAg levels and the wild-type precore sequence were significant factors in univariate analysis. In multivariate analysis, no HBV infections in third-degree or closer relatives (RR 2.22, $p = 0.006$), interferon treatments (RR 3.15, $p < 0.001$), and HBeAg-negative status (RR 3.75, $p < 0.001$) were significant factors.

Thus, there were differences in factors predictive of the HBsAg loss between untreated and treated patients. Remarkably, age and HBsAg titer were independent factors in untreated patients, whereas family history and negative HBeAg were independent factors in treated patients. Since this work studied patients who were followed for a long time (>15 years), age and HBsAg titer were factors for clearance of HBsAg in untreated patients. Treated patients, in contrast, would have included more patients with HBeAg, with a good response to antiviral treatment, as well as those without family history who would have been infected with HBV with a sorter duration than those with family history. In other words, most untreated patients were those with favorable clinical course, in whom HBsAg titer gradually decreased and eventually lost it with time. In fact, there would be many such patients, the majority of whom do not visit hospitals and are unaware of HBV infection, who may have unapparent liver disease. Treated patients, on the other hand, would have had higher risks for cirrhosis and HCC, owing to elevated ALT/AST levels; this risk is especially high for patients with a family history of HBV [21]. Therefore, patients with family history would not be able to easily lose HBsAg.

In treated patients, IFN led to HBsAg loss more effectively than other treatments [RR 2.13, $p < 0.001$ (Table 7)]. The immunomodulatory activity of IFN, which is not shared by nucleot(s)ide analogues, would have accelerated the immune response to HBV required for the seroclearance of HBsAg. Of the 333 patients who received IFN, 190 (57 %) were treated with IFN multiply. In them, seroclearance of HBsAg was achieved in 49 of the 190 (26 %) patients with multiple IFN treatments in comparison with 41 of the 143 (29 %) with single IFN treatment. Owing to indications for IFN, patients who received IFN tended to be younger, without previous treatments and higher HBV DNA as well as ALT levels. They might have increased the rate of HBsAg loss that was higher with IFN than other treatments.

Since this is a retrospective cohort study of patients visiting our hospital for more than 15 years, and there has been so much innovation in the treatment of chronic hepatitis B during that period, treated and untreated patients have different backgrounds at the baseline. Hence, treated patients had higher ALT and HBV DNA levels with severer liver disease than untreated patients (Table 3). This might have been responsible, at least in part, for the failure in finding differences in the rate of HBsAg loss between untreated and treated patients (Fig. 2). Future studies will be aimed at analyzing contributing factors in treated and matched controls. This will allow us to analyze factors contributing to HBsAg seroclearance in the treatment of patients with chronic hepatitis B.

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