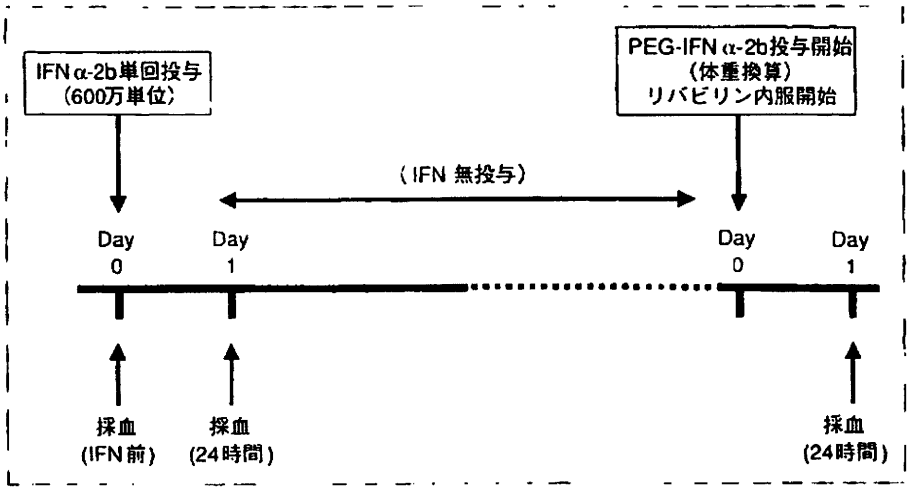


図1 インターフェロン抵抗性試験とペグインターフェロン・リバビリン併用療法

## 結　果

全107例中29例(27.1%)で治療中ペグインターフェロンを，49例(45.8%)でリバビリンを減量したが中止例はなかった．治療中の血中HCV RNAの陰性化はRVRが6例(5.6%)，EVR40例(37.4%)，投与終了時の血中HCV RNA陰性化例77例(72.0%)であり，最終的な治療効果としてはSVR39例(36.5%)，再燃(relapse)例38例(35.5%)，NR30例(28.0%)であった．

HCVの各領域のアミノ酸変異の検討では，Core領域70番のアミノ酸はarginine(Arg)70例(65.4%)・glutamine(Gln)29例(27.1%)であり，残り8例中5例はhistidine，3例はArgとGlnのmixed typeであった．Core領域91番のアミノ酸ではleucine(Leu)76例(71.0%)，methionine(Met)29例(27.1%)であり，残りの2例はLeuとMetのmixed typeであった．E1領域の139番のアミノ酸ではthreonine(Thr)55例(51.4%)，alanine(Ala)40例(37.4%)であり，残り12例中serineが2例，ThrとAlaのmixed typeが4例，deletionが4例あり，ほかの2例では領域が増幅できなかった．一方，NS5A領域のISDRの変異については，HCV-Jとの比較で変異数が2以下のものをwild type，3以上のものをnon-wild typeとすると，wild type64例(59.8%)，non-wild type43例(40.2%)であった．なお，いずれの変異も年齢・性・肝線維化の程度と関連を認めなかった．

各領域のアミノ酸による投与前開始前のウイルス量の比較を図2に示す．インターフェロン抵抗性とされているNS5A領域のISDRのwild typeでnon-wild typeに比べ有意に高く(2076±1213KIU/ml vs. 1291±830KIU/ml：$P$=0.0004)，今までの報告と一致した．一方，Core領域70番のアミノ酸での比較ではインターフェロン抵抗性とされているCore 70-Gln症例でArgに比べ投与前開始前のウイルス量はむしろ低かった(1410±895KIU/ml vs. 1943±1204KIU/ml：$P$=0.0260)．Core領域91番とE1領域139番のアミノ酸については投与前開始前のウイルス量に差はみられなかった．

次にインターフェロン感受性試験後のウイルス量の低下をみると(図3)，通常のインターフェロンα投与24時間後の血中HCV RNA量はCore領域70番のアミノ酸がArgの症例でGlnの症例に比べ有意に低下した(1.50±0.45 $\log_{10}$ vs. 0.95±0.69 $\log_{10}$, $P$<0.0001)．また，ペグインターフェロン・リバビリン併用療法開始後24時間の時点でも血中HCV RNA量はCore領域70番のアミノ酸がArgの症例でGlnの症例に比べ有意に低下した(1.36±0.52 $\log_{10}$ vs. 0.97±0.57 $\log_{10}$, $P$=0.0025：図4)．一方，Core領域91番・E1領域139番・NS5A領域ISDRのアミノ酸とインターフェロン感受性試験後のウイルス量の低下，ペグインターフェロン・リバビリン併用療法開始後24時間の時点でのウイルス量の低下との間には関連は認められなかった．

各領域のアミノ酸変異によるRVR・EVRおよび

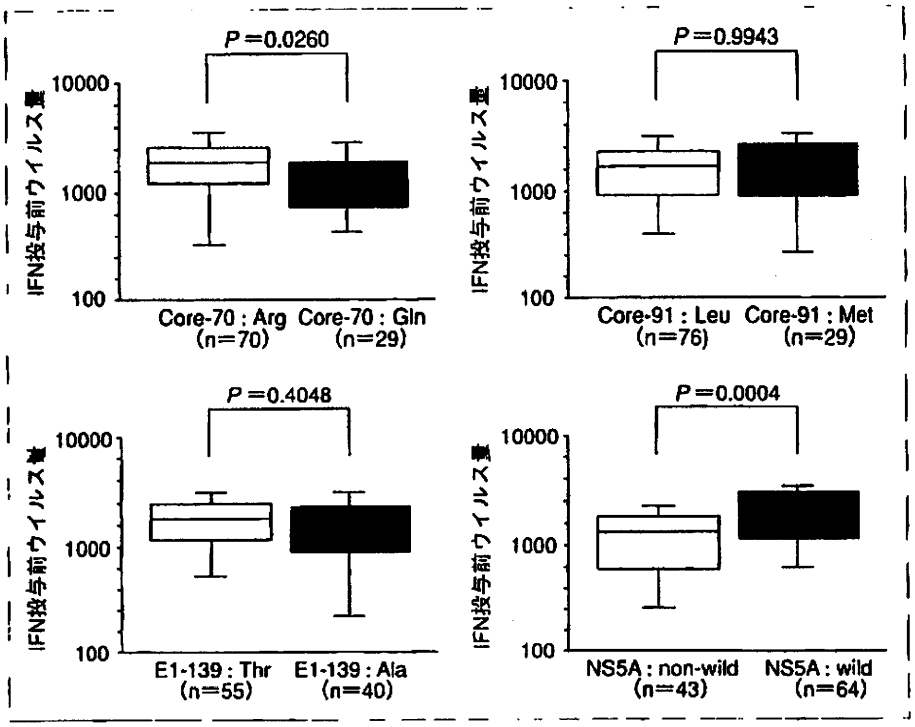


図2　HCV Core・E1・NS5A領域のアミノ酸変異と治療前のHCV RNA量（amplicor monitor法・version 2.0：KIU/ml）

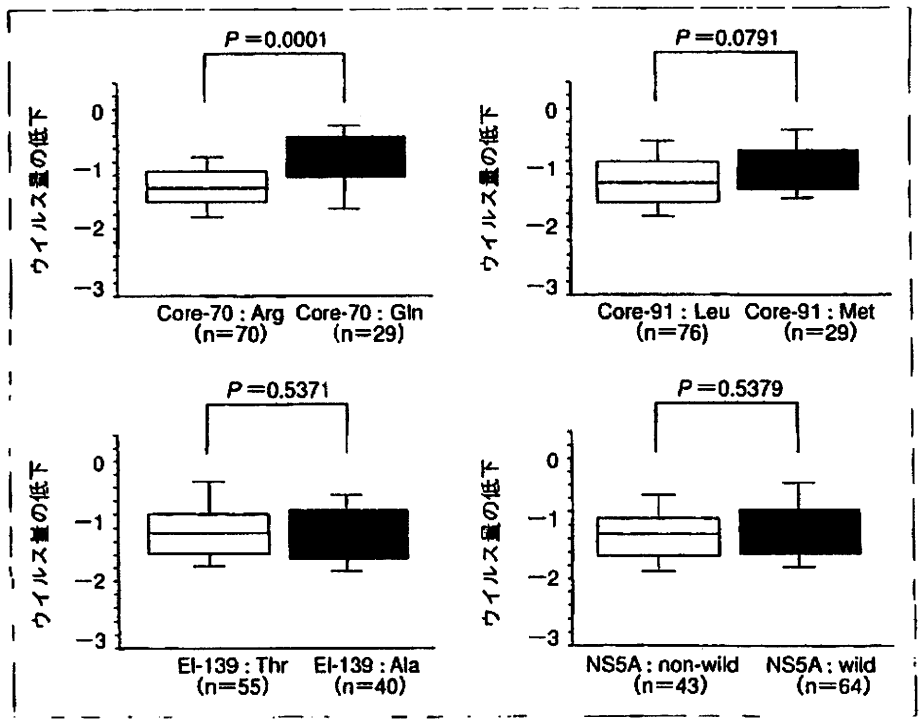


図3　HCV Core・E1・NS5A領域のアミノ酸変異とインターフェロン抵抗性試験によるHCV RNAの低下（$\log_{10}$）

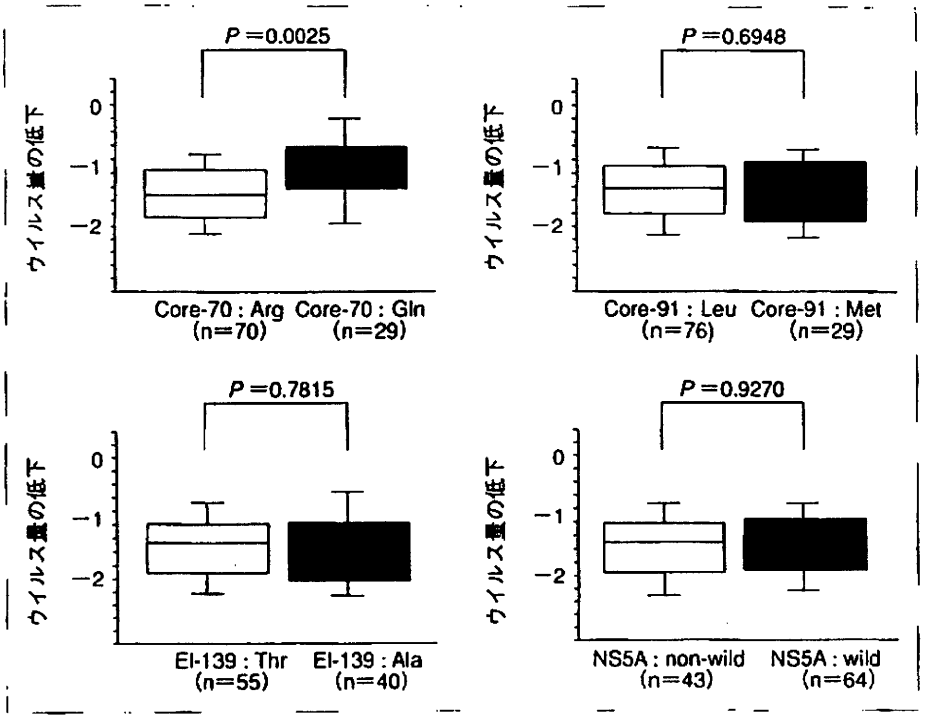


図4 HCV Core・E1・NS5A領域のアミノ酸変異とペグインターフェロン・リバビリン併用療法開始24時間後のHCV RNAの低下($log_{10}$)

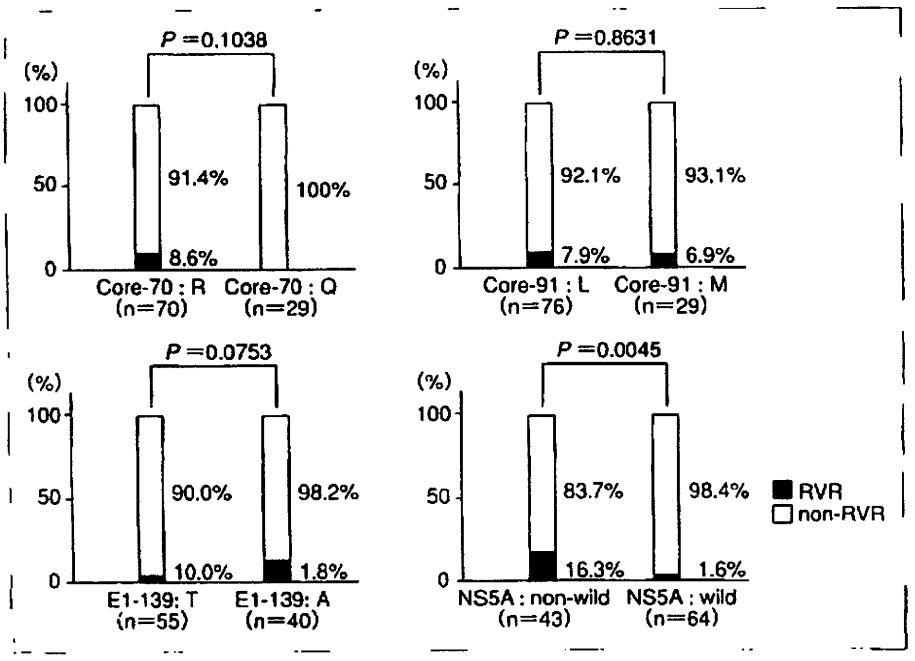


図5 HCV Core・E1・NS5A領域のアミノ酸変異とrapid virologic response

最終的な治療効果を図5〜7に示す．RVR症例は6例のみであったがISDRの非wild typeにおいてwild typeより有意に多いという結果が得られた($P$=0.0045)．EVRについてはCore領域70番のアミノ

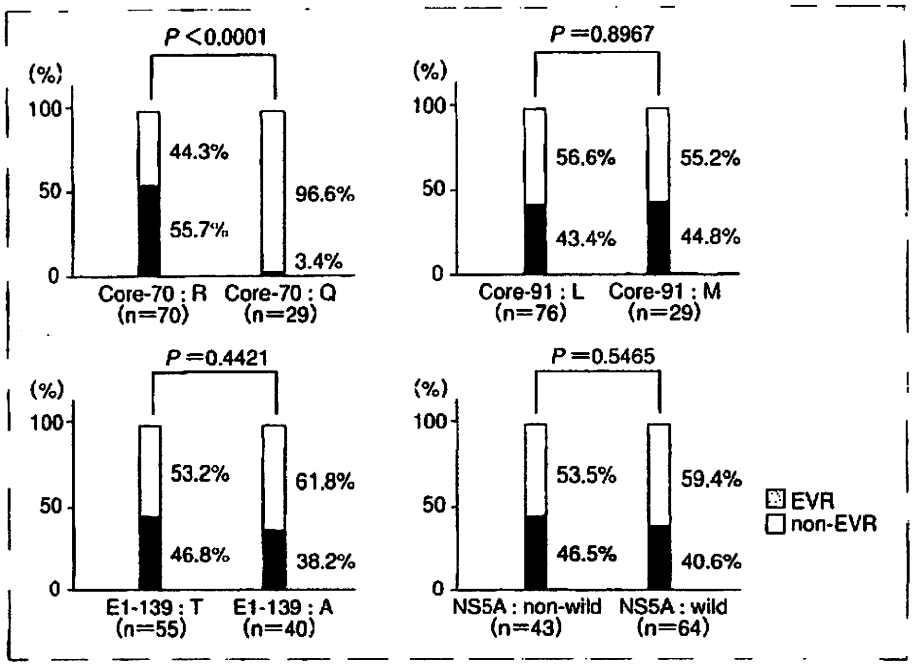


図6　HCV Core・E1・NS5A領域のアミノ酸変異とearly virologic response

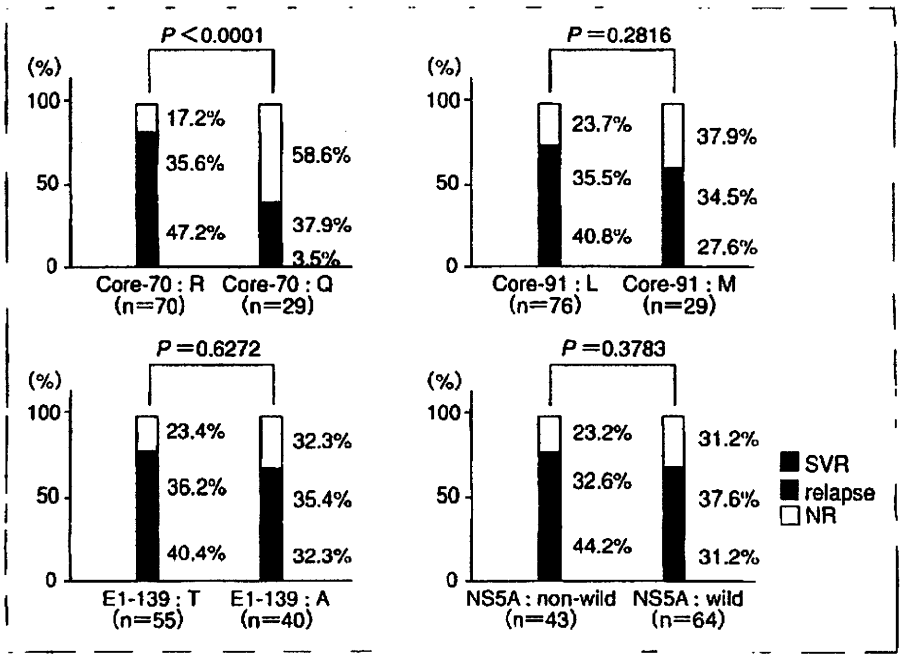


図7　HCV Core・E1・NS5A領域のアミノ酸変異とtreatment outcome

酸により有意差をもってEVR率に差がみられ（$P<0.0001$），同部位のアミノ酸がArgの症例では50%以上がEVRになったのに対し，Glnの症例でEVRになったのはわずか1例であった．最終的な治療効果においてもCore領域70番のアミノ酸は治療効果に強く影響しており（$P<0.0001$），同部位のアミノ酸がArgの症例では50%近くの症例がSVRとなり，対照的にGlnの症例では50%以上がNRで

表3 Early virologic responseに関与する因子(多変量解析)

| Factor | | parameter estimate | standerd error | X | Odds ratio (95% confidence interval) | *P value* |
|---|---|---|---|---|---|---|
| ・年齢 | 1：≦60歳 | | | | 1 | |
| | 2：>60歳 | 0.4870 | 0.3236 | 2.27 | 2.6487(0.7693～10.048) | 0.1323 |
| ・性別 | 1：男性 | | | | 1 | |
| | 2：女性 | −0.4370 | 0.4363 | 1.00 | 0.4173(0.0651～2.1643) | 0.3165 |
| ・体重 | 1：≦60kg | | | | 1 | |
| | 2：>60kg | −0.6198 | 0.4282 | 2.09 | 0.2895(0.0459～1.4281) | 0.1478 |
| ・肝組織像 | 1：A0/A1 | | | | 1 | |
| -activity | 2：A2/A3 | −0.0797 | 0.4175 | 0.04 | 0.8527(0.1614～4.5255) | 0.8487 |
| ・肝組織像 | 1：F0/F1 | | | | 1 | |
| -fibrosis | 2：F2/F3 | 0.0177 | 0.4419 | 0.00 | 1.0360(0.1791～6.0891) | 0.9681 |
| ・Core-70 | 1：Arg | | | | 1 | |
| | 2：Gln | −1.9777 | 0.5990 | 10.90 | 0.0191(0.0008～0.1314) | 0.0010 |
| ・Core-91 | 1：Leu | | | | 1 | |
| | 2：Met | −0.1308 | 0.4518 | 0.08 | 0.7699(0.1239～4.6302) | 0.7722 |
| ・E1-139 | 1：Thr | | | | 1 | |
| | 2：Ala | −0.3073 | 0.3290 | 0.87 | 0.5408(0.1442～1.9603) | 0.3503 |
| ・NS5A-ISDR | 1：wild | | | | 1 | |
| | 2：non-wild | 0.4002 | 0.3336 | 1.44 | 2.2266(0.6143～8.6856) | 0.2303 |

表4 Sustained virologic responseに関与する因子(多変量解析)

| Factor | | Parameter estimate | Standard error | X | Odds ratio (95% confidence interval) | *P value* |
|---|---|---|---|---|---|---|
| ・年齢 | 1：≦60歳 | | | | 1 | |
| | 2：>60歳 | 0.2172 | 0.3114 | 0.49 | 1.5441(0.4544～5.3670) | 0.4854 |
| ・性別 | 1：男性 | | | | 1 | |
| | 2：女性 | −0.6361 | 0.4263 | 2.23 | 0.2802(0.0443～1.3663) | 0.1356 |
| ・体重 | 1：≦60kg | | | | 1 | |
| | 2：>60kg | −0.5240 | 0.4129 | 1.61 | 0.3507(0.0593～1.6447) | 0.2045 |
| ・肝組織像 | 1：A0/A1 | | | | 1 | |
| -activity | 2：A2/A3 | 0.2470 | 0.4220 | 0.34 | 1.6388(0.3167～9.2242) | 0.5584 |
| ・肝組織像 | 1：F0/F1 | | | | 1 | |
| -fibrosis | 2：F2/F3 | −0.4120 | 0.4451 | 0.86 | 0.4387(0.0702～2.4515) | 0.3546 |
| ・Core-70 | 1：Arg | | | | 1 | |
| | 2：Gln | −1.6373 | 0.5636 | 8.44 | 0.0378(0.0019～0.2322) | 0.0037 |
| ・Core-91 | 1：Leu | | | | 1 | |
| | 2：Met | −0.2967 | 0.4388 | 0.46 | 0.5524(0.0870～2.9801) | 0.4989 |
| ・E1-139 | 1：Thr | | | | 1 | |
| | 2：Ala | −0.3015 | 0.3262 | 0.85 | 0.5471(0.1481～1.9691) | 0.3553 |
| ・NS5A-ISDR | 1：wild | | | | 1 | |
| | 2：non-wild | 0.4233 | 0.3294 | 1.65 | 2.3317(0.6553～8.9412) | 0.1988 |

あった．EVRおよび最終的な治療効果においてCore領域91番・E1領域139番・NS5A領域ISDRのアミノ酸による比較では有意な差は認められなかった．

EVR・SVR・NRに対して年齢・性・体重・組織像(activityおよびfibrosis)と各領域のアミノ酸変異を因子として多変量解析を行うと，EVR・SVR・NRいずれの場合においてもCore領域70番のアミノ酸変異のみが独立して有意に関与する因子であった(表3～5)．

## 考　察

NS5A-ISDRのアミノ酸配列とインターフェロン単独療法の効果との関係が報告されて以来，HCVの各領域のアミノ酸配列とインターフェロンを中心とした抗HCV療法との関連を検討した報告が多くみられるが，今回の検討では最近報

表5　Null-responseに関与する因子(多変量解析)

| Factor | Parameter estimate | Parameter estimate | Standard error | X | Odds ratio (95% confidence interval) | *P value* |
|---|---|---|---|---|---|---|
| ・年齢 | 1：≦60歳 | | | | 1 | |
| | 2：>60歳 | 0.0351 | 0.3118 | 0.01 | 1.0727(0.3058～3.6504) | 0.9104 |
| ・性別 | 1：男性 | | | | 1 | |
| | 2：女性 | 0.1000 | 0.4186 | 0.06 | 1.2214(0.2408～6.8212) | 0.8112 |
| ・体重 | 1：≦60kg | | | | 1 | |
| | 2：>60kg | 0.1958 | 0.3919 | 0.25 | 1.4795(0.3210～7.2819) | 0.6173 |
| ・肝組織像 | 1：A0/A1 | | | | 1 | |
| -activity | 2：A2/A3 | −0.0167 | 0.4121 | 0.00 | 0.9672(0.1748～4.7639) | 0.9678 |
| ・肝組織像 | 1：F0/F1 | | | | 1 | |
| -fibrosis | 2：F2/F3 | −0.0421 | 0.4253 | 0.01 | 0.9193(0.1665～4.9935) | 0.9212 |
| ・Core-70 | 1：Arg | | | | 1 | |
| | 2：Gln | 0.9716 | 0.3214 | 9.14 | 6.9812(2.0852～26.906) | 0.0025 |
| ・Core-91 | 1：Leu | | | | 1 | |
| | 2：Met | 0.3489 | 0.4105 | 0.72 | 2.0091(0.3841～10.144) | 0.3954 |
| ・E1-139 | 1：Thr | | | | 1 | |
| | 2：Ala | 0.5052 | 0.3400 | 2.21 | 2.7469(0.7453～11.201) | 0.1373 |
| ・NS5A-ISDR | 1：wild | | | | 1 | |
| | 2：non-wild | −0.0605 | 0.3304 | 0.03 | 0.8860(0.2339～3.2396) | 0.8547 |

告されたCore領域70番のアミノ酸変異がHCVのインターフェロンへの感受性，およびペグインターフェロン・リバビリン併用療法への反応と治療効果に大きく関与していることが示された．Core領域70番のアミノ酸がGlnの症例ではArgの症例に比べてインターフェロン感受性試験における血中のHCV RNAの低下率は有意に低く，インターフェロンに対する抵抗性を示唆した．われわれの検討ではインターフェロン単回投与24時間後，もしくはペグインターフェロン・リバビリン併用療法開始24時間後のウイルス量の低下の悪さはペグインターフェロン・リバビリン併用療法におけるNRと強く関連しており[20]，したがってCore領域70番のアミノ酸変異はペグインターフェロン・リバビリン併用療法に対するNRとの強い関連が考えられていたが，今回の検討ではSVRに対してもきわめて強い関連がみられた．井田らの報告ではCore領域のアミノ変異は70番と91番がともにmutant(GlnとMet)であるいわゆるdouble mutant症例がペグインターフェロン・リバビリン併用療法に対し強い抵抗性を示すとされるが，Core領域70番のアミノ酸変異は単独でも現行のペグインターフェロン・リバビリン併用療法の効果に対して強く影響しているようである．

一方，NS5A領域のISDRの変異はペグインターフェロン・リバビリン併用療法におけるRVRとの関連が示唆された．RVR症例は最終的なSVRを予測するもっとも強い因子とされる[21][22]．今回の検討ではISDRの変異のSVRへの関与は認められなかったが，今回の検討は症例がgenotype 1Bの高ウイルス量症例に限られており，低ウイルス量症例も含めてHCV genotype 1B全体の検討ではISDRの変異がSVRに関与する可能性がある．また，ISDRの変異に関しては，従来の報告通り投与前のHCV RNA量との関連が示された．インターフェロンを用いた抗HCV療法においてはgenotypeがもっとも強く治療効果に関与するが，同一のgenotypeの場合には治療前のHCV RNA量は重要な効果規定因子である．さらに最近のreal-time PCR法を用いたHCV RNA定量の導入によりその重要性は増している．今回の検討ではHCV RNA量の評価はamplicor monitor法によっているが，real-time PCRでのHCV RNA量の評価によりペグインターフェロン・リバビリン併用療法におけるISDR変異の位置づけも変わるかもしれない．

## おわりに

今回，HCV genotype 1Bの高ウイルス量症例に限りHCVの4か所のアミノ酸変異とインター

フェロンへの感受性，ペグインターフェロン・リバビリン併用療法の効果との関連を検討した．今回の検討ではCore領域70番のアミノ酸変異がきわめて強く治療効果と関連していた．しかしそのメカニズムは不明であり，今後の研究が待たれる．またgenotype 1B，および他のgenotypeにおいても上記以外のHCVのさまざまな領域のアミノ酸変異とインターフェロン療法の効果との関連が現在も研究されており，われわれは今後もこれらの研究結果を慎重に見極めていく必要があろう．

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CLINICAL STUDIES

# Eight-week regimen of antiviral combination therapy with peginterferon and ribavirin for patients with chronic hepatitis C with hepatitis C virus genotype 2 and a rapid virological response

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chronic hepatitis C – genotype 2 – peginterferon – ribavirin – sustained virological response

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## Abstract

*Background:* It remains unclear how we can shorten the treatment duration of antiviral combination therapy with peginterferon and ribavirin for patients with chronic hepatitis C virus (HCV) genotype 2 infection who achieved a rapid virological response (RVR). *Aim:* We compared the efficacy of antiviral combination therapy with peginterferon and ribavirin for 8 vs. 24 weeks for the treatment of patients with HCV genotype 2 infection and with RVR. *Methods:* Sixty-one patients were enrolled. Serum HCV RNA was not detected at 4 weeks after the start of treatment in 32 patients with an RVR. These 32 patients were randomly assigned to 8-week ($n = 15$) or 24-week ($n = 17$) treatment regimens. Patients in the 8-week group who relapsed underwent a 24-week retreatment. *Results:* No significant difference in patient characteristics was observed between the 8- and the 24-week treatment groups. A sustained virological response (SVR) was seen in five of 15 patients (33.3%) in the 8-week treatment group and 14 of 17 (82.4%) in the 24-week treatment group; the rate was significantly higher in the 24-week treatment group ($P = 0.0140$). Nine of 10 relapsed patients in the 8-week treatment group underwent a 24-week retreatment, and seven achieved an SVR. *Conclusion:* An 8-week regimen of combination antiviral therapy with peginterferon and ribavirin yielded an increase in the relapse rate, indicating the limitation of a reduction of treatment below 12 weeks in patients with genotype 2, after RVR.

The currently recommended treatment regimen for patients with chronic hepatitis C who are infected with hepatitis C virus (HCV) genotype 2 is combination therapy comprising weekly administration of peginterferon and daily administration of ribavirin for 24 weeks (1). Approximately 80% of patients have a sustained virological response (SVR) with this regimen (2, 3). Increasing the duration of treatment from 24 to 48 weeks does not appear to increase the rate of SVR (2–4). Several studies have attempted to shorten the treatment period for this patient population. SVR rates of 80–85% were observed with just 12–16 weeks of treatment in patients in whom serum HCV RNA was undetectable within 4 weeks after the start of therapy (5–8). These results suggest that patients with HCV genotype 2 infection and a rapid virological response (RVR, undetectable serum HCV RNA by 4 weeks after the start of therapy) may be treated for $< 24$ weeks. However, the results of a recent large clinical trial reported a lower overall SVR rate with a 16-week treatment regimen than with the standard 24-week treatment regimen in patients with HCV genotype 2 or 3, although the results suggested that the difference in SVR rate was smaller in patients with an RVR (9).

Antiviral therapy with peginterferon and ribavirin can cause various adverse effects, some of which can be severe. Adverse effects reportedly increase with the length of treatment (10). Investigation into further shortening of the treatment period is therefore important in terms of adverse effects and medical economy. In the present study, we evaluated the efficacy of an 8-week regimen of antiviral therapy with peginterferon and ribavirin for patients with HCV genotype 2 and an RVR.

## Patients and methods

### Patients

A total of 61 patients with chronic HCV genotype 2 infection who underwent antiviral combination therapy with peginterferon and ribavirin were enrolled in this study during the period January – June 2006. The patient characteristics are listed in Table 1. Patients comprised 24 men and 37 women with a mean age of $56.7 \pm 11.2$ years. Forty patients had no history of interferon therapy (naïve cases), and 21 patients had previously undergone interferon therapy (retreatment cases). HCV genotype was 2a in 46 patients and 2b in 15 patients, and the pretreatment HCV RNA concentration was $1919 \pm 1618 \times 10^3$ IU/ml by a quantitative polymerase chain reaction assay (Amplicor GT-HCV Monitor, version 2.0; Roche Molecular Systems, Pleasanton, CA, USA). All patients had a pretreatment serum HCV RNA concentration of $> 100 \times 10^3$ IU/ml because the addition of ribavirin to peginterferon is not allowed by Japanese National Medical Insurance for patients with a pretreatment HCV RNA concentration $\leq 100 \times 10^3$ IU/ml. Liver histology was evaluated before antiviral therapy in specimens obtained by a fine-needle biopsy. According to METAVIR scoring (11), the activity grade was A0 in one patient, A1 in 39, A2 in 13

and A3 in four, and the fibrosis grade was F0 in three patients, F1 in 39, F2 in 11 and F3 in four. Liver biopsy was not performed for four patients.

### Study design

All patients were given peginterferon α-2b (Schering-Plough, Osaka, Japan) weekly and ribavirin (Schering-Plough) daily. For peginterferon, patients weighing ≤ 45 kg were given 60 μg, those > 45 kg and ≤60 kg were given 80 μg, those >60 kg and ≤75 kg were given 100 μg, those >75 kg and ≤90 kg were given 120 μg and those >90 kg were given 150 μg according to the manufacturer's recommendations. The ribavirin dosage was also adjusted according to body weight. Patients ≤60 kg were given 600 mg, those >60 kg and ≤80 kg were given 800 mg and those >80 kg were given 1000 mg according to the manufacturer's recommendations.

Serum HCV RNA was measured 4 weeks after the start of therapy. Patients with detectable serum HCV RNA underwent the standard 24-week treatment. Patients with no detectable serum HCV RNA were randomly assigned to one of two groups; one group underwent the 8-week treatment, and the other underwent the standard 24-week treatment. When relapse occurred after completion of treatment in the 8-week group, it was recommended that the patients undergo retreatment with the same dose of peginterferon α-2b and ribavirin for an additional standard 24 weeks.

All patients were monitored during the treatment period and for an additional 6 months after the end of treatment. They underwent weekly outpatient evaluations during the first 2 months and monthly evaluations during the rest of the treatment period and during the 6-month follow-up period. Patients underwent a physical examination, complete blood count, laboratory tests and serum HCV RNA measurement. Serum HCV RNA was measured using a COBAS TaqMan HCV test (Roche Molecular Systems); the detection limit was 15 copies/ml. SVR was defined as a continuous absence of serum HCV RNA up to 6 months after the completion of therapy.

**Table 1.** Clinical characteristics of the study patients ($n = 61$)

| | |
|---|---|
| Age (years) | 56.7 ± 11.2 |
| Gender (female/male) | 37 (60.7)/24 (39.3) |
| History of interferon therapy (naïve/retreatment) | 40 (65.6)/21 (34.4) |
| History of transfusion (no/yes) | 44 (72.1)/17 (27.9) |
| Alanine aminotransferase (IU/L) | 51.4 ± 56.4 |
| Aspartate aminotransferase (IU/L) | 45.7 ± 49.2 |
| γ-glutamyl transpeptidase (IU) | 58.7 ± 123.7 |
| Alkaline phosphatase (IU/L) | 270.5 ± 147.9 |
| Albumin (g/dl) | 4.30 ± 0.33 |
| Total bilirubin (mg/dl) | 0.65 ± 0.24 |
| White blood cell count (/μl) | 5001 ± 1602 |
| Haemoglobin (g/dl) | 14.0 ± 1.5 |
| Platelet count (× $10^3$/μl) | 19.5 ± 6.5 |
| Body weight (kg) | 59.1 ± 10.9 |
| Liver histology – activity (A0/A1/A2/A3)* | 1 (1.8)/39 (68.4)/ 13 (22.8)/4 (7.0) |
| Liver histology – fibrosis (F0/F1/F2/F3)* | 3 (5.3)/39 (68.4)/ 11 (19.3)/4 (7.0) |
| HCV genotype (2a/2b) | 46 (75.4)/15 (24.6) |
| HCV RNA concentration (× $10^3$ IU/ml) | 1919 ± 1618 |

Percentages are shown in parentheses.

*Liver biopsy was not performed in four patients.

HCV, hepatitis C virus.

### Statistical analysis

Quantitative values are shown as mean ± standard deviation. Between-group differences were analysed using the $\chi^2$ test. Differences in quantitative values between two groups were analysed using the Mann–Whitney *U*-test. All *P* values were two tailed, and $P < 0.05$ was accepted as being statistically significant.

The study protocol was approved by the hospital ethics committee and was carried out in compliance with the Helsinki declaration. Written informed consent was obtained from each patient before enrolment in this study.

## Results

One patient dropped out of this study within 4 weeks after the start of treatment because of severe general malaise (Fig. 1). Therefore, serum HCV RNA at 4 weeks after the start of therapy was measured in 60 patients. Serum HCV RNA was detected in 28 (46.7%) patients and was not detected in 32 (53.3%) patients (RVR). The characteristics of RVR and non-RVR patients are listed in Table 2. A history of transfusion was most prevalent and the HCV RNA concentration was the lowest in RVR patients. The 32 RVR patients were randomized at 4 weeks after the start of therapy: 8-week group ($n = 15$) and 24-week group ($n = 17$, Fig. 1). Patients in the 8-week group underwent an additional 4 weeks of treatment. Patients in the 24-week group underwent an additional 20 weeks of treatment. No difference was found in the background characteristics between these two groups. The single dose of peginterferon and ribavirin was reduced in one and five of 17 patients, respectively, in the 24-week group, whereas no patient required reduction of the dose of peginterferon or ribavirin in the 8-week group. In non-RVR patients, three of 28 patients required reduction of the peginterferon dose, and six of 28 patients required reduction of the ribavirin dose during the treatment period. No patient required discontinuation of peginterferon or ribavirin during the treatment regimen and no patient dropped out of this study during the treatment or follow-up periods.

In patients who achieved an RVR, the SVR rate was 82.4% (14 of 17 patients) in the 24-week group and 33.3% (five of 15 patients) in the 8-week group. The SVR rate was significantly lower in the 8-week group than that in the 24-week group ($P = 0.0140$). Nine of 10 patients with relapse after treatment in the 8-week group subsequently underwent retreatment with the standard 24-week regimen (one patient declined retreatment). The SVR rate in this retreatment group was 77.8% (seven of nine patients). The SVR rate of the entire 8-week group, when including seven SVR patients by retreatment, was 80.0% (12 of 15 patients). The SVR rate between the 8- and the 24-week groups was similar when patients with an SVR by retreatment were included in the 8-week group ($P = 0.8650$). The SVR rate of patients without an RVR (non-RVR) was 53.6% (15 of 28 patients), which was between that of the 24-week group with an RVR and that of the 8-week group.

The characteristics and outcomes of patients in the 8-week group are listed in Table 3. There was no difference in patient

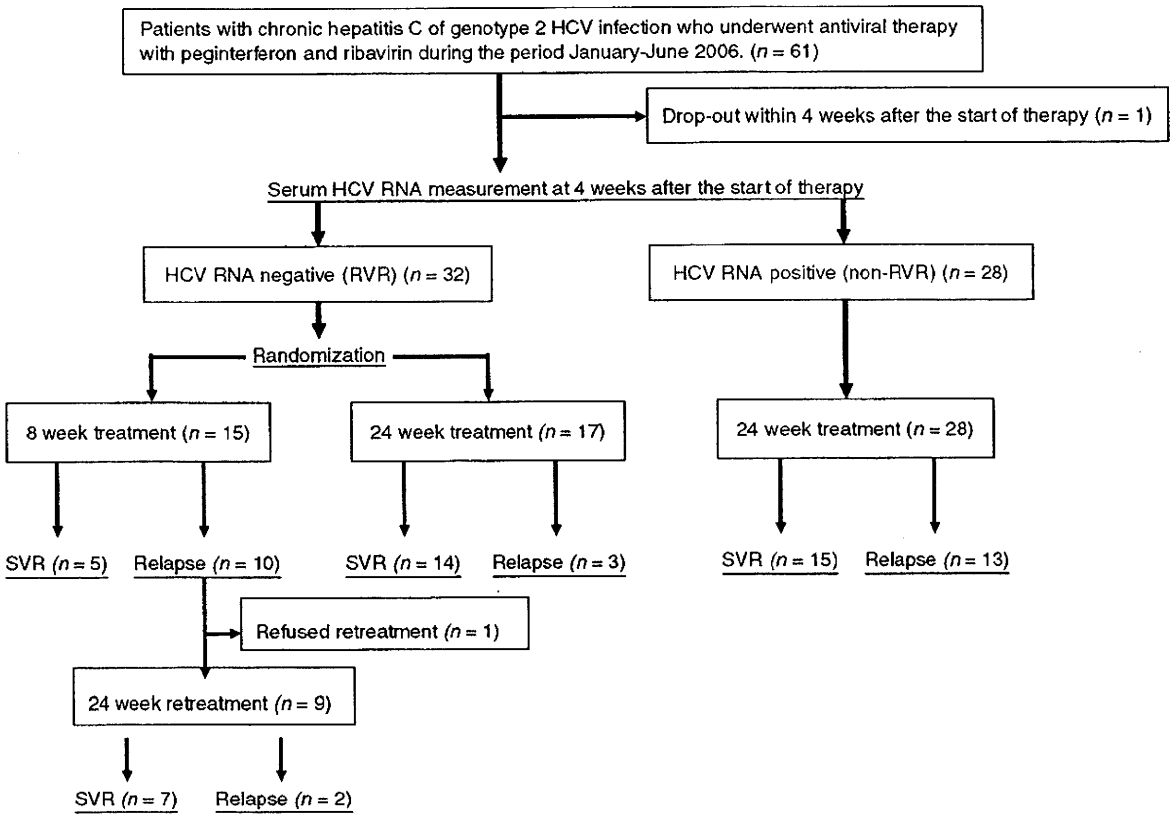


**Fig. 1.** Schematic of the trial profile. Serum hepatitis C virus (HCV) RNA was negative in 32 patients (rapid virological responders), and they were assigned to the 8-week regimen (15 patients) or the standard 24-week regimen (17 patients). Nine of 10 relapsers in the 8-week group underwent retreatment with the standard 24-week regimen. SVR, sustained virological response; RVR, rapid virological response.

age, gender, liver histology, HCV genotype, pretreatment HCV RNA concentration or detection of serum HCV RNA at 2 weeks after the start of therapy between patients with an SVR and those who relapsed. Serum HCV RNA was detected at 4 weeks after the end of therapy in all patients with relapse, with the exception of one patient with serum HCV RNA detected at 8 weeks after the end of therapy. Nine of 10 relapsers underwent retreatment within 1–6 months after the end of the initial treatment. The HCV RNA concentration just before retreatment was higher than that before the initial treatment in four patients, and was lower than that before the initial treatment in five patients. Two patients relapsed after retreatment, and the other seven patients achieved an SVR. One patient required reduction of the peginterferon dose, and two patients required reduction of the ribavirin dose during retreatment. No patient required discontinuation of peginterferon or ribavirin during either the initial treatment or the retreatment period.

## Discussion

Shortening the period of antiviral therapy with peginterferon and ribavirin is important in terms of adverse effects and medical costs. A shortened regimen may be less expensive and better tolerated by the majority of patients. Studies on shorten treatment performed so far are different in terms of the design, the characteristics of the population and the virological methods used; these differences may account for the different relapse rates reported so far after an abbreviated course (7–9). Moreover, the possibility to further reduce to an 8-week treatment duration had not been investigated so far.

In the present study, we evaluated an 8-week regimen for patients with HCV genotype 2 and RVR. It has been reported recently that antiviral therapy is more beneficial for patients with HCV genotype 2 than for those with HCV genotype 3 (4, 6). In addition, achievement of an RVR is a reported indicator of the highest likelihood of SVR in response to short-duration therapy. Patients who were assigned to the 8-week regimen were, therefore, the population with the highest likelihood of achieving an SVR.

The results of the present study clearly showed that reduction of the treatment duration to 8 weeks is not sufficient as an antiviral therapy for patients with chronic infection of HCV genotype 2 (2a or 2b), at least in patients with pretreatment HCV RNA concentrations $> 100 \times 10^3$ IU/ml, even though an RVR is achieved. A marked increase in the rate of relapse after the completion of treatment was observed in patients in the


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**Table 2.** Characteristics of rapid virological responders and non-rapid virological responders*

| | Rapid virological responders ($n=32$) | Non-rapid virological responders ($n=28$) | *P* value |
|---|---|---|---|
| Gender (years) | 55.6 ± 12.7 | 57.8 ± 9.6 | 0.7553 |
| Sex (female/male) | 20 (62.5)/12 (37.5) | 16 (57.1)/12 (42.9) | 0.8741 |
| History of interferon therapy (naïve/retreatment) | 19 (59.4)/13 (40.6) | 21 (75.0)/7 (25.0) | 0.3142 |
| History of transfusion (no/yes) | 2 (6.3)/30 (93.7) | 16 (57.1)/12 (42.9) | 0.0004 |
| Alanine aminotransferase (IU/L) | 49.3 ± 57.7 | 54.4 ± 56.8 | 0.9409 |
| Aspartate aminotransferase (IU/L) | 46.3 ± 54.8 | 45.6 ± 43.9 | 0.2354 |
| γ-glutamyl transpeptidase (IU) | 64.3 ± 149.4 | 53.7 ± 90.7 | 0.6037 |
| Alkaline phosphatase (IU/L) | 244.7 ± 99.6 | 302.0 ± 187.8 | 0.1848 |
| Albumin (g/dl) | 4.35 ± 0.26 | 4.25 ± 0.41 | 0.2368 |
| Total bilirubin (mg/dl) | 0.67 ± 0.25 | 0.63 ± 0.23 | 0.7411 |
| White blood cell count (/μl) | 4961 ± 1269 | 5052 ± 1963 | 0.9882 |
| Haemoglobin (g/dl) | 13.9 ± 1.3 | 14.1 ± 1.7 | 0.6944 |
| Platelet count ( × $10^3$/μl) | 19.0 ± 7.1 | 20.0 ± 6.0 | 0.3660 |
| Body weight (kg) | 56.6 ± 9.2 | 62.2 ± 12.1 | 0.1044 |
| Liver histology – activity (A0/A1/A2/A3)† | 1 (3.3)/22 (73.4)/6 (20.0)/1 (3.3) | 0/17 (65.4)/6 (23.1)/3 (11.5) | 0.4997 |
| Liver histology – fibrosis (F0/F1/F2/F3)† | 2 (6.6)/22 (73.3)/5 (16.7)/1 (3.3) | 1 (3.9)/16 (61.5)/6 (23.1)/3 (11.5) | 0.5526 |
| HCV genotype (2a/2b) | 27 (84.4)/5 (15.6) | 18 (64.3)/10 (35.7) | 0.1352 |
| HCV RNA concentration (× $10^3$ IU/ml) | 1197 ± 979 | 2713 ± 1848 | 0.0002 |
| Treatment duration (8 weeks/24 weeks) | 15/17 | 0/28 | |

Percentages are shown in parentheses.

*Rapid virological responders, serum HCV RNA was negative at 4 weeks after the start of therapy; non-rapid virological responders, serum HCV RNA remained positive at 4 weeks after the start of therapy.

†Liver biopsy was not performed in four patients.

HCV, hepatitis C virus.

8-week group compared with reported rates with treatment periods of 12–16 weeks (5, 6, 8); the relapse rate in RVR patients was 0% in those on a 16-week regimen (8), 9.5% in those on a 14-week regimen (5), 9.2% in those on a 12-week regimen (6) and 66.7% in those on the 8-week regimen in the present study. (The study of the 14-week regimen involved patients with undetectable HCV RNA at 8 weeks after the start of therapy in addition to RVR patients.) This marked increase in the relapse rate in the 8-week group strongly indicates the limitations of shortening the treatment period in patients with HCV genotype 2, even when an RVR is achieved. Although the appropriate duration of combination therapy for patients with HCV genotype 2 remains controversial (5–9), shortening the treatment period to 8 weeks is definitely insufficient as an antiviral therapy.

The very low SVR rate in the 8-week group could be accounted for, in part, by the higher pretreatment HCV RNA concentration. Our study did not include patients with a pretreatment HCV RNA concentration $\leq 100 \times 10^3$ IU/ml because only peginterferon monotherapy is allowed by Japanese National Medical Insurance for this patient population. SVR rates reportedly decrease in inverse proportion to pretreatment HCV RNA concentrations (2, 3, 12), and patients with a low pretreatment HCV RNA concentration, as well as those with RVR, reportedly have the highest likelihood of an SVR in response to short-duration therapy (9). Evaluation of the 8-week regimen in patients with HCV genotype 2 and with a low pretreatment HCV RNA concentration ($\leq 100 \times 10^3$ IU/ml) is necessary. Another reason for the very low SVR rate in the 8-week group could be the slightly lower dose of ribavirin used, in comparison with that in previous reports. The dosage of ribavirin was decided according to the manufacturer's recommendations for Japanese patients, which was slightly lower than that for patients in Western countries. The increase in the ribavirin dose, therefore, might have increased the SVR rate in this patient population.

In summary, an 8-week regimen of antiviral combination therapy with peginterferon and ribavirin yielded a very high relapse rate, indicating the limitation of shortened treatment in patients with HCV genotype 2 and who achieved an RVR. However, as well as the report by Mangia *et al.* (6), retreatment of relapsers with the standard 24-week regimen yielded a high SVR rate comparable to that in patients treated initially with the standard 24-week regimen. Reduced durations of therapy may, therefore, be reasonable in patients who experience adverse events and are unlikely to tolerate 24 weeks of therapy as initial treatment. Currently, in patients with HCV genotype 2 and 3 infection and with RVR, a 12-week treatment regimen remains the shortest duration proven to maintain an acceptable rate of relapse as a short course of antiviral combination therapy with peginterferon and ribavirin (6). Further studies will be necessary to investigate the appropriate treatment duration with a sufficient SVR rate and with less adverse effect and less medical expense.

**Table 3.** Characteristics and outcomes of patients receiving the 8-week regimen

| Patient | Age (years) | Gender | Liver histology | HCV genotype | HCV RNA* concentration | HCV RNA at 2 weeks | Result | Time to relapse after the end of treatment (weeks) | Retreatment | Time to retreatment after the end of treatment (months) | HCV RNA† concentration | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 65 | M | A1/F1 | 2a | 4400 | Positive | Relapse | 4 | Yes | 2 | 1900 | SVR |
| 2 | 64 | F | Not done | 2b | 2500 | Positive | Relapse | 4 | Yes | 1 | 2100 | Replace |
| 3 | 29 | F | A1/F1 | 2b | 660 | Negative | SVR | | | | | |
| 4 | 65 | M | A2/F1 | 2b | 120 | Positive | SVR | | | | | |
| 5 | 28 | M | A1/F1 | 2a | 1800 | Positive | SVR | | | | | |
| 6 | 63 | F | A2/F2 | 2a | 1300 | Positive | Relapse | 4 | Yes | 2 | 590 | SVR |
| 7 | 59 | F | A2/F2 | 2a | 160 | Positive | Relapse | 4 | No (refused) | | | |
| 8 | 53 | M | A2/F2 | 2a | 1100 | Positive | SVR | | | | | |
| 9 | 46 | F | A1/F1 | 2a | 1000 | Positive | Relapse | 4 | Yes | 2 | 1300 | SVR |
| 10 | 58 | F | A1/F1 | 2a | 2100 | Positive | Relapse | 4 | Yes | 1.5 | 1400 | SVR |
| 11 | 66 | F | A1/F1 | 2a | 110 | Negative | SVR | | | | | |
| 12 | 68 | F | A1/F1 | 2a | 1400 | Positive | Relapse | 4 | Yes | 1.5 | 1700 | Replace |
| 13 | 21 | F | A1/F1 | 2a | 110 | Negative | Relapse | 8 | Yes | 6 | 70 | SVR |
| 14 | 68 | M | A1/F1 | 2a | 1000 | Positive | Relapse | 4 | Yes | 1 | 1200 | SVR |
| 15 | 51 | F | A1/F1 | 2b | 550 | Negative | Relapse | 4 | Yes | 3 | 2200 | SVR |

*Before initial treatment ($\times 10^3$ IU/ml).

†Before retreatment ($\times 10^3$ IU/ml).

No patient required reduction of peginterferon or ribavirin during the initial treatment.

Patient 6 required reduction of the peginterferon dose, and patients 4 and 15 required reduction of the ribavirin during retreatment.

No patient required discontinuation of peginterferon or ribavirin during initial treatment or retreatment.

HCV, hepatitis C virus; SVR, sustained virological response.

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# Long-Term Follow-Up of Patients With Hepatitis C With a Normal Alanine Aminotransferase

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An attempt was made to identify factors influencing the cumulative probability of an increased alanine aminotransferase (ALT) level and hepatocarcinogenesis in hepatitis C patients with a normal ALT level initially. A total of 398 consecutive patients with a normal ALT level initially for 6 months or more and follow-up period longer than 3 years during the period January 1995 to December 2004 were included. Patients were classified by ALT level into three groups: Group A (3–20 IU/L), Group B (21–30 IU/L), and Group C (31–35 IU/L). Factors associated with the cumulative probability of increased ALT level and hepotocarcinogenesis were evaluated. Women in groups B and C and men in Group C showed high cumulative probabilities of increased ALT levels. Factors associated with increased ALT were a high ALT level (Group B, relative risk; 1.758 [95% confidence interval: 1.290–2.392], $P < 0.001$, Group C, 3.328 [2.256–4.909], $P < 0.001$), high lactate dehydrogenase level (2.352 [1.445–3.829], $P = 0.001$), or low total cholesterol level (1.957 [1.330–2.882], $P = 0.001$). Factors associated with incidence of hepatocellular carcinoma were increased age (3.088 [1.025–9.308], $P = 0.045$), high ALT level (Group C, 5.803 [1.530–22.066], $P = 0.010$), and high total bilirubin level (8.309 [2.235–30.888], $P = 0.002$). In patients with hepatitis C with a normal ALT level initially, an ALT level of 21–35 IU/L is a risk factor for an increased ALT level and hepatocarcinogenesis. ***J. Med. Virol. 81:446–451, 2009.*** © 2009 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis C; hepatitis C virus; normal alanine aminotransferase; hepatocarcinogenesis; long-term follow-up

## INTRODUCTION

Hepatitis C virus (HCV) results in numerous complications including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Disease progression and clinical manifestations are heterogeneous, and the underlying mechanisms are not understood fully [Alberti et al., 1999; Marcellin, 1999; Alberti, 2005]. Approximately 30% of patients with chronic HCV infection show persistently normal alanine aminotransferase (ALT) levels, but the majority of these patients have some degree of histologic liver damage, which is usually mild [Marcellin et al., 1997; Tassopoulos, 1999; Bacon, 2002].

ALT activity is the laboratory marker that is used most extensively for the evaluation of liver disease [Craxi and Almasio, 1996; Pratt and Kaplan, 2000]. However, particularly in the case of chronic hepatitis C, ALT measurement often fails to identify patients with minimal to mild necroinflammatory activity [Zanella et al., 1995; Prati et al., 1996; Puoti et al., 1997]. Thus, determination of the true normal range of ALT activity is important for screening of large populations and for the recognition of occult liver abnormalities.

Recently, combination therapy with pegylated interferon and ribavirin has produced sustained virologic response rates of 53–63% in patients with chronic hepatitis C infection and an increased ALT level [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004]. Zeuzem et al. [2004] reported that pegylated interferon plus ribavirin combination therapy was safe and effective in patients with persistently normal ALT levels.

Many studies have provided evidence that, although the majority of HCV carriers with a normal ALT level have minimal hepatic changes, a subgroup may have active and progressive liver disease that is difficult to predict based on clinical or biochemical parameters [Puoti, 2003; Alberti et al., 2004]. The association

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between ALT level and prognosis is not established clearly in HCV carriers with normal ALT levels. The aim of this retrospective study was to identify factors influencing the cumulative probability of an increased ALT level and hepatocarcinogenesis in patients with a normal ALT level initially.

## MATERIALS AND METHODS

### Patients

Five thousand three hundred consecutive patients positive for anti-HCV antibody visited the Department of Gastroenterology of Ogaki Municipal Hospital during the period from January 1995 to December 2004. The long-term prognosis of patients with a normal ALT level initially was evaluated in these cases. Normal ALT was defined at our institution as 3–35 IU/L. All patients included in this study fulfilled the following criteria: (1) positive for anti-HCV by a second- or third- generation enzyme-linked immunosorbent assay, (2) no evidence of hepatitis B virus infection, (3) exclusion of other causes of chronic liver disease (alcohol, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, or Wilson's disease), (4) detectable HCV-RNA and normal ALT level for longer than 6 months, (5) follow-up period longer than 3 years, (6) no evidence of hepatocellular carcinoma for at least 3 years from the start of the follow-up period, (7) no interferon treatment, and (8) measurement of ALT more than twice in 1 year. A total of 398 consecutive patients fulfilled these criteria.

All patients were followed-up at least every 6 months. During each follow-up examination, platelets, ALT, aspartate aminotransferase (AST), gamma glutamyl transpeptidase (gamma-GTP), prothrombin time (PT), total bilirubin, cholinesterase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, albumin, and total cholesterol were measured. The patients were classified into the following three groups according to initial ALT values: Group A (3–20 IU/L), Group B (21–30 IU/L), and Group C (31–35 IU/L). In some cases, the HCV genotype was determined and quantitation of HCV-RNA (Amplicor 2, Roche Diagnostics K.K., Tokyo, Japan) was undertaken. To detect early-stage hepatocellular carcinoma, ultrasonography (US), computed tomography (CT), and measurement of tumor markers (alpha-fetoprotein [AFP], *Lens culinaris* agglutinin-reactive AFP, des-γ-carboxyprothrombin) were carried out in all patients at least every 6 months. The median follow-up period was 8.8 years (range: 3.0–14.7 years). The total number of blood examinations was 21,259, and the median number was 23 (range: 6–241).

### Statistical Analysis

Statistical analysis was performed with the Statistical Program for Social Science (SPSS 11.5 for Windows, SPSS Japan, Inc., Tokyo, Japan). Continuous variables are expressed as median (range). The Kruskal–Wallis test was used to assess continuous variables with a skewed distribution, and the chi-square test was used to assess categorical variables. Actuarial analyses of cumulative increased ALT levels and hepatocarcinogenesis were performed by the Kaplan–Meier method, and differences were tested with the log-rank test. Bonferroni correction was performed for multiple comparisons. The Cox proportional hazard model and forward selection method were used for univariate and multivariate analyses. Statistical significance was defined as $P < 0.05$.

## RESULTS

Baseline patient characteristics are listed in Table I for the three groups. Viral concentration, AST, total

TABLE I. Patient Characteristics[a]

| | Group A (n=180) | Group B (n=165) | Group C (n=53) | p |
|---|---|---|---|---|
| Age (years) | 61 (15–89) | 60 (11–93) | 58 (23–79) | NS |
| Sex (women/men) | 109/71 | 93/72 | 21/32 | 0.0265 |
| Genotype (type 1/type 2) | 30/28 | 57/32 | 19/12 | NS |
| Viral concentration (KIU/ml) | 440 (0.5–3,500) | 570 (0.5–7,700) | 960 (9.1–4,800) | 0.0082 |
| Measurement frequency of ALT | 17 (6–118) | 25 (6–241) | 27 (6–101) | <0.0001 |
| AST (IU/L) | 20 (10–60) | 26 (15–58) | 32 (15–121) | <0.0001 |
| Platelets ($10^4$/mm$^3$) | 19.9 (5.8–58.8) | 18.5 (4.5–31.8) | 16.9 (4.9–32.5) | 0.0018 |
| PT (%) | 100 (26–140) | 99 (22–145) | 102 (58–125) | NS |
| Gamma-GTP (IU/L) | 18 (6–175) | 22 (6–174) | 29 (12–475) | NS |
| Total bilirubin (mg/dl) | 0.4 (0.2–2.1) | 0.5 (0.2–2.7) | 0.5 (0.3–2.0) | 0.0027 |
| Cholinesterase (IU/L) | 276 (34–622) | 280 (65–710) | 279 (34–710) | NS |
| ALP (IU/L) | 230 (84–3458) | 246 (110–768) | 220 (84–659) | 0.0091 |
| LDH (IU/L) | 174 (92–567) | 182 (106–763) | 183 (107–360) | 0.0300 |
| Total protein (g/dl) | 7.1 (4.5–8.0) | 7.2 (5.6–9.0) | 7.4 (5.3–8.4) | 0.0037 |
| Albumin (g/dl) | 4.1 (2.0–4.8) | 4.2 (2.6–4.9) | 4.2 (2.8–4.7) | NS |
| Total cholesterol (mg/dl) | 177 (66–289) | 174 (72–301) | 164 (66–301) | NS |

ALT, alanine aminotransferase, Group A (ALT, 3–20 IU/L), Group B (ALT, 21–30 IU/L), Group C (ALT, 31–35 IU/L); AST, aspartate aminotransferase; PT, prothrombin time; Gamma-GTP, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.
[a]Continuous variables are quoted as median (range).

bilirubin, LDH, and total protein were significantly higher in Groups B and C compared to Group A. Platelets were significantly lower in Groups B and C compared to Group A. ALP was significantly higher in Group B compared to Groups A and C.

### Incidence of Increased ALT Level

Factors associated with an increased ALT level are listed in Table II. Male sex (risk ratio: 1.389 [95% confidence interval: 1.062–1.817], $P = 0.016$), high ALT level (Group B, 1.732 [1.297–2.346], $P < 0.001$; Group C, 3.400 [2.318–4.896], $P < 0.001$), high AST level (1.675 [1.020–2.750], $P = 0.042$), high total bilirubin level (2.257 [1.287–3.961], $P = 0.005$), high LDH level (2.151 [1.340–3.452], $P = 0.002$), low albumin level (1.712 [1.621–2.717], $P = 0.022$), and low total cholesterol level (2.217 [1.513–3.247], $P < 0.001$) were significantly associated with increased ALT by univariate analysis. The 3-, 5-, and 10-year cumulative incidences of increased ALT were 22.9%, 33.2%, and 48.1%, respectively, in Group A, 37.6%, 50.4%, and 70.3% in Group B, and 69.4%, 73.6%, and 82.9% in Group C. Increased ALT occurred at higher rates in Groups B and C than in Group A and occurred at a higher rate in Group C than in Group B ($P < 0.001$, Fig. 1). Among women, there was a significant difference between Group A and Groups B and C in the cumulative incidence of increased ALT ($P = 0.021$, $P = 0.036$, respectively, Fig. 2), but there was no significant difference between Group B and Group C. In contrast, among men, there were significant differences in the cumulative incidence of increased ALT between Groups A and B and Group C ($P < 0.001$, Fig. 3), but there was no significant difference between Group A and Group B.

TABLE II. Factors Associated With an Increased ALT Level (Univariate Analyses)

| | Relative risk (95% CI) | P |
|---|---|---|
| Age | | |
| ≤60 years | 1 | 0.879 |
| >60 years | 0.979 (0.748–1.282) | |
| Sex | | |
| Women | 1 | 0.016 |
| Men | 1.389 (1.062–1.817) | |
| Genotype | | |
| Type 1 | 1 | 0.783 |
| Type 2 | 1.054 (0.726–1.529) | |
| Viral concentration | | |
| ≤100 KIU/ml | 1 | 0.783 |
| >100 KIU/ml | 1.030 (0.678–1.523) | |
| ALT | | |
| <20 IU/L | 1 | <0.001 |
| 21–30 IU/L | 1.732 (1.297–2.346) | <0.001 |
| 31–35 IU/L | 3.400 (2.318–4.986) | <0.001 |
| AST | | |
| ≤40 IU/L | 1 | 0.042 |
| >40 IU/L | 1.675 (1.020–2.750) | |
| Platelets | | |
| $\geq 12.0\times10^4/mm^3$ | 1 | 0.113 |
| $<12.0\times10^4/mm^3$ | 1.030 (0.678–1.523) | |
| PT | | |
| <70% | 1 | 0.958 |
| ≥70% | 0.984 (0.546–1.774) | |
| Gamma-GTP | | |
| ≤56 IU/L | 1 | 0.667 |
| >56 IU/L | 0.894 (0.537–1.489) | |
| Total bilirubin | | |
| ≤1.2 mg/dl | 1 | 0.005 |
| >1.2 mg/dl | 2.257 (1.287–3.961) | |
| Cholinesterase | | |
| ≤431 IU/L | 1 | 0.099 |
| >431 IU/L | 0.723 (0.491–1.063) | |
| LDH | | |
| ≤250 IU/L | 1 | 0.002 |
| >250 IU/L | 2.151 (1.340–3.452) | |
| Total protein | | |
| ≥6.5 g/dl | 1 | 0.644 |
| <6.5 g/dl | 1.136 (0.661–1.955) | |
| Albumin | | |
| ≥3.5 g/dl | 1 | 0.022 |
| <3.5 g/dl | 1.712 (1.621–2.717) | |
| Total cholesterol | | |
| ≥130 mg/dl | 1 | |
| <130 mg/dl | 2.217 (1.513–3.247) | <0.001 |

CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PT, prothrombin time; Gamma-GTP, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

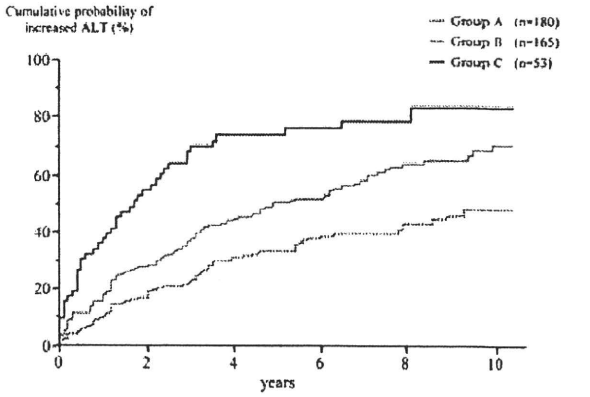


Fig. 1. Incidence of increased ALT for different initial ALT levels. Patients were classified into three groups according to ALT level: Group A (3–20 IU/L), Group B (21–30 IU/L), and Group C (31–35 IU/L). ALT increases at greater rates in Groups B and C compared to the increase in Group A and occurred at a greater rate in Group C compared to that in Group B ($P < 0.001$).

Factors associated with increased ALT analyzed by the Cox proportional hazard model and the forward selection method are listed in Table III. High ALT level (Group B, 1.758 [1.290–2.392], $P < 0.001$; Group C, 3.328 [2.256–4.909], $P < 0.001$), high LDH level (2.352 [1.445–3.829], $P = 0.001$), and low total cholesterol level (1.957 [1.330–2.882], $P = 0.001$) were factors associated significantly with an increased ALT level.

### Incidence of Hepatocellular Carcinoma

Hepatocellular carcinoma occurred in 16 of 398 patients (4%) in this follow-up study. The 3-, 5-, and 10-year cumulative incidences of hepatocellular carcinoma were none, none, and 4%, respectively, in Group A, none, 1.3%, and 4.3% in Group B, and none, 7.8%, and

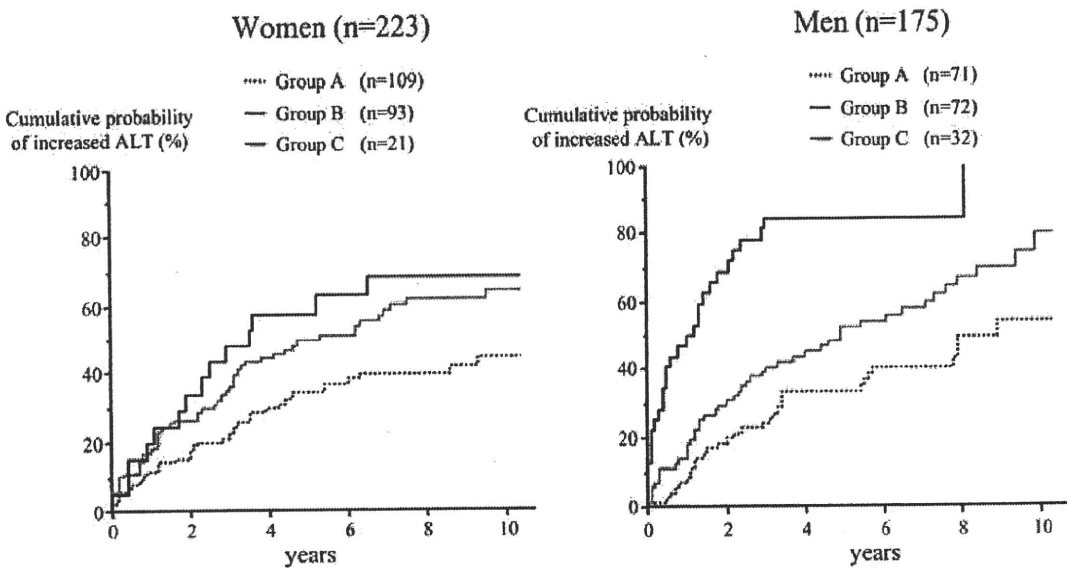


Fig. 2. Relation between sex and incidence of increased ALT. In women (left), the cumulative incidence of increased ALT was significantly different between Group A and Groups B and C ($P = 0.021$, $P = 0.036$) but not between Group B and Group C. In men (right), the cumulative incidence of increased ALT differed significantly between Groups A and B and Group C ($P < 0.001$) but not between Group A and Group B.

14.4% in Group C. The cumulative incidence of hepatocellular carcinoma differed significantly between Group A and Group C ($P = 0.017$, Fig. 3) but not between Group A and Group B, or between Group B and Group C. Factors associated with the incidence of hepatocellular carcinoma analyzed by Cox proportional hazards modeling and the forward selection method are listed Table IV. Increased age (3.088 [1.025–9.308], $P = 0.045$), a high ALT level (Group C, 5.803 [1.530–22.066], $P = 0.010$), and high total bilirubin level (8.309 [2.235–30.888], $P = 0.002$) were factors associated significantly with the incidence of hepatocellular carcinoma.

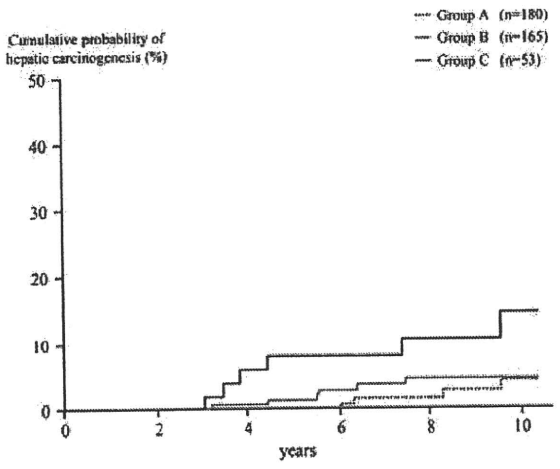


Fig. 3. Incidence of hepatic carcinogenesis in HCV carriers with a normal ALT level initially. The cumulative incidence of hepatic carcinogenesis differed significantly between Group A and Group C ($P = 0.017$) but not between Group A and Group B or between Group B and Group C.

## DISCUSSION

Criteria for the subgroup of HCV patients with a persistently normal ALT level differ with respect to the most appropriate cut-off for defining ALT normality in patients infected chronically with HCV and the most appropriate time frame and algorithm for defining a persistently normal ALT level. The current upper limit for normal ALT in patients with HCV is approximately 40 IU/L (range: 30–50 IU/L) [Alberti et al., 1992; Zanella et al., 1995; Prati et al., 1996; Pratt and Kaplan, 2000]. Studies have suggested that the normal values currently used clinically may be low [Piton et al., 1998; Hayashi et al., 2000; Prati et al., 2002]. Prati et al. [2002] reported that the upper limit for normal ALT was 19 IU/L in women and 30 IU/L in men after excluding patients with undiagnosed HCV infection and those with behavioral risk for blood-borne disease. In this study,

TABLE III. Factors Associated With an Increased ALT Level (Multivariate Analysis)

| | Relative risk (95% CI) | *P* |
|---|---|---|
| ALT | | |
| <20 IU/L | 1 | <0.001 |
| 21–30 IU/L | 1.758 (1.292–2.392) | <0.001 |
| 31–35 IU/L | 3.328 (2.256–4.909) | <0.001 |
| LDH | | |
| ≤250 IU/L | 1 | 0.001 |
| ≥250IU/L | 2.352 (1.445–3.829) | |
| Total cholesterol | | |
| ≥130 mg/dl | 1 | 0.001 |
| <130 mg/dl | 1.957 (1.330–2.882) | |

CI, confidence interval; ALT, alanine aminotransferase; LDH, lactate dehydrogenase.

TABLE IV. Factors Associated With Hepatic Carcinogenesis (Multivariate Analysis)

| | Relative risk (95% CI) | P |
|---|---|---|
| Age (years) | | |
| ≤60 | 1 | 0.045 |
| >60 | 3.088 (1.025–9.308) | |
| ALT | | |
| <20 IU/L | 1 | 0.017 |
| 21–30 IU/L | 1.515 (0.415–5.530) | 0.529 |
| 31–35 IU/L | 5.803 (1.530–22.066) | 0.010 |
| Total bilirubin | | |
| ≤1.2 mg/dl | 1 | 0.002 |
| >1.2 mg/dl | 8.309 (2.235–30.888) | |

CI, confidence interval; ALT, alanine aminotransferase.

there was a significant difference in the cumulative incidence of increased ALT between Group A (ALT 3–20 IU/L) and Groups B (21–30 IU/L) and C (31–35 IU/L) among women. In contrast, among men, there were significant differences between Groups A and B and Group C. This is an interesting and important finding, suggesting that the upper limit for a normal ALT level should be revised to 30 IU/L for male patients and 20 IU/L for female patients with HCV infection. These data are consistent with the healthy limits in women and men proposed by Prati et al. [2002].

A persistently normal ALT level in patients with chronic hepatitis C has been defined generally as consecutive measurements within the normal range during a 6-month period [Marcellin et al., 1997; EASL International Consensus Conference on Hepatitis C, 1999]. In this study, patients with a normal ALT level for at least 6 months were selected according to this definition.

The natural course of HCV infection in patients with a normal ALT level is not understood fully. HCV patients with an initially and persistently normal ALT level may show disease progression on long-term follow-up as a consequence of transient ALT flares or of persistent biochemical reactivation of their liver disease. The incidence of ALT flares and/or of durable reactivation reported in HCV patients with a normal ALT level initially is variable and ranges from 15% to 27.5%, mainly as a consequence of different inclusion criteria and follow-up periods [Ohmiya et al., 2000; Tsuji et al., 2001; Puoti et al., 2002; Okanoue et al., 2005]. In the present study, the 3-, 5-, and 10-year cumulative incidences of increased ALT were 35.3%, 45.8%, and 62.5%, respectively. Thus, the longer the follow-up period, the more the incidence increased. Factors associated with increased incidence of increased ALT were gender, ALT, AST, total bilirubin, LDH, albumin, and total cholesterol level. Among these, the ALT level was associated most strongly with increased ALT. However, Puoti et al. [2002] reported that baseline ALT levels (<20 IU/L vs. >21 IU/L) did not correlate with the incidence of ALT flares. Further research is necessary to reconcile these data.

Hepatocellular carcinoma occurred in 16 of 398 patients (4%) in the present study. Factors associated with hepatic carcinogenesis were increased age, gender, platelets, ALT, ALP, total cholesterol, and albumin in patients with abnormal ALT level [Kumada et al., 2007]. Thus, it is important to maintain a normal or low ALT level to prevent hepatic carcinogenesis.

Recently, the results of the first large multinational trial of a pegylated interferon plus ribavirin combination regimen in patients with a persistently normal ALT level became available [Zeuzem et al., 2004]. A total of 491 patients were assigned at random to three groups: either 24 or 48 weeks of treatment with pegylated interferon alfa-2a plus 800 mg/day ribavirin, or no treatment for 72 weeks. The overall sustained response rates were 30% and 52% in patients treated for 24 and 48 weeks, respectively, and the treatment outcome was equal in patients with an increased ALT level initially [Manns et al., 2001; Fried et al., 2002]. Median ALT levels were decreased consistently from the baseline level in treatment responders, confirming that, in many HCV patients with a normal ALT level according to current cut-off limits, ALT levels were in fact abnormal, reflecting possible underlying liver disease, and were normalized following successful antiviral therapy to what might be considered a true healthy level.

In conclusion, Groups B and C of women with ALT levels of 21–35 IU/L (corresponding to Groups B and C), Group C of men with ALT levels of 31–35 IU/L (corresponding to Group C) should be treated, taking into consideration patient age, motivation, and the possibility of complications. Maintenance of a low ALT level may prevent hepatic carcinogenesis, even in patients with ALT levels in the normal range.

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