Age-Related Response to Interferon Alfa Treatment in Women vs Men With Chronic Hepatitis C Virus Infection

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Background: Interferon alfa is used widely for patients with chronic hepatitis C virus (HCV) infection. Little is known, however, of the relationship between patients’ sex and the effectiveness of interferon alfa treatment in these patients.

Methods: We treated 311 patients (199 men and 112 women) with human lymphoblastoid interferon (6 million units subcutaneously every day for 2 weeks and 3 times a week for 22 weeks) and observed them for an additional 6 months. Serum HCV RNA levels and genotype were tested by polymerase chain reaction before treatment. A liver biopsy was also done. For the purposes of this study, a complete response was defined as the elimination of HCV RNA for at least 6 months after the termination of treatment.

Results: The rate of complete response was 27.1% for men and 24.1% for women. With multiple logistic regression analysis, the HCV RNA level (P < .001), genotype (P < .001), patients’ sex (P < .05), and the interaction between sex and age were associated with a complete response to interferon alfa. The rate of complete response was 33.3% in men aged 39 years and younger, 25.0% in men aged 40 years and older, 75.0% in women aged 39 years and younger, and 15.6% in women aged 40 years and older. The odds ratio by group was 1.00, 0.72, 4.38, and 0.21, respectively.

Conclusions: Our finding that women aged 39 years and younger are responsive to interferon alfa treatment suggests that hormonal activity, in particular the level of estrogen, may be associated with the sustained elimination of HCV.

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PATIENT AND METHODS

PATIENTS

We studied 311 Japanese patients (199 men and 112 women) with chronic HCV infection who were treated with IFN-α at Kyushu University Hospital, Fukuoka, Japan, from April 1, 1992, to March 31, 1995. Of these 311, 102 (32.8%) had a history of blood transfusion, but none had a history of alcohol or drug abuse or homosexuality. None of the women had estrogen replacement treatment. All patients had the antibody to HCV by the second-generation assay and did not have the hepatitis B surface antigen or the antibody to the human immunodeficiency virus. Before treatment, a liver biopsy was done for all patients. In all patients during the 6-month period, HCV RNA was detected before treatment. The HCV RNA of genotype 1b was found in 224 (72.0%), genotype 2a in 58 (18.6%), and genotype 2b in 29 (9.3%). The HCV RNA levels of serum RNA (logarithmic transformed copy numbers per 50 µL of serum) ranged from 2 to 7 copies per 50 µL.

METHODS

Paired blood specimens were obtained every 2 weeks during the treatment, every 4 weeks for at least 3 months before entry, and 6 months after the cessation of treatment. All serum specimens were separated and stored at −20°C until tested for antibody to HCV, HCV RNA, HCV RNA genotype, and HCV RNA levels. Antibodies to HCV (HCV EIA II, Abbott Laboratories, North Chicago, Ill)13 were examined using enzyme-linked immunosorbent assay. Assays for hepatitis B surface antigen and human immunodeficiency virus antibody were done using commercial serological tests.

TREATMENT REGIMEN

The patients were given subcutaneous injections of natural IFN-α (human lymphoblastoid interferon, Sumyferon, Sumitomo Co, Tokyo, Japan). A dose of 6 million units was given daily for the first 2 weeks, then 3 times a week for the next 22 weeks. The total dose given was 480 million units during a 24-week period. This schedule of IFN-α treatment is currently in wide use in Japan. None of the patients had the hepatitis B surface antigen or the antibody to the human immunodeficiency virus. Before treatment, a liver biopsy was done for all patients. In all patients during the 6-month period, HCV RNA was detected before treatment. The HCV RNA of genotype 1b was found in 224 (72.0%), genotype 2a in 58 (18.6%), and genotype 2b in 29 (9.3%). The HCV RNA levels of serum RNA (logarithmic transformed copy numbers per 50 µL of serum) ranged from 2 to 7 copies per 50 µL.

DEFINITION OF RESPONSE TO IFN-α

In this study, a complete response was defined as negative results on HCV RNA tests by the end of the scheduled treatment and sustained negativity beyond 6 months; partial response as negative results on HCV RNA tests by the end of the scheduled treatment, but the virus reappeared during the 6-month follow-up; and no response when HCV RNA was not eliminated at any time during the observation period.

HCV RNA BY POLYMERASE CHAIN REACTION

Ribonucleic acid was extracted from 50 µL of serum by Sepa Gene RV (Sanko Junyaku, Tokyo, Japan), and complementary DNA was synthesized using random primers and reverse transcriptase (Super Script II; GIBCO BRL, Gaithersburg, Md). The HCV RNA was detected by 2-stage polymerase chain reaction (PCR) using primers from the 5’-noncoding region of the HCV genome, as previously described.14

GENOTYPE OF HCV RNA

The HCV RNA genotype was determined by 2-stage PCR using universal and type-specific primers from the putative C gene of the HCV genome with a modification of the method of Okamoto et al15 and Hayashi et al.16 The genotype nomenclature was based on the system proposed by Simmonds et al.17

HCV RNA LEVEL BY COMPETITIVE PCR

The level of HCV RNA was determined by means of competitive PCR using a modification of the methods of Hayashi et al16 and Kato et al.18 By recombinant PCR, we obtained mutant HCV RNA with the EcoRI site in the 5’-noncoding region. This mutant HCV complementary DNA fragment was cloned into the pGEM-4z vector, which is a cloning and transcription vector (Promega Corp, Madison, Wis). In vitro RNA transcription from PvuI-digested pGEM-4z was done using T7 RNA polymerase (Riboprobe Gemini System II; Promega Corp) according to the manufacturer’s instructions. Primers and a probe were constructed for the 5’-noncoding region. The amplified products were analyzed by electrophoresis after the digestion of mutant HCV RNA by EcoRI, mutant HCV RNA being demonstrated at 106 and 112 base pairs and HCV RNA from patients at 218 base pairs. The size of the PCR product for each patient was compared with that of the diluted mutant HCV RNA.

STATISTICAL ANALYSIS

Statistical analysis was performed using a commercially available software package (BMDP Statistical Software Inc, Los Angeles, Calif) for the IBM 3090 system computer.19 The BMDP 4F program (ie, 2-way and multiway frequency tables, measures of association, and the log-linear model) was used for the χ² test with the Bonferroni correction for multiple comparison.

The BMDP program LR was used for the stepwise logistic regression analysis, which was done to evaluate the relationship between the clinical features and the proportion of patients with chronic HCV infection responding completely to IFN-α treatment. All categorical clinical features (ie, sex [male=0, female=1], age [39 years=0, ≥40 years=1], serum alanine aminotransferase level [≤99 IU/L=0, ≥100 IU/L=1], histological features [chronic persistent hepatitis=0, chronic active hepatitis=1, severe chronic active hepatitis=2, and cirrhosis=3], and HCV RNA genotype [1b=0, 2a=1, 2b=2]) were handled as dichotomous variables. Using this method, the most significant associated variable was entered into the model. After adjusting for that variable, the next most significant variable was added to the model. This procedure was continued until no more variables met the entry criteria. A P value of .05 or less was considered statistically significant.
(27.1%) had a complete response, 68 (34.2%) had a partial response, and 77 (38.7%) had no response; of 112 women, 27 (24.1%) had a complete response, 55 (49.1%) had a partial response, and 30 (26.8%) had no response. The frequency of a partial response was significantly higher in women than in men ($P<.01$), but the frequencies of a complete response and no response did not differ between the sexes (Table 1).

To clarify the effectiveness of IFN-α treatment, properties of HCV and patient characteristics were compared based on sex (Table 2). No significant difference was found in the number of patients having a complete response by sex, based on pretreatment levels of alanine aminotransferase and histological features of the liver. Those who had a complete response included 37 (16.5%) of 224 patients with genotype 1b, 33 (56.9%) of 58 with genotype 2a, and 11 (37.9%) of 29 with genotype 2b. Significant differences were noted in the number of complete responses between genotypes 1b and 2a ($P<.001$) and between 1b and 2b ($P<.01$). No significant differences were noted in the number of patients with a complete response by sex and genotype. The frequency of a complete response was significantly higher in patients with HCV RNA levels of less than 4 by competitive PCR (65.1%) than in those with levels greater than 5 (16.1%) ($P<.001$). Of 144 patients with HCV RNA levels above 6, only 6.9% had a complete response. No significant difference was found, however, in the number of patients with a complete response by sex and HCV RNA level.

To search for predictive factors that would influence the response to IFN-α treatment, we used multiple logistic regression analysis. The HCV RNA level ($P<.001$), genotype ($P<.001$), sex ($P=.05$), and interaction between sex and age were associated with a complete response to natural IFN-α. As the HCV RNA level decreased, there was a tendency for the probability of a complete response to increase. For patients with genotypes 2a and 2b, there was an increased probability of having a complete response. Moreover, a significant correlation was found when women were grouped by age (Table 3).

In the men, the rate of complete response ranged from 20.0% to 47.4% for those younger than 60 years and decreased to 10.6% in those aged 60 years and older. A complete response in women ranged from 71.4% to 80.0% in those aged 39 years and younger, 25.0% in the 40- to 44-years age group, 16.7% to 18.2% in the 45- to 59-years age group, and 8.6% in those aged 60 years and older (Figure). The rate of complete response was significantly higher in women (80%) than in men (20%) in the 35- to 39-year-old group ($P<.05$), but there were no sex differences in other age groups. The rate of response to IFN-α treatment was remarkably decreased in women 40 years of age and older, but it did not decrease with aging in the men.

The outcome of IFN-α treatment for these 311 patients based on sex and age is shown in Table 4. Patients were separated into 4 groups by sex and age: group 1 consisted of men younger than 40 years; group 2, men older than 40 years; group 3, women younger than 40 years; and group 4, women older than 40 years. The rate of complete response in each group was 33.3%, 25.0%, 75.0%, and 15.6%, respectively, significantly higher in group 3 than in groups 1 ($P<.01$), 2 ($P<.001$), and 4.

### Table 1. Outcome of Interferon Alfa Treatment by Sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of Patients</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>No Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>199</td>
<td>54 (27.1)</td>
<td>68 (34.2)*</td>
<td>77 (38.7)</td>
</tr>
<tr>
<td>Women</td>
<td>112</td>
<td>27 (24.1)</td>
<td>55 (49.1)*</td>
<td>30 (26.8)</td>
</tr>
<tr>
<td>Total</td>
<td>311</td>
<td>81 (26.0)</td>
<td>123 (39.5)</td>
<td>107 (34.4)</td>
</tr>
</tbody>
</table>

*Men vs women, $P<.01$.

### Table 2. Clinical Features of Patients With Chronic Hepatitis C Virus (HCV) Infection

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Men (n=199)</th>
<th>Women (n=112)</th>
<th>Total (N=311)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td><strong>Alanine aminotransferase level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤99 IU/L</td>
<td>137 (36)</td>
<td>85 (19)</td>
<td>222 (55)</td>
</tr>
<tr>
<td>≥100 IU/L</td>
<td>62 (18)</td>
<td>27 (8)</td>
<td>89 (26)</td>
</tr>
<tr>
<td><strong>Histological</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPH</td>
<td>24 (7)</td>
<td>20 (6)</td>
<td>44 (13)</td>
</tr>
<tr>
<td>CAH</td>
<td>89 (25)</td>
<td>43 (14)</td>
<td>132 (39)</td>
</tr>
<tr>
<td>Severe CAH</td>
<td>64 (19)</td>
<td>26 (5)</td>
<td>90 (24)</td>
</tr>
<tr>
<td>LC</td>
<td>22 (3)</td>
<td>23 (2)</td>
<td>45 (5)</td>
</tr>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>148 (72)</td>
<td>76 (40)</td>
<td>224 (71)</td>
</tr>
<tr>
<td>2a</td>
<td>30 (19)</td>
<td>28 (14)</td>
<td>58 (18)</td>
</tr>
<tr>
<td>2b</td>
<td>21 (11)</td>
<td>8 (3)</td>
<td>29 (9)</td>
</tr>
<tr>
<td><strong>HCV-RNA level (10^6 copies/50 µL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4</td>
<td>35 (24)</td>
<td>28 (17)</td>
<td>63 (41)</td>
</tr>
<tr>
<td>≥5</td>
<td>164 (30)</td>
<td>84 (10)</td>
<td>248 (40)</td>
</tr>
</tbody>
</table>

*CPH indicates chronic persistent hepatitis; CAH, chronic active hepatitis; and LC, liver cirrhosis.
†Genotypes 1b vs 2a, $P<.001$.
‡Genotypes 1b vs 2b, $P<.01$.
§HCV RNA level ≤4 vs ≥5, $P<.001$. 
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times that of group 2, and 20 times that of group 4. The odds ratio of group 3 was 4 times that of group 1, 6 times that of group 1 than in group 4 (P<.05). The odds ratio of group 3 was 4 times that of group 1, 6 times that of group 2, and 20 times that of group 4.

In the present study of the effectiveness of IFN-α treatment for patients with chronic HCV infection, focused on patient’s sex, we obtained evidence that age was associated with response to the treatment in women, but not in men. Although there was no significant male-female difference in the overall rate of complete response to IFN-α treatment, being a younger woman (<40 years) was a favorable marker for successful treatment. This study also confirmed that the HCV RNA genotype and the HCV RNA level are important factors of response to IFN-α treatment.6,8–10 To exclude bias, the relation between the outcome of treatment and patients’ sex was investigated, based on these HCV markers.

Histological features are also useful predictive markers. In this study, the response rate was low in patients with advanced liver disease, especially in women. This is consistent with the results of multiple logistic regression analysis showing that older women had a low response because most patients with advanced liver disease were 40 years of age and older. The duration of hepatitis was not correlated with the response to IFN-α treatment in a previous study,6 but the relationship is difficult to determine because the onset of hepatitis is uncertain in many cases. Therefore, we excluded it and other factors such as a history of blood transfusion, antibody to c100 (which is the first-generation assay for antibody to HCV), and antibody to GOR (which is isolated from the plasma of a chimpanzee infected with a human non-A, non-B hepatitis agent) that did not correlate with the response to IFN-α treatment.

In contrast to previous studies in which only the biochemical response was assessed, this study defined a complete response as a sustained return to normal serum aminotransferase levels and the disappearance of HCV RNA by PCR. Because serum aminotransferase levels return to normal in some patients, either during treatment or within 6 months, those with a complete response in the previous studies may have had HCV RNA in the serum and thus were at risk of the reactivation of chronic hepatitis C. It therefore, we defined a complete response as the elimination of HCV RNA from the serum that was sustained for 6 months after the cessation of IFN-α treatment. This study indicates that HCV RNA is more easily eliminated from younger women than older women.

The relationship between the response to IFN-α treatment and aging is controversial. Other investigators reported that older age was not an unfavorable marker for IFN-α treatment,21,22 whereas Garson et al23 reported that patients who had a complete response were significantly younger than those who had no response. Moreover, Horiike et al22 reported that elderly patients with a low level of HCV RNA respond well to IFN-α treatment. Their studies were done on a small series, and they defined a response to IFN-α treatment as the return of aminotransferase levels to normal after the cessation of treatment, with no attention given to the status of HCV RNA. We obtained clear findings that an older age (≥40 years) was one of the unfavorable markers of the elimination of HCV RNA from the serum of patients with chronic HCV infection.

Of interest was the finding that the response to IFN-α treatment was better in women than in men among patients younger than 40 years and that the response decreased remarkably in women aged 40 years and older. Why HCV appears to act differently in younger and older women, however, is unclear. During perimenopause (from age 40 years), ovulation can be erratic, and plasma go-
nadotropin levels frequently reach menopausal level, even when plasma estrogen levels are within the menstrual range.21,23 These data suggest that the decreased rate of a complete response to IFN-α treatment may correspond with increases in estrogen levels. Interleukin 1, associated with an inflammatory response, is stimulated by low concentrations of estrogen and progesterone. A low concentration of estrogen allows peripheral blood monocytes to secrete more interleukin 1.26 The spontaneous production of interleukin 1β by peripheral mononuclear cells has been shown to be significantly higher in patients with chronic hepatitis C than in healthy control subjects, then decreased in those with a complete response after the administration of IFN-α.27 This cytokine production may alter the effectiveness of IFN-α treatment in perimenopausal and menopausal women with chronic HCV infection.

Because women do have a response to IFN-α treatment, as shown in Table 1, and in this study HCV RNA was eliminated during treatment more often in women than in men, hormonal activity, in particular the level of estrogen, may be associated with the sustained elimination of HCV in patients undergoing IFN-α treatment for HCV infection. Hormone levels should be measured before treatment. A pertinent subject for investigation would be the results of a combination of estrogen replacement therapy28 and IFN-α therapy for older women.

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REFERENCES


