CLINICO-PATHOLOGICAL PREDICTIVE FACTORS OF RESPONSE TO INTERFERON THERAPY IN CHRONIC HEPATITIS C

Paninee Thavarungkul[1], Kan Toriyama[2], Masako Kusuda[2], Hodaka Kamito[3], Masa-aki Kamito[4], Toshihiro Nakayama[4], Yuji Kato[3] and Hideyo Itakura[2]

[1]Institute of Pathology, Department of Medical Services, Rajvithi Hospital, Rajvithi Rd, Bangkok 10400, Thailand; [2]Department of Pathology, Institute of Tropical Medicine, Nagasaki University, Sakamoto 1-12-4, Nagasaki 852; [3]First Department of Internal Medicine, School of Medicine, Nagasaki University, Sakamoto 1-7-1, Nagasaki 852; [4]Kamito Hospital, Joei-machi 30-2, Nagasaki 852, Japan

Abstract. We performed a clinico-pathological study to determine which pre-treatment factors could predict the response to interferon (IFN) therapy in 55 Japanese patients with chronic hepatitis C. Responses to the IFN therapy were evaluated as sustained response, relapse and non-response by the presence or absence of serum hepatitis C virus (HCV) RNA during the course of treatment and at least 6-months post-treatment. The numbers of sustained response, relapse and non-response were 16 (29.0%), 25 (45.5%) and 14 (25.5%), respectively. Eight out of 16 sustained response cases (50%) showed HCV genotype III. Eight among 10 patients with HCV genotype III (80%) were sustained responders. HCV genotypes were found to be correlated with the response to the IFN therapy ($p < 0.0001$). None of the histological features, the types of the IFN therapy and other clinical factors showed significant differences. These findings suggest that outcome of the IFN therapy in chronic hepatitis C can be predicted by a virological factor, and that HCV genotype III is a useful predictor of a favorable outcome.

INTRODUCTION

Since the introduction of routine donor blood screening for hepatitis B virus (HBV), the incidence of HBV infection has decreased and hepatitis C virus (HCV) has been the major cause of non-A, non-B post-transfusion and sporadic hepatitis in many countries (Choo *et al*, 1989; Kuo *et al*, 1989). Acute HCV infection will develop chronic hepatitis C in approximately 50% of cases and progress to cirrhosis and hepatocellular carcinoma with a high tendency (Koretz *et al*, 1980; Realdi *et al*, 1982; Kiyosawa *et al*, 1990; Di Bisceglie *et al*, 1991). Interferon (IFN) is at the present the most recommended treatment for chronic hepatitis C to reduce disease activity and improve liver histology (Hoofnagle, 1994). However, many studies showed that not all the cases of chronic hepatitis C responded to IFN therapy (Diodati *et al*, 1994; Reichard *et al*, 1994; Poynard *et al*, 1995). It is said that several factors, such as the schedules of the IFN therapy, HCV genotypes and pre-treatment liver histology relate to the response to IFN therapy (Tsubota *et al*, 1993; 1994, Lam *et al*, 1994; Hayashi *et al*, 1994; Yoshioka *et al*, 1995).

In this study we investigated which factors could play the main role to predict the response to IFN therapy in the patients with chronic hepatitis C.

MATERIALS AND METHODS

Fifty-five Japanese patients with chronic hepatitis C who received IFN therapy from 1992 to 1994 were studied retrospectively. We reviewed histological specimens in the Department of Pathology, Institute of Tropical Medicine, Nagasaki University and the clinical data of the patients from Kamito Hospital, Nagasaki City.

Hepatitis C virus infection was proved by the presence of anti HCV antibodies, using a second generation radioimmunoassay kit (Ohtsuka assay Co Ltd, Japan) and HCV RNA was detected by polymerase chain reaction method. HCV genotypes were classified in 49 cases among 55 patients, according to the method of Okamoto *et al* (1992). The patients with chronic hepatitis from other etiology were excluded.

The liver biopsy specimens were fixed in 10% neutral buffer formalin and embedded in paraffin. The 3-4 microns thick sections were stained with hematoxylin and eosin, Azan-Mallory and silver impregnation for reticulin fiber. The biopsy speci-

mens were evaluated by two pathologists (PT and KT) without the knowledge of the clinical data. The histological features were classified according to the international criteria as chronic persistent hepatitis (CPH), mild or severe chronic hepatitis (CAH) or liver cirrhosis (LC), (Bianchi *et al*, 1977). Histological activity index (HAI) was graded by using scoring system of Knodell *et al* (1981). Two other histological lesions, interlobular bile duct damage (BDD) and lymphoid aggregates (LA) in the portal tracts, which are useful pathological parameters in the diagnosis of chronic hepatitis C were also evaluated as presence or absence (Lefkowitch *et al*, 1993; Graf *et al*, 1994).

The patients were randomly treated with natural IFN alpha (Sumiferon, Sumitomo Pharmaceutical Co Ltd, Tokyo, Japan) or IFN alpha 2a (Canferon, Takeda Pharmaceutical Co Ltd, Tokyo, Japan; Roferon, Japan Roche Co Ltd, Tokyo, Japan), 6MU subcutaneously injected daily for 2 weeks and then 3 times a week for 22 weeks or IFN alpha 2b (Intron A, Schering-Plough Co Ltd, Osaka, Japan), 6MU subcutaneously injected daily for 4 weeks and then 3 times a week for 20 weeks. All the patients were monitored the presence or absence of serum HCV RNA at 2nd, 4th, 12th and 24th weeks during treatment and at 3rd, 6th and 12th months during post-treatment follow up.

The response to IFN was defined as a sustained response when serum HCV RNA was undetectable during the course of treatment and for at least 6-months in the post-treatment period. Relapse was defined when serum HCV RNA was undetectable during the course of treatment and become detectable when the treatment was finished. Non-response was defined when serum HCV RNA was detected during the course of treatment and at the end of treatment. The patients were divided into 3 groups according to the response to the therapy. Sex, age, clinical history, laboratory data and histological features of liver biopsy specimens were analyzed among these groups.

Table 1

Comparison of pre-treatment clinical features, laboratory data and therapy among chronic hepatitis C patients.

	Sustained response	Relapse	Non response
Number of patients	16 (29.0%)	25 (45.5%)	14 (25.5%)
Male	12 (35.3%)	15 (44.1%)	7 (20.6%)
Female	4 (19.0%)	10 (47.6%)	7 (33.3%)
Blood transfusion			
+	5 (27.8%)	10 (55.6%)	3 (16.7%)
-	11 (29.7%)	15 (40.6%)	11 (29.7%)
HCV genotype			
II	5 (13.9%)[a]	22 (61.1%)	9 (25.0%)
III	8 (80.0%)[a]	1 (10.0%)	1 (10.0%)
IV	2 (66.7%)	1 (33.3%)	0 (0%)
Therapy regimen			
IFN alfa	0 (0%)	1 (25.0%)	3 (75.0%)
IFN alfa 2 a	6 (31.6%)	7 (36.8%)	6 (31.6%)
IFN alfa 2 b	10 (31.3%)	17 (53.1%)	5 (15.6%)
Age (years)[b]	53.50 ± 7.72	54.75 ± 10.91	60.00 ± 5.35
SGOT (IU/L)[b]	53.81 ± 24.11	57.24 ± 30.53	59.14 ± 30.66
SGPT (IU/L)[b]	69.75 ± 44.47	68.96 ± 42.15	69.79 ± 40.50
Platelet ($\times 10^4$)[b]	13.11 ± 3.70	12.72 ± 3.51	12.32 ± 2.72

[a] $p < 0.0001$ by chi-square test
[b] Mean ± SD

Statistical analysis of the results was carried out by chi-square test, Fisher's exact test and Student's *t*-test. A statistical significance was defined as a p-value < 0.05.

RESULTS

Pre-treatment clinical features and laboratory data of chronic hepatitis C patients with IFN therapy appear in Table 1. Fifty-five patients were divided into 3 groups according to the presence or absence of serum HCV RNA after IFN administration. Sixteen (29.0%), 25 (45.5%) and 14 (25.5%) out of 55 patients showed sustained response, relapse and non-response, respectively. Forty-nine out of 55 patients were examined for HCV genotypes. There were 36 patients with HCV genotype II, 10 with HCV genotype III and 3 with HCV genotype IV. Eight out of 16 sustained cases (50%) showed HCV genotype III. Eight among 10 patients with HCV genotype III (80%) were sustained responders. HCV genotype correlated with the response of IFN therapy ($p < 0.0001$). Other factors such as sex, age, history of blood transfusion, types of IFN, SGOT, SGPT and platelet levels showed no significant difference.

Table 2 shows a comparison of pre-treatment liver histology with the response to IFN therapy. One out of 6 patients with CPH (16.7%) and 15 out of 46 patients with mild CAH (32.6%) were sustained responders. Two out of 6 patients with CPH (33.3%) and 12 out of 46 patients with mild CAH (26.1%) showed no response. Three out of 6 patients with CPH (50.5%). 19 out of 46 patients with mild CAH (41.3%) and three patients with liver cirrhosis were in the relapsed group. No statistical difference was found in liver histology among these 3 groups.

According to Knodell's HAI scores (Table 3), total HAI score, periportal score, lobular score, portal score and fibrosis score gave no correlation with the outcome of IFN therapy.

Table 4 shows a comparison of the pre-treatment BDD and LA among chronic hepatitis C patients. Among 55 patients, 35 (63.6%) and 14 cases (25.5%) showed BDD and LA, respectively. All cases of LA were accompanied by BDD. No correlation was

Table 2

Comparison of pre-treatment histological findings among chronic hepatitis C patients.

	Sustained response	Relapse	Non response
CPH	1 (16.7%)	3 (50.0%)	2 (33.3%)
Mild CAH	15 (32.6%)	19 (41.3%)	12 (26.1%)
Severe CAH	0	0	0
LC	0	3 (100%)	0

Table 3

Comparison of pre-treatment histological activity index among chronic hepatitis C patients.

	Sustained response (Mean ± SD)	Relapse (Mean ± SD)	Non response (Mean ± SD)
Total HAI score	6.00 ± 1.83	5.76 ± 1.94	5.36 ± 1.78
Periportal score	0.94 ± 0.25	0.88 ± 0.33	0.93 ± 0.27
Lobular score	1.00 ± 0.00	0.96 ± 0.02	0.93 ± 0.27
Portal score	1.94 ± 1.12	1.76 ± 1.05	1.64 ± 1.08
Fibrosis score	2.13 ± 1.02	2.16 ± 1.18	1.86 ± 1.03

Table 4

Comparison of pre-treatment bile duct damages and lymphoid aggregates among chronic hepatitis C patients.

	Sustained response	Relapse	Non response
BDD (+)	4 (19.0%)	12 (57.1%)	5 (23.8%)
BDD (+) with LA (+)	6 (42.9%)	6 (42.9%)	2 (14.3%)
BDD (-) and LA (-)	6 (30.0%)	7 (35.0%)	7 (35.0%)

found between the outcome of IFN therapy and these two characteristic lesions of HCV infection.

DISCUSSION

We analyzed which pre-treatment factors could predict the response of IFN therapy among 55 patients with chronic hepatitis C. Although in several previous reports, normalization of serum alanine aminotransferase (ALT) level was employed as the indicator of effectiveness of IFN therapy, serum HCV RNA and inflammatory change in the liver can be found in anti-HCV positive patients with normal serum ALT (Alberti *et al*, 1992; Naito *et al*, 1994). For this reason, we defined the response to interferon therapy as elimination of serum HCV RNA instead of normalization of serum ALT levels.

With this definition, among 55 patients, sustained response, relapse and non-response to IFN therapy were 16 (29.0%), 25 (45.5%) and 14 cases (25.5%), respectively. Among 16 sustained responders, 8 cases (50.0%) showed HCV genotype III and 8 out of 10 patients with HCV genotype III (80%) showed sustained responses. Our findings support other reports that patients with HCV genotype III had significantly higher response rate to IFN therapy (Hayashi *et al*, 1994; Saitoh *et al*, 1994; Tsubota *et al*, 1993; 1994, Yoshioka *et al*, 1995). Although this genotype-dependent response is a question, the latest reports suggested that the nucleotide and amino acid sequences of the HCV genome may influence the sensitiveness to IFN (Weiner *et al*, 1992; Okada *et al*, 1992).

Lau *et al* (1993) found that chronic hepatitis C patients who had history of blood transfusion exhibited a correlation of HCV viremia with unfavorable response to IFN therapy, using serum ALT levels as an indicator of effectiveness of IFN therapy. In our study, we found no difference in response rate between the post-transfusion and sporadic cases of chronic hepatitis C.

In correlating age and histological findings with the outcome of treatment, several different results have been reported, using the same definition of response (presence or absence of HCV RNA). Hayashi *et al* (1994) reported that age is an important marker of effective interferon treatment of patients with chronic HCV infection. In contrast, Saitoh *et al* (1994) and our study showed no significant correlation of age with the outcome of treatment. On histological findings, Saitoh *et al* (1994) found that the more severe the liver disease of the patients, the less response there was to IFN. Hayashi *et al* (1994) and our study showed no correlation of histological findings with the outcome of IFN therapy.

According to the HAI scoring system, fibrosis was reported to be significantly related to the success of IFN therapy (Hayashi *et al*, 1994). However, with the same definition of response to IFN therapy, we found HAI could not be used to predict the response to IFN treatment.

BDD and LA, which are useful pathological parameters in the diagnosis of liver disease caused by HCV (Lefkowitch *et al*, 1993; Graf *et al*, 1994), showed no important role in predicting the response to IFN therapy in our study. Lau *et al* (1993) showed the opposite results. However in their study, they defined the response as the normalization of serum ALT levels.

In summary, by using a virological factor (presence or absence of serum HCV RNA) to determine

the response to IFN therapy, sustained responders were 16 out of 55 patients (29.0%). HCV genotypes were a useful predictor among various variables and the patients with HCV genotype III had higher sustained response rates.

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