J Gastroenterol 1996; 31:69–74

Journal of Gastroenterology

Assessment of histological features and outcome of interferon therapy in chronic hepatitis C

NAOKI KUMAGAI,[1] SHIGERU KURAMOCHI,[3] KYOUKO TODA,[2] NAOTO IWABUCHI, KANJI TSUCHIMOTO,
[1] SATOSHI TSUNEMATSU, HIDETSUGU SAITO, TOSHIO MORIZANE, MASAHARU TSUCHIYA, and HIROMASA ISHII[4]

[1] Department of Internal Medicine and [2] Biomedical Laboratory, Kitasato Institute Hospital, 5-9-1 Shirokane, Minato-ku, Tokyo, 108 Japan
[3] Department of Pathology and [4] Department of Internal Medicine, School of Medicine, Keio University, Tokyo, 160 Japan

Abstract: The correlation between the histological features of liver biopsy specimens before interferon (IFN) treatment and the clinical effect of IFN administration on chronic hepatitis C was investigated. A study of the relation between several histological features that were graded in 60 liver biopsy specimens from chronic hepatitis C patients before IFN treatment disclosed that the grade of portal fibrosis was positively correlated with the grade of other inflammatory features, including piecemeal necrosis and portal and lobular inflammation. The degree of portal fibrosis adversely affected the rate of normalization of ALT levels in chronic hepatitis C during and after IFN treatment. We reexamined 36 liver biopsy specimens that showed a moderate degree of portal fibrosis, and found that the degree of piecemeal necrosis was inversely correlated with the extent of lymphoid follicle formation in the portal tracts. During IFN therapy, the group of chronic hepatitis C patients who showed marked piecemeal necrosis and less lymphoid follicle formation in the liver specimens had a poor response to IFN treatment, whereas another group that showed marked lymphoid follicle formation and little piecemeal necrosis in the liver specimens had a good response to IFN. These relationships gradually disappeared after the completion of IFN treatment.

Key words: hepatitis C, interferon therapy, liver histology

Offprint requests to: N. Kumagai
(Received for publication on Mar. 20. 1995; accepted on June 23, 1995)

Introduction

Since the cloning and sequencing of hepatitis C virus (HCV)[1] was performed and since the effectiveness of interferon (IFN) administration in patients with chronic hepatitis C has been well established by a variety of clinical studies,[2–4] IFN treatment for chronic hepatitis C has been employed vigorously. As clinical data accumulated, we recognized the variety of responses to IFN among patients with chronic hepatitis C. Interferon is known to have antiviral, antiproliferative, and immunomodulatory properties; it has also been reported that prolonged α-interferon therapy may lead to immune disorders. Indeed, several investigators have warned of the adverse effects of IFN in autoimmune hepatitis with anti-HCV antibody.[5–7] Although certain characteristic morphological features have been ascribed to chronic hepatitis of differing origins,[8–11] chronic hepatitis C has, more or less, the features characteristic of immune reactions between lymphocytes and HCV-infected hepatocytes.[12–14] These findings tempted us to investigate the correlation between liver histological features and the clinical outcome of IFN treatment in chronic hepatitis C.

Patients and methods

Patients

Sixty patients with histologically proven chronic active hepatitis were studied retrospectively. All patients had anti-HCV antibodies detected by second-generation enzyme immunoassay (Ortho Diagnostics, Raritan, N.J.) and all were negative for serological markers of HBV infection (absence of HBsAg and anti-HBc antibody).

All patients were given 6 million units of recombinant interferon-α-2a by intramuscular injection every day for 14 days and then three times a week for

22 weeks. All patients were seen monthly during and after therapy. All serum samples were stored at −80°C and thawed immediately before use. Initial and monthly serum samples were tested for biochemical markers and anti-HCV antibodies. Initial and selected serum samples during and after therapy were also tested for HCV RNA by the polymerase chain reaction (PCR).[15–16] All patients showed abnormal ALT values and HCV-RNA was detected in their sera before IFN treatment. Patients with normal ALT values and no HCV-RNA in their sera 6 months after IFN treatment were judged as being in complete remission.

Informed consent was obtained from all patients.

Methods

All patients underwent a liver biopsy before treatment. All specimens were obtained by percutaneous needle biopsy, performed with a biopsy needle. Formalin-fixed, paraffin-embedded sections were stained with hematoxylin and eosin, elastica-van Gieson, azan, and reticulin stains. Changes in the 60 biopsy specimens were graded in terms of 19 different histological features, as described by Bach et al.,[14] with minor modifications. Grading was classified on a scale of 0–4, based on the severity of the lesion (0, absent; 1, mild; 2, mild-moderate; 3, moderate; and 4, severe). Portal fibrosis was graded on a scale of 0–4 (0, absent; 1, mild, bridging fibrosis −; 2, moderate, bridging fibrosis +, portal-portal linkage; 3, transition to cirrhosis, portal-portal and portal-central linkage; and 4, cirrhosis). Portal inflammation, piecemeal necrosis, bile duct damage and/or loss, proliferation of bile ductules and lobular inflammation and/or necrosis were graded on a scale of 0–4. Piecemeal necrosis (p.n.) was graded on a scale of 0–4 (1, p.n. involving less than 50% of the circumference of some portal tracts; 2, p.n. involving more than 50% of the circumference of most portal tracts; 3, p.n. involving almost the entire cicumference of the portal tracts; and 4, marked p.n. involving the entire circumference of the portal tracts). Other features examined included the presence of steatosis, activation of sinusoidal lining cells, formation of lymphoid follicles in the portal tracts, hepatocellular dysplasia, multinucleated giant hepatocytes, oncocytic hepatocytes, broad areas of parenchymal collapse, and iron deposition and rosette formation of periportal hepatocytes. Lymphoid follicles formation in the portal tracts were graded on a scale of 0–4 (1, lymphocytes aggregating and forming a vague nodulation; 2, lymphocytes aggregating and forming a clear nodulation—primary follicle; 3, lymphoid follicles with equivocal germinal centers; and 4, lymphoid follicles with unequivocal germinal centers).

Statistical analysis

Correlation between variables was evaluated by single linear-regression analysis. Statistical significance for the linear regression was determined from Pearson's tables of critical values. χ^2 analysis with Yate's correction was used to compare dichotomous variables. Differences between groups of patients were analyzed with Student's unpaired *t*-test (two-tailed). *P* Values less than 0.05 were considered significant.

Results

We first studied the correlation between five characteristic histological features of all liver biopsy specimens obtained from patients with chronic hepatitis C. As shown in Table 1, the degree of piecemeal necrosis was positively correlated with the degree of both portal and lobular inflammation. The grades of portal fibrosis were positively correlated with the degree of these inflammatory features, except for the degree of lymphoid follicle formation within the portal tracts. The degree of lymphoid follicle formation within the portal tracts was not correlated with the degree of these features, except for portal inflammation. Therefore the feature of lymphoid follicle formation in the portal tracts was considered to be a factor relatively independent of the other histological features.

As portal fibrosis seemed to be an important feature reflecting many histological aspets, we determined the influence of the degree of portal fibrosis on the responsiveness to IFN treatment in chronic hepatitis C. The degree of portal fibrosis was inversely correlated with the normalization of ALT in the patients treated with IFN (Table 2). Next, to investigate the clinical influence of histological features other than portal fibrosis, we analyzed the liver histological features in 36 patients who showed a moderate degree of portal fibrosis (i.e., grade 2). There was an inverse correlation between the grade of piecemeal necrosis and the degree of lymphoid follicle formation in the portal tracts (Table 3).

When the clinical outcome was analyzed according to the grade of the above two histological features, the degree of piecemeal necrosis was found to have a bad effect on IFN therapy, whereas the degree of lymphoid follicle formation in the portal tracts had a good effect on IFN therapy, although the differences between each group were not significant. Although these phenomena were observed during treatment, the tendency disappeared after treatment (Table 4).

To confirm the tendency, we selected a group of patients whose liver histology showed piecemeal necrosis dominance (group A) and another group

Table 1. Correlation between histological features of liver biopsy specimens in chronic hepatitis

	Portal fibrosis	Piecemeal necrosis	Portal inflammation	Lobular inflammation	Lymphoid follicle formation in portal tracts
Portal fibrosis	—	0.679*	0.520*	0.550*	NS
Piecemeal necrosis	0.679*	—	0.512*	0.532*	NS
Portal inflammation	0.520*	0.512*	—	0.363**	0.454*
Lobular inflammation	0.550*	0.532*	0.363**	—	NS
Lymphoid follicle formation in portal tracts	NS	NS	0.454*	NS	—

Numbers indicate correlation coefficients (examined by single linear regression analysis)
* $P < 0.001$; ** $P < 0.01$; NS, Not significantly correlated

Table 2. Changes in serum ALT levels in patients with chronic hepatitis C treated with IFN: Influence of the degree of portal fibrosis

		No. of patients with normal ALT levels at			Complete remission[a]
		12 Weeks	24 Weeks	48 Weeks	
	Grade				
Portal fibrosis	1	9/11 (82%) *	9/10 (90%) *	7/10 (70%)	7/10 (70%) *
	2	22/36 (61%)	19/35 (54%)	23/35 (66%)	15/35 (43%)
	3	5/13 (38%)	3/12 (25%)	6/12 (50%)	4/12 (33%)

* $P > 0.05$ (evaluated by χ^2 analysis)
[a] Complete remission means both normal ALT levels and no HCV-RNA in the serum

Table 3. Correlation between histological features of liver biopsy specimens in chronic hepatitis C with grade 2 portal fibrosis

	Piecemeal necrosis	Portal inflammation	Lobular inflammation	Lymphoid follicle formation in portal tracts
Piecemeal necrosis	—	NS	NS	−0.332**
Portal inflammation	NS	—	NS	0.462*
Lobular inflammation	NS	NS	—	NS
Lymphoid follicle formation in portal tracts	−0.332**	0.462*	NS	—

* $P < 0.01$; ** $P < 0.05$; NS, Not significantly correlated
Numbers indicate correlation coefficients (examined by single linear regression analysis)

Table 4. Changes in serum ALT levels in chronic hepatitis C with grade 2 portal fibrosis: Influence of the degree of piecemeal necrosis and lymphoid follicles

		No. of patients with normal ALT levels at			Complete remission
		12 Weeks	24 Weeks	48 Weeks	
	Grade				
Piecemeal necrosis	1	4/5 (80%)	3/5 (60%)	5/5 (100%)	2/5 (40%)
	2	15/22 (68%)	13/21 (62%)	12/21 (57%)	9/21 (43%)
	3–4	3/9 (33%)	3/9 (33%)	6/9 (67%)	4/9 (44%)
Lymphoid follicle formation in portal tracts	0	5/11 (45%)	4/11 (37%)	8/11 (73%)	6/11 (55%)
	1	7/12 (58%)	6/11 (55%)	6/11 (55%)	4/11 (36%)
	2	7/10 (70%)	7/10 (70%)	6/10 (60%)	4/10 (40%)
	3–4	3/3 (100%)	2/3 (67%)	2/3 (67%)	1/3 (33%)

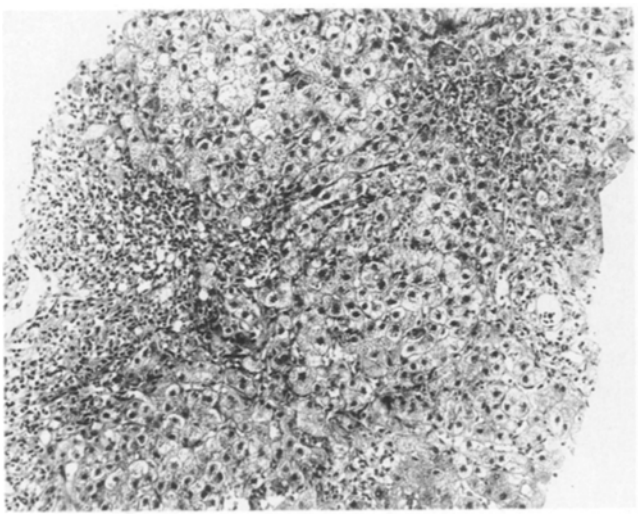

Fig. 1. Photomicrograph of liver biopsy from a patient seropositive for anti-HCV antibodies. Periportal fibrosis is mild and piecemeal necrosis is prominent along the portal tract. Lymphocytic infiltration in the portal tract is diffuse and lymphoid follicles are not seen, i.e., portal fibrosis (grade 1), piecemeal necrosis (grade 3), and lymphoid follicle formation in portal tracts (grade 0). H&E, × 105

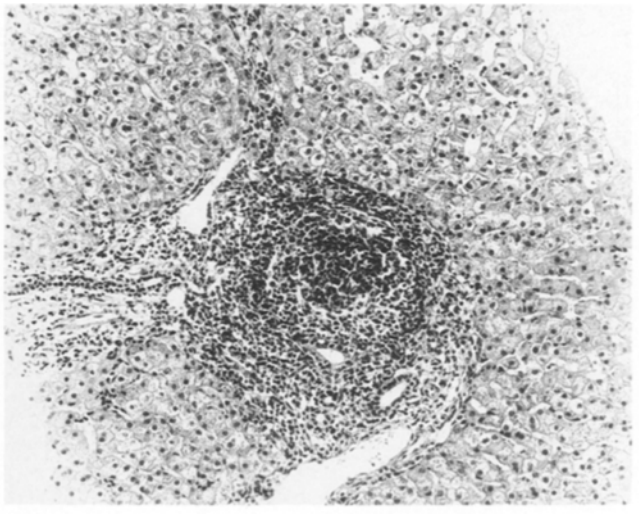

Fig. 2. Photomicrograph of part of a liver biopsy specimen from another patient with chronic active hepatitis C. Periportal fibrosis is slight and piecemeal necrosis is limited to a small segment of the portal tract. A lymphoid follicle with a germinal center is formed in the portal tract, i.e., portal fibrosis (grade 1), piecemeal necrossi (grade 1), and lymphoid follicle formation in portal tracts (grade 4). H&E, × 105

whose liver histology showed lymphoid follicle dominance (group B) from all cases of chronic hepatitis C, irrespective of the grade of portal fibrosis, according to the following criteria: group A, in which the grade of piecemeal necrosis was 3–4 and the grade of lymphoid follicle formation in the portal tracts was 0–1 (Fig. 1) and group B, in which the grade of piecemeal necrosis was 1–2 and the grade of lymphoid follicle formation 2–4 (Fig. 2). The clinical findings before IFN therapy in the two groups are shown in Table 5. No difference was found in clinical findings, liver function parameters, or grade of portal fibrosis between groups A and B, except for the values of the zinc sulfate turbidity test (ZTT). The ZTT values in group A were significantly higher than those in group B, whereas γ-globulin and IgG values were not different. The clinical outcome of IFN therapy in these two groups is presented in Table 6. During the IFN treatment, group B showed a good response (80% of the patients showed normalized ALT in their sera), while only 25% of the patients in group A showed normalized ALT levels ($P < 0.025$). However, the difference in clinical effects between the two groups was insignificant after the treatment and the percentage

Table 5. Clinical findings in groups A and B

Characteristics	Group A ($n = 12$)	Group B ($n = 15$)	P Value[c]
Sex, M/F	8/4	9/6[a]	NS
Age (years)	47.4 ± 7.3[b]	44.5 ± 8.5[b]	NS
Baseline ALT levels (IU/l)	132.3 ± 78.0[b]	114.1 ± 86.5[b]	NS
ZTT (KU)	16.3 ± 4.8[b]	11.6 ± 4.1[b]	$P = 0.01$
γGl (g/dl)	1.59 ± 0.52[b]	1.27 ± 0.29[b]	NS
IgG (mg/dl)	2277 ± 608[b]	2041 ± 420[b]	NS
Grade of portal fibrosis	2.5 ± 0.8[b]	2.1 ± 0.6[b]	NS

Normal ranges in our laboratory: ALT, 0–35 U/l; ZTT, 1.5–14.0 KU; γGl, 0.7–1.9 g/dl; IgG, 1015–2108 mg/dl

ZTT, Zinc sulfate turbidity test

[a] Data expressed as no. male/no. female

[b] Data expressed as means ± SD

[c] P Values less than 0.05 were considered significant (evaluated by χ^2 analysis[a] or Student's unpaired t-test[b])

NS, Not significantly different

Table 6. Comparison of changes in ALT levels between piecemeal necrosis-dominant group and lymphoid follicle-dominant group

	No. of patients with normal ALT levels at			Complete remission
	12 Weeks	24 Weeks	48 Weeks	
Group A[a]	3/12 (25%)	3/12 (25%)	10/12 (83%)	7/12 (58%)
Group B[b]	12/14 (79%) *	10/14 (71%) **	10/14 (71%) NS	7/14 (50%) NS

Differences were evaluated by χ^2 analysis. $^*P < 0.025$; $^{**}P < 0.05$; NS Not significantly different

[a] Group A, Piecemeal necrosis, grade 3–4; lymphoid follicle formation in portal tracts, grade 0–1

[b] Group B, Piecemeal necrosis, grade 1–2; lymphoid follicle formation in portal tracts, grade 2–4

of complete remissions was almost the same in the two groups 6 months after the treatment. When the serum was examined for HCV-RNA by the RT-PCR method (Table 7), 7 out of 12 patients in group A (59%) showed no HCV-RNA in their sera during IFN therapy, however serum ALT remained abnormal in 5 of the 7. In these 5 patients, the ALT levels were normalized after the completion of IFN therapy. The percentage of patients who had both normalized ALT levels and were negative for HCV-RNA in serum during IFN treatment was 17% in group A and 57% in group B. After IFN treatment, these percentages were 58% in group A and 50% in group B.

There were no significant correlations between the clinical outcome of IFN therapy and the 14 characteristic histological features of liver biopsy specimens examined, other than the five features noted above.

Discussion

The histological features of chronic hepatitis C have been studied extensively.[17–22] Lymphoid aggregates in portal tracts, sometimes with germinal centers, damage of the bile duct epithelium, and micro- or macrovesicular steatosis of hepatocytes appear to be unique to chronic hepatitis C compared with chronic hepaititis B. A comparative analysis of the histological features of chronic hepatits C and autoimmune chronic hepatitis has also been reported,[14] in which the features more commonly observed in chronic hepatitis C were bile duct damage, bile duct loss, steatosis, and lymphoid cell follicles within portal tracts. In contrast, severe lobular necrosis and inflammation, piecemeal necrosis, multinucleated hepatocytes, and broad areas of parenchymal collapse were seen more often in autoimmune chronic hepatitis.

On the basis of the pathological findings in chronic hepatitis, we analyzed and graded 19 pathological features in the liver specimens from patients with chronic hepatitis C biopsied before IFN treatment. Characteristic morphological features known to be unique to chronic hepatitis C were frequently observed in our study. Examination of the relationships among these pathological features disclosed that the grade of portal fibrosis was well correlated with the grade of other inflammatory features, including piecemeal necrosis and lobular and portal inflammation. These findings indicate that the grade of portal fibrosis also reflects the current status of hepatic inflammation.

We divided the 60 chronic hepatitis C patients into four groups according to the grade of portal fibrosis and investigated the relationship of this feature to the clinical results of IFN treatment. The degree of portal fibrosis was found to be inversely correlated with a beneficial response to IFN therapy, a finding consistent

Table 7. Changes in serum ALT levels and HCV-RNA in groups A and B during and after treatment

		During treatment		After treatment	
		HCV-RNA		HCV-RNA	
	ALT	(−)	(+)	(−)	(+)
Group A (n = 12)	normal	2 (17%)	1 (8%)	7 (58%)	3 (25%)
	abnormal	5 (42%)	4 (33%)	0 (0%)	2 (17%)
Group B (n = 14)	normal	8 (57%)	3 (21%)	7 (50%)	3 (21%)
	abnormal	3 (21%)	0 (0%)	2 (14%)	2 (14%)

with previous reports.[21,23] Portal fibrosis is a common phenomenon in chronic hepatitis of differing etiologies and is regarded as an end-result of necro-inflammatory activity.

We investigated the pathological features, other than portal fibrosis, that correlated with responsiveness to IFN therapy. To eliminate the influence of portal fibrosis, we analyzed the pathological features in 36 liver specimens with the same degree of portal fibrosis (grade 2). Study of the relationship among these pathological features disclosed that the grade of piecemeal necrosis was inversely correlated with that of lymphoid follicle formation within the portal tracts. Piecemeal necrosis is thought to reflect an immunoreaction between lymphocytes and hepatocytes and is seen more often in autoimmune chronic hepatitis than in chronic hepatitis C.[14] On the other hand, the formation of lymphoid follicles within the portal tracts is seen more frequently in chronic hepatitis C than in autoimmune chronic hepatitis, although its pathological significance remains obscure.[12,14] Interestingly, the degree of piecemeal necrosis had an adverse effect on the responsiveness to IFN therapy, while the degree of lymphoid follicle formation within the portal tracts showed a beneficial effect on IFN treatment. However, these relationships were limited to the period of IFN therapy, and afterwards the relationships gradually disappeared, suggesting that direct exposure of the patients to IFN is required for the presence of these phenomena. One possible explanation for these findings is as follows: Although the antiviral effects of IFN are always advantageous for normalization of serum transaminase, the other functions of IFN, including enhancement of the interaction between lymphocytes and hepatocytes, sometimes induce persistently abnormal ALT activity, even after the disappearance of serum HCV-RNA. Indeed, a discrepancy between the biochemical and virological responses to IFN was frequently observed in patients whose pathological features showed marked piecemeal necrosis.

These findings indicate that an adverse effect of IFN on hepatitis in some populations of chronic hepatitis C patients should be taken into consideration when prolonged IFN treatment for sustained disappearance of the HCV virus is considered.

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