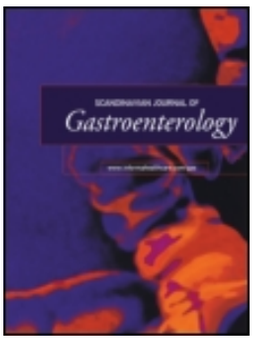

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Influence of Viral Genotype and Level of Viremia on the Severity of Liver Injury and the Response to Interferon Therapy in Spanish Patients with Chronic C Infection

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Background: We wanted to investigate the influence of viral genotype on the severity of liver injury and response to interferon and whether the level of viremia differs in accordance with genotype, severity of liver disease, and response to interferon in patients with hepatitis C virus (HCV) infection. *Methods:* We studied 118 patients with HCV-related liver disease. HCV genotypes were determined with a line probe assay, and serum HCV RNA levels with a competitive reverse transcription polymerase chain reaction assay. *Results:* HCV type 1b was the most prevalent genotype (88%). It was present in 100% of cirrhotic patients, with or without hepatocellular carcinoma (HCC), but only in 78% of patients with chronic hepatitis ($P < 0.001$). The response to interferon was better in patients infected with non-1b HCV genotypes ($P = 0.002$). In a multivariate analysis non-1b HCV genotypes and a low hepatic fibrosis correlated with a favorable response to interferon. Among patients with chronic hepatitis those infected with HCV type 1b were older ($P < 0.001$), and age was the only independent factor associated with HCV type 1b. Viremia levels differed neither between genotypes nor in response to interferon and was significantly lower in patients with cirrhosis and HCC. *Conclusions:* HCV 1b was associated with more severe liver disease and a worse response to interferon therapy. Non-1b genotypes and a lower liver fibrosis were the only independent predictors of a favorable response to interferon. Levels of HCV viremia differed neither among different genotypes nor in response to interferon and decreased with advanced liver disease.

Key words: Chronic hepatitis C; hepatitis C virus; hepatitis C virus genotype; interferon therapy; viremia

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Hepatitis C virus (HCV) infection has been shown to be the cause of most cases of non-A, non-B hepatitis (1). Some patients with chronic HCV infection progress slowly to cirrhosis and hepatocellular carcinoma (2, 3). Because the most serious consequences of HCV infection may not become apparent for many years, it is important to identify those patients infected with HCV who are at greatest risk for progressive liver disease and who may benefit most from early treatment with interferon (IFN) therapy.

Since the discovery of HCV, nucleotide sequence analyses of many strains of the viral RNA have shown substantial variability among the different isolates (4, 5). These variations fall into a series of specific patterns, which have been classified into several genotypes (6–8). The clinicopathologic correlation of specific genotypes is currently the subject of active investigation. Recent reports have suggested an association between some genotypes and a more severe liver disease (9, 10) and the lack of response to IFN treatment (11, 12). It has also been suggested that the level of viral replication in HCV infection might correlate with the response to IFN therapy (13). However, it is still unclear whether the level of HCV viremia varies with the severity of liver disease and with the specific HCV genotype.

In the present study we have analyzed the virologic variables, clinical manifestations, histologic severity of liver disease, and response to IFN therapy in a group of 118 Spanish patients with type-C chronic liver disease. The aims were to investigate 1) whether some specific HCV genotypes lead to a more severe liver injury and to a low response to IFN-α treatment; 2) the relationship between HCV genotypes and the level of viral replication; and 3) whether the amount of HCV RNA in serum differs with the severity of liver disease and the response to IFN.

PATIENTS AND METHODS

Patients

We prospectively studied 118 patients with type-C chronic liver disease who were referred to our hospital between January 1992 and December 1994 and fulfilled the inclusion

Table I. Clinical characteristics and virologic features of patients on the basis of stage of chronic hepatitis C virus (HCV) infection

Feature	HCC and cirrhosis (n = 29)	Cirrhosis (n = 26)	Chronic hepatitis (n = 63)
Sex			
Male	22	16	43
Female	7	10	20
Age (years)	65.9 ± 7.9	63.4 ± 10	41.3 ± 11.7
Epidemiologic risk			
Transfusion	7	6	14
IVDU	—	—	3
Unknown	22	20	46
ASAT, U/l	126.5 ± 82.2	69.1 ± 63.7	98.7 ± 70.2
ALAT, U/l	88.5 ± 54.3	54.2 ± 36.3	135.6 ± 99.8
HCV genotype			
1a	—	—	4
1b	29	25	49
3	—	—	7
4	—	—	3
Unclassified	—	1	—

HCC = hepatocellular carcinoma; IVDU = intravenous drug use; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase.

criteria: biopsy-proven liver disease, detectable HCV RNA in serum, human immunodeficiency virus antibody negative, and exclusion of other forms of liver diseases (autoimmune hepatitis, primary biliary cirrhosis, Wilson's disease, drug-induced liver disease, and negative serologic markers of hepatitis B virus infection, such as hepatitis B surface antigen (HBsAg) and anti-hepatitis B core antigen (HBc), tested by enzyme immunoassay (Abbott Laboratories). Patients were divided into three groups: group 1 consisted of 29 patients with histologically confirmed hepatocellular carcinoma (HCC) associated with liver cirrhosis; group 2 consisted of 26 patients with liver cirrhosis and no evidence of HCC; and group 3 consisted of 63 patients with chronic hepatitis, who showed increased serum alanine aminotransferase (ALAT) levels for more than 6 months. Chronic hepatitis was scored on the basis of the histologic activity index described by Knodell et al. (14). Periportal necrosis, intralobular inflammation, and portal inflammation were analyzed as a 'necro-inflammatory index', and hepatic fibrosis was compared separately. Thirty patients (25%) had identifiable risk factors for acquiring HCV infection: 27 patients had received blood transfusions, and 3 patients had been intravenous drugs abusers. In the other 88 patients no apparent source of HCV infection could be identified.

Recombinant IFN-α-2b (Intron A, Schering-Plough) was administered to 51 of the 63 consecutive patients with chronic hepatitis, at a dose of 5 MU subcutaneously 3 times weekly for 12 months. Twelve patients refused to receive antiviral therapy. All patients had persistently increased serum ALAT levels (more than 1.5 times the upper limit of the normal range). Patients were monitored for serum levels of ALAT and aspartate aminotransferase (ASAT), total protein, albumin, and bilirubin, and erythrocyte, leukocyte, and thrombocyte counts. The patients were divided into two groups on the basis of the response to IFN therapy: 26 patients were considered responders to IFN because ALAT levels returned to normal after therapy, and 25 patients were considered nonresponders because their ALAT levels did not return to normal by the end of IFN.

Methods

Serologic tests. All patients had anti-HCV antibodies as detected by a second-generation enzyme immunoassay (EIA II) (Ortho, Raritan, N.J., USA).

Amplification of HCV by polymerase chain reaction and genotyping. The serum samples were rapidly separated, aliquoted, and stored at −70°C until analyzed. HCV RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) in duplicate, in accordance with the manufacturer's instructions (Amplicor-HCV Test, Roche, Nutley, N.J., USA), using conserved biotinylated primers localized in the 5′ noncoding region of the viral genome. The amplified product was hybridized with a specific nucleic acid probe and was detected by color formation.

HCV genotypes were determined in all patients with a line probe assay (LiPA), which is based on type-specific sequence variations in the 5′ untranslated region. LiPA analysis enables differentiation of subtypes of types 1 and 2 into types 1a, 1b and 2a, 2b, respectively. Others types defined in our study included types 3 and 4. The LiPA assay was performed in accordance with the manufacturer's instructions (Innogenetics, Zwijnaarde, Belgium).

Quantitative detection of serum HCV RNA. Levels of circulating HCV RNA were measured in 82 patients from whom serum specimens were available, using a competitive RT-PCR assay amplified in the 5′ noncoding region (Amplicor HCV Monitor, Roche). This competitive assay combines RT and PCR and is based on coamplification of the target RNA with known amounts of synthetic mutated RNA. The detection limit of this test is 10^3 to 10^7 copies HCV/ml.

Table II. Comparison of clinicopathologic and virologic features of 51 patients with chronic hepatitis according to their response to interferon-α

Characteristics	Responders (n = 26)	Nonresponders (n = 25)	Statistical analysis (P)
Male/female	17:9	17:8	>0.05
Age (years)	39.7 ± 8.4	46.5 ± 9.6	0.03
Epidemiologic risk			>0.05
Parenteral	10	4	
Unknown	16	21	
ASAT, U/l	78.4 ± 45.6	109 ± 66.8	>0.05
ALAT, U/l	147.2 ± 98	187.3 ± 86.4	>0.05
Histologic scores			
Necroinflammatory	7.8	9.0	0.01
Hepatic fibrosis	1.68	2.45	0.005
HCV genotypes			0.002
1b	16 (41%)	23 (59%)	
Non-1b	10 (83%)	2 (17%)	
Viremia level (copies/ml × 10^5)	12.2 ± 8.5	17.3 ± 6.8	> 0.05

See Table I for definition of abbreviations.

Statistical analysis

The chi-square test or Fisher's exact test was used to compare between-group frequencies. Where appropriate, age and laboratory values were compared by means of Student's test or ANOVA test. If data were not normally distributed, Wilcoxon's signed rank test and Wilcoxon's rank sum test were used. Independent factors associated with the presence of HCV genotype 1b and those associated with the response to IFN-α therapy in patients with chronic hepatitis were studied, using a stepwise logistic regression model. In both models the P value for entrance limit had to be less than 0.10. Univariate and multivariate analyses were done, using SAS statistical software (SAS Institute Inc, Cary, N.C., USA). The level of significance for all tests was $P < 0.05$.

RESULTS

Prevalence of HCV genotypes

Among 118 patients studied, the commonest genotype was HCV 1b (88%), whereas type 3 was found in 6%, type 1a in 3%, and type 4 in 3% of patients (Table I). None was infected with 2a or 2b genotypes. Coinfection with two distinct HCV genotypes was not observed in our study. HCV genotype could not be determined in one patient with cirrhosis, so he was excluded from further clinicopathologic analysis.

Because the number of patients infected with genotypes 1a, 3 and 4 was too small to enable a relevant statistical analysis, these three groups were combined (non-1b HCV types), and data were compared with those in patients infected with HCV type 1b.

HCV genotypes and severity of liver disease

HCV type 1b was found more frequently in patients with severe liver disease (Table I): all patients (54 of 54) with cirrhosis, with or without associated HCC, were infected with HCV type 1b, whereas type 1b was detected in only 49 of 63 (78%) patients with chronic hepatitis (P = 0.00001). Furthermore, infection with HCV 1b was associated with a 32-fold increase in the probability of having liver cirrhosis, with or without hepatocellular carcinoma associated, compared with infection by other genotypes.

HCV genotypes and response to IFN-α therapy

Among the 63 patients with chronic hepatitis, 51 received

Table III. Clinical and pathologic variables of patients with chronic hepatitis: univariate analysis

Characteristics	1b HCV genotypes (n = 49)	Non-1b HCV genotypes (n = 14)	Statistical analysis (P)
Male/female	36:13	7:7	>0.05
Age (years)	46 ± 11	34 ± 9.2	0.0001
Epidemiologic risk			>0.05
Transfusion	11	3	
IVDU	—	3	
Unknown	38	8	
ASAT, U/l	103.8 ± 65.3	71 ± 15.8	>0.05
ALAT, U/l	123.3 ± 95.7	149.4 ± 18.1	>0.05
Histologic scores			
Necroinflammatory	8.25	7.00	=0.06
Hepatic fibrosis	2.08	1.50	=0.06
Viremia level (copies/m × 10^5)	21 ± 8.5	14 ± 5.9	>0.05

See Table I for definition of abbreviations.

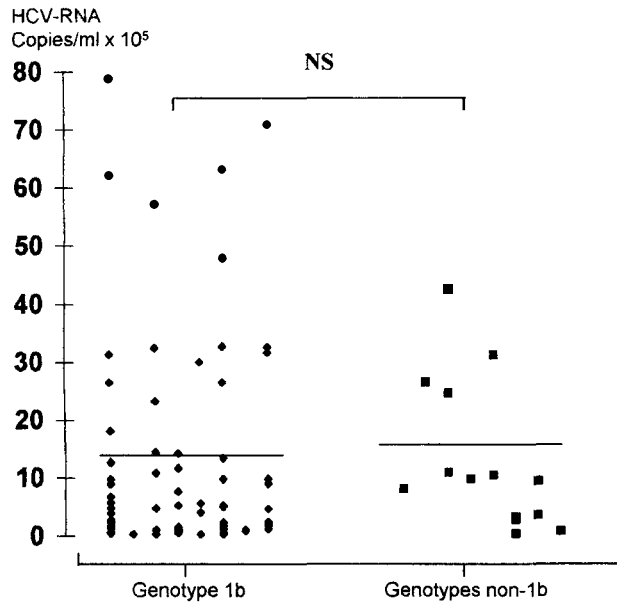

Fig. 1. Serum hepatitis C virus (HCV) RNA concentration in patients with 1b and non-1b HCV genotypes. Bars indicate arithmetic means. NS = not significant.

IFN-α-2b (Table II). Twenty-six were considered responders, and 25 nonresponders. Responders were younger than nonresponders (39.7 ± 8.4 years versus 46.5 ± 9.6 years; $P = 0.03$). The necroinflammatory index before IFN therapy was lower in responders than in nonresponders (7.8 versus 9; $P = 0.01$), and the hepatic fibrosis score was higher in nonresponders (2.45 versus 1.68; $P = 0.005$). No difference was seen between these two groups in sex, mode of transmission of infection, or mean ALAT value.

The response to IFN-α was more favorable in patients infected with non-1b HCV genotypes (10 of 12; 83%) than in those infected with HCV type 1b (16 of 39; 41%) ($P = 0.002$). All patients with types 3 and 4 responded to IFN therapy.

Clinical and pathologic features of patients with chronic hepatitis infected with different HCV genotypes

As shown in Table III, patients with chronic hepatitis infected with HCV 1b genotype were older than the others. There were no significant differences in the mode of acquisition of HCV infection between patients with HCV genotype 1b and those infected by other genotypes. However, type 1b was often seen in patients who had had transfusions (11 of 14; 79%), and the three patients who had used intravenous drugs were infected with HCV non-1b genotypes. Similarly, we found no relationship between the genotype and serum ALAT and ASAT levels.

Scores for periportal necrosis and intralobular and portal inflammation (necroinflammatory index) did not differ significantly among patients with HCV genotype 1b (8.25) and those with non-1b HCV types (7.0) ($P = 0.06$). Similarly, we noted no significant difference with regard to hepatic fibrosis between type 1b (2.08) and the other types (1.5) ($P = 0.06$).

Quantification of serum HCV RNA

The mean titer of HCV RNA did not differ significantly between patients infected with HCV type 1b and those infected with other HCV genotypes ($13.5 \times 10^5 \pm 12.4 \times 10^5$ copies/ml; $n = 68$, compared with $13.9 \times 10^5 \pm 13.2 \times 10^5$ copies/ml; $n = 14$; $P = 0.95$) (Fig. 1). When the amounts of HCV RNA were estimated among patients with chronic hepatitis, there were also no differences between HCV type 1b and non-1b genotypes.

The mean titer of HCV RNA in patients with hepatocellular carcinoma was significantly lower than that in patients with chronic hepatitis ($4.7 \times 10^5 \pm 6.7 \times 10^5$ copies/ml; $n = 24$, compared with $18.7 \times 10^5 \pm 12.4 \times 10^5$ copies/ml; $n = 37$; $P = 0.01$) (Fig. 2). Although the mean value of viremia in patients with cirrhosis ($12.8 \times 10^5 \pm 17.8 \times 10^5$ copies/ml; $n = 21$) was lower than that of patients with chronic hepatitis and higher than that of those with HCC, these differences were not statistically significant.

Serum levels of HCV RNA were measured in 26 patients who received IFN-α-2b therapy, 14 responders and 12 nonresponders. We have not found significant differences between these groups in the level of HCV viremia before treatment ($12.2 \times 10^5 \pm 11.7 \times 10^5$ copies/ml, compared with $17.3 \times 10^5 \pm 13.2 \times 10^5$ copies/ml; $P = 0.4$).

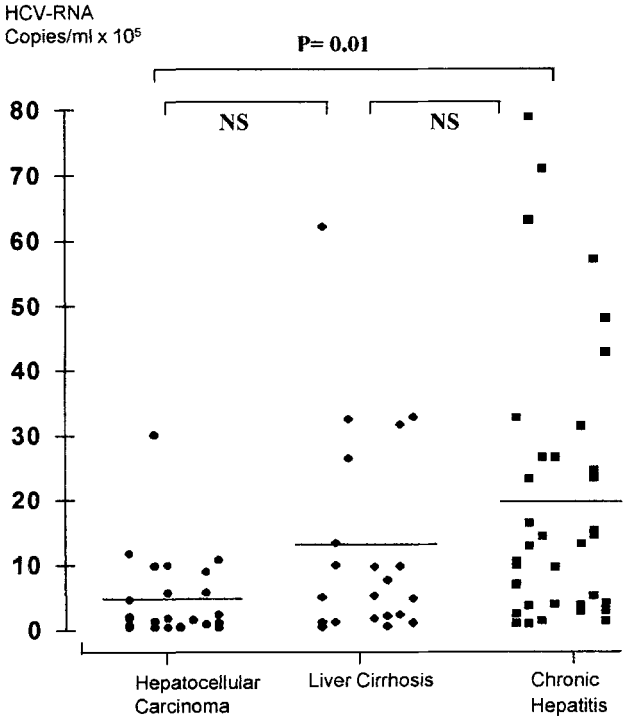

Fig. 2. Viremia levels in patients with hepatocellular carcinoma, liver cirrhosis, and chronic hepatitis. Bars indicate arithmetic means. NS = not significant; HCV = hepatitis C virus.

Table IV. Factors associated with hepatitis C virus (HCV) genotype and with response to interferon-α in patients with chronic hepatitis: multivariate analysis

Variable	Odds ratio	(95% CI)*
Association with genotype 1b		
Age	1.1	(1 to 1.2)
Association with response to interferon		
Non-1b HCV genotypes	5.4	(0.97 to 30)
Hepatic fibrosis score	0.4	(0.2 to 0.9)

* CI = confidence interval.

Multivariate analysis

Age, hepatic fibrosis score, necroinflammatory index, and level of viremia in patients with chronic hepatitis were selected as independent variables in a logistic regression analysis with HCV genotype as the dependent variable. As shown in Table IV, age was the only independent factor associated with HCV type 1b.

With regard to independent factors associated with the response to IFN-α-2b therapy, non-1b HCV genotypes and a low hepatic fibrosis score correlated with a favorable response to the treatment. Because few samples were tested, circulating levels of HCV RNA were not included in the analysis.

DISCUSSION

Our present investigation establishes the high prevalence (88%) of HCV type 1b among Spanish patients with type-C chronic liver disease. HCV genotype 1b has also been found to be the predominant genotype in France, Italy, and Japan (15, 16). Infection with more than one genotype ('mixed infections') was not observed in our population, which is in accordance with previous reports (17, 18).

HCV type 1b was even more prevalent among patients with severe liver disease. Similar results were reported by Nousbaum et al. (15) in Italian and French patients with chronic liver diseases. Takada et al. (9) in Japan found an increased frequency of HCV type II (1b) infection in patients with hepatocellular carcinoma. Moreover, the seven Spanish patients with cirrhosis and HCC included in this study were also infected with HCV type II (1b). However, some authors have found that patients with type-2 HCV were more likely to have severe liver disease (12, 17). Other authors have not found significant differences in the distribution of any HCV type at any disease stage (19).

Our findings suggest that HCV type 1b exerts a distinct and more severe cytopathogenic effect than the rest of the genotypes. However, we also observed an independent association between HCV type 1b infection and older age. In contrast, non-1b genotypes were most often identified in young patients. It is therefore possible that HCV non-1b types might also lead to more severe liver disease because a long time is required for the development of cirrhosis and hepatocellular carcinoma. Recent reports have shown genomic mutations of HCV during the course of infection, particularly in the hypervariable E1 and E2 envelope encoding regions (20). Changes in the E1 and E2 genes alter the antigenicity of the virus, to enable 'immune escape' from neutralizing antibodies. It is possible that this mechanism has selected HCV type 1b to be the genotype most 'resistant' to the host immune response.

Both serum HCV RNA levels and HCV genotypes have been reported to predict the complete and sustained response to IFN therapy (11, 13, 15, 21–23). Previous reports have also shown that IFN therapy was less effective in patients with advanced liver fibrosis (24, 25). In our study serum HCV RNA levels were similar in responders and nonresponders to IFN, but because few cases were tested, this variable was not included in the multivariate analysis. In this multivariable regression analysis the only independent predictors of complete response to IFN were HCV genotype and liver fibrosis score. The infection with non-1b genotypes was associated with an increased chance of response to therapy. Thus, type-1b patients were more likely to be resistant to IFN therapy. Indeed, recent investigations determined that HCV genotype was the most important factor for predicting IFN response (26, 27). Matsumoto et al. (22) observed that the response to IFN was significantly higher in patients with genotypes III (2a) and IV (2b) than in those with genotype II (1b), even when patients with HCV RNA concentrations lower than 10^5 copies/ml were analyzed. These findings may suggest that the difference in response to IFN between genotypes is not simply due to a difference in HCV RNA concentration. Why HCV genotype would influence the susceptibility to IFN remains to be determined. It may be that the degree of heterogeneity in the hypervariable region within HCV E2/NS1 glycoprotein may affect the response to IFN and be closely associated with genotype (28).

We could not find any significant correlation between HCV genotypes and specific routes of transmission. Recent studies have shown that genotypes 1a and 3 are almost exclusively detected in patients with a history of intravenous drug abuse (29), and HCV type 1b is frequently found in patients receiving hemodialysis or renal transplants (30).

The relationship between genotype, level of HCV viremia, and the severity of liver disease remains unclear. We found no significant differences in the amount of serum HCV RNA between patients with genotype 1b and those with other genotypes. This finding agrees with previous reports (15, 26), although in other studies (11, 12) HCV type 2 was associated with lower levels of viremia than type 1. We observed that the mean titer of HCV RNA of patients with HCC associated with cirrhosis was significantly lower than that of patients with chronic hepatitis. The level of HCV viremia was lower in patients with cirrhosis than in patients with chronic hepatitis and higher than in patients with HCC, but the difference was not significant. Recent investigations of the relationship between the level of viral replication in chronic HCV infection and the stage of liver injury offer contradictory results (15, 31–33). Our study suggests that replication of

HCV occurs even in advanced stages of liver disease, although it seems to decrease with time.

In summary, HCV type 1b was, by far, the most predominant genotype in Spanish patients with chronic C infection. It was associated with more severe liver disease and a worse response to IFN therapy. Non-1b genotype and a lower liver fibrosis score were the only independent predictors of response to IFN treatment. No differences in HCV viremia levels were found among different HCV genotypes. However, viral load decreased in advanced liver disease. Levels of HCV viremia have been shown not to differ significantly between those patients who responded to IFN therapy and those who did not.

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