Gut 1993; **34**: 1714–1717

Prediction of the response of chronic hepatitis C to interferon alfa: a statistical analysis of pretreatment variables

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Abstract

Pretreatment variables that could predict the response of chronic hepatitis C to interferon alfa treatment have not been fully assessed. Eighteen baseline variables were evaluated in a series of 100 consecutive patients treated with a 12 month course of interferon alfa. For the purposes of this study, response was defined as the return to normal of aminotransferase activities before the third month of treatment. Seventy per cent of the patients responded to treatment. Six variables were associated with an increased likelihood of response assessed by univariate analysis. With stepwise multiple regression analysis assessment, however, only three variables remained independently predictive of response: low γ glutamyltransferase (γGT) activities ($p<0.001$), absence of obesity ($p=0.005$), and absence of cirrhosis ($p=0.01$). The response rate in patients with γGT activities <0·66 μkat/l (n=55) was 78% and 60% in patients with values >0·66 μkat/l (n=45) ($p=0.048$). Response was attained in 75% of non-obese patients (n=80), compared with only 50% of obese patients (n=20) ($p=0.03$). Finally, 80% of patients without cirrhosis (n=76) responded, while among those with cirrhosis (n=24) the response rate was only 37% ($p<0.001$). All 23 patients without cirrhosis, <40 years old, and with γGT activities <0·66 μkat/l responded to treatment, while only 28·5% of 14 patients with cirrhosis, >40 years old, and with γGT activities >0·66 μkat/l responded to interferon alfa ($p<0.001$). Those findings may be useful when evaluating interferon alfa trials and it is suggested that this treatment should be applied early in the course of chronic hepatitis C.

(*Gut* 1993; **34**: 1714–1717)

Interferon alfa has been assessed in the treatment of chronic hepatitis C in several trials.[1-6] In most studies normalisation of aminotransferase activities has been attained in about 50% of the patients and relapses after stopping interferon alfa are usually seen in half of the responders.[6] Pretreatment parameters that might predict response to interferon alfa have not been clearly determined. In some trials predictive parameters of response were not found.[1 2 5 7] Other studies have suggested, however, that the absence of cirrhosis and a younger age predicted response to interferon alfa.[8-12] Different criteria when defining response to interferon alfa were used in these reports, which might account for the discrepancy. The knowledge of factors predictive of response in chronic hepatitis C has a remarkable clinical interest, as was the case in chronic hepatitis B.[13]

This study was aimed at assessing several pretreatment clinical variables as predictors of response to interferon alfa, in a series of 100 consecutive patients treated with a 12 month course of lymphoblastoid interferon alfa. Univariate analysis was followed by a stepwise multiple regression analysis to evaluate the independent predictive value of each variable.

Patients and methods

PATIENTS

One hundred patients with chronic hepatitis C consecutively treated with the same schedule of lymphoblastoid interferon alfa (Wellferon, Wellcome Research Foundation, Beckhenham, UK) were included in this study. Eighty four of them had participated in a randomised controlled trial of 12 month interferon alfa *v* no treatment[4] and 16 patients were treated with the same dose and schedule in an uncontrolled study. All patients were treated between 1988 and 1991. Interferon alfa was given in a stepdown schedule: 3 million units (MU) daily for two months, 3 MU three times per week for three months, and 1·5 MU three times per week for seven months. Before treatment all patients had a clinical evaluation, including a medical history with special attention to epidemiological data, analytical blood tests, and a percutaneous liver biopsy performed within three months before treatment. Other causes of chronic hepatitis were carefully excluded. All patients, except three, had anti-HCV antibodies by a second generation enzyme immunoassay (Ortho Diagnostic Systems, Raritan, NJ). In all cases aminotransferase activities had been raised for at least six months before starting treatment and the liver biopsy showed chronic hepatitis with or without cirrhosis. Patients with anti-HIV antibodies and other serious medical illnesses were excluded. All patients were instructed to stop alcohol intake at least six months before starting treatment. The trials were approved by the Local Ethics Committee and written informed consent was obtained from all patients.

As well as pretreatment variables, neutralising anti-lymphoblastoid interferon alfa antibiodies were assessed by a specific bioassay in a sample collected during the last week of treatment in 15 of 30 patients who did not respond to treatment to investigate their potential influence in non-responsiveness. The bioassay is based on the protective effect of lymphoblastoid interferon alfa on monkey kidney V3 cells challenged with

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Accepted for publication 22 April 1993

Semliki Forest virus and was performed as described elsewhere.[14] Purified lymphoblastoid interferon alfa of standard potency and control anti-serum samples (kindly supplied by Wellcome Research Foundation, Beckenham, UK) were used to confirm the specificity and sensitivity of the assay.

DEFINITION OF RESPONSE

Response was defined as a complete normalisation of aminotransferase activities before the third month of treatment and persisting at least until the fifth month of the interferon alfa course. The initiation of the response beyond the third month of treatment was not seen in any case. For the present analysis those patients who had responded initially and relapsed when the interferon dose was tapered to 1·5 MU three times per week at five months were considered responders. All other patients, including 16 whose alanine aminotransferase (ALT) activities decreased by more than 50% but did not return to normal, were considered non-responders. Our definition of response was intended to assess the capacity of interferon alfa for inducing a biochemical remission of the disease.

PRETREATMENT VARIABLES

Eighteen pretreatment variables were assessed in each patient: age, sex, history of acute hepatitis and transfusions, presence of obesity defined by a body weight >20% the ideal weight (determined with an age/sex based standard table), and the estimated time of evolution considered as the interval between an obvious percutaneous risk factor (for example, transfusion) or the first detected ALT rise and the start of treatment, were all recorded from the clinical history of each patient. Liver biopsy specimens were classified into two groups by an experienced liver pathologist: chronic hepatitis (either persistent (five cases) or active (71 cases)) and active cirrhosis. Presence of fatty degeneration of hepatocytes was also assessed. Before starting treatment ALT (normal range: 0·10–0·48 μkat/l), alkaline phosphatase (normal range: 1·21–3·45 μkat/l), γ glutamytransferase (γGT) (normal range: 0·08–0·63 μkat/l), albumin, and γ globulin values, as well as neutrophil and platelet counts, were determined in each case. Procollagen type III aminoterminal peptide, a marker of fibrosis and inflammatory activity,[15] was assessed using a commercial radioimmunoassay kit (RIA-gnost PIIIP, Behringwerke AG, Marburg, Germany). Anti-nuclear and anti-smooth muscle antibodies were assessed by indirect immunofluorescence and titres higher than 1/80 were considered positive.

STATISTICS

Univariate analysis was applied to each variable to assess its association with response to interferon alfa treatment. χ^2 and Fisher's exact tests were used for dichotomous variables, and Student's *t* test for continuous variables. Time of evolution and γGT were logarithmically transformed before analysis. Cathegorical explanatory variables were transformed to 0/1 dummy variables.[16] Stepwise multiple linear regression analysis (Stat View SE+graphics, 1988 Abacus Concepts Inc, Berkeley, CA) was subsequently applied to all variables to assess their independent predictive value. Statistical significance was assumed where $p \leq 0{\cdot}05$ and all p values are two tailed.

Results

UNIVARIATE ANALYSIS

Seventy per cent of the patients responded to treatment (as defined in the methods section), while the remaining 30% did not. Table I shows the results of the univariate analysis. Six variables were found to be significantly associated with treatment response. Response to interferon alfa was significantly more frequent in the young ($p<0{\cdot}001$), in patients without cirrhosis ($p<0{\cdot}001$), and in patients with low γGT values ($p=0{\cdot}003$). The remaining three predictive variables were less strongly associated with response to interferon alfa (Table I). Absence of obesity, male sex, and a high platelet count predicted response to interferon alfa. Only 37% of the patients with cirrhosis (n=24) responded to interferon alfa, while in 80% of the patients with chronic hepatitis (n=76) aminotransferase activities returned to normal ($p<0{\cdot}001$). The response rate among patients <45 years (n=39) was 92%, while it decreased to 56% in patients >45 years (n=61) ($p<0{\cdot}001$). Patients with γGT values ≤0·66 μkat/l had a response rate of 78%, while response was obtained in only 60% of the patients with higher γGT values ($p=0{\cdot}048$). All patients (n=23) without cirrhosis, <40 years, and with γGT values <0·66 μkat/l responded to interferon alfa while only 64% of non-cirrhotic patients, >53 years or with γGT values >1·00 μkat/l (n=28), or both responded to this treatment. In contrast, the response rate was only 28·5% among patients with cirrhosis, >40 years and with γGT values >0·66 μkat/l (n=14).

When univariate analysis was applied to the patients divided by liver histological tests into

TABLE I *Univariate analysis of pretreatment variables*

Variable	*Responders (n=70)*	*Non-responders (n=30)*	*p Value*
Age (y)*	42·7	53·5	<0·001
Sex (M/F)	46/24	13/17	0·04
Obesity (%)	14	33	0·03
Acute hepatitis (%)	30	27	NS
Transfusions (%)	53	40	NS
Time of evolution (months)*	46·6	58·7	NS
ALT (μkat/l)*[a]†	2·66	2·36	NS
Alkaline phosphatase (μkat/l)*[b]†	2·61	2·36	NS
γGT (μkat/l)*[c]†	0·61	0·91	0·003
Neutrophils (10^9/l)*	3·08	2·70	NS
Platelets (10^9/l)*	179	152	0·02
Albumin (g/l)*	42·9	41·1	NS
γ Globulins (g/l)*	13·7	15·3	NS
PIIIP (ng/ml)*[d]†	18·7	21·9	NS
ANA positive (%)	9	3	NS
SMA positive (%)	17	13	NS
Chronic hepatitis/cirrhosis	61/9	15/15	<0·001
Fatty degeneration (%)	34	50	NS

*Results presented as means. †Normal ranges: (a) 0·10–0·48 μkat/l, (b) 1·21–3·45 μkat/l, (c) 0·08–0·63 μkat/l, (d) 6–12 ng/mL. ALT=alanine aminotransferase; γGT=glutamyltransferase; PIIIP=procollagen aminoterminal peptide; ANA=anti-nuclear antibodies; SMA=anti-smooth muscle antibodies.

TABLE II *Univariate analysis of pretreatment variables in patients with chronic hepatitis*

Variable	Responders (n=61)	Non-responders (n=15)	p Value
Age (y)*	40·9	51·6	0·008
Sex (M/F)	40/21	6/9	NS
Obesity (%)	16	47	0·01
Acute hepatitis (%)	33	20	NS
Transfusions (%)	56	40	NS
Time of evolution (months)*	42·9	59·0	NS
ALT (μkat/l)*	2·66	2·38	NS
Alkaline phosphatase (μkat/l)*	2·55	2·16	NS
γGT (μkat/l)*	0·56	0·90	0·03
Neutrophils (10^9/l)*	3·12	2·92	NS
Platelets (10^9/l)*	188	158	0·05
Albumin (g/l)*	43·3	42·7	NS
γ Globulins (g/l)*	13·5	13·4	NS
PIIIP (ng/ml)*	17·8	16·6	NS
ANA positive (%)	7	7	NS
SMA positive (%)	16	7	NS
Fatty degeneration (%)	34	47	NS

*Results presented as means.
Abbreviations the same as in Table I.

two groups (chronic hepatitis/cirrhosis), among patients with cirrhosis (n=24), none of the variables predicted response, while among patients without cirrhosis (n=76), young age, absence of obesity, low γGT values, and a high platelet count were all associated with response (Table II).

Neutralising anti-lymphoblastoid interferon alfa antibodies were detected in only one of 15 patients tested who did not respond (7%).

STEPWISE MULTIPLE LINEAR REGRESSION ANALYSIS

Stepwise multiple regression analysis showed that γGT values (p<0·001), obesity (p=0·005) and histological tests (p=0·01) were the only independently predictive variables. Low γGT values, the absence of obesity, and the absence of cirrhosis were all independently associated with a positive response to interferon alfa. The remaining pretreatment variables found predictive on univariate analysis added no further improvement in discrimination. Total body weight did not influence the response to treatment because the mean weight of responders and non-responders was similar (kg; responders: mean (SEM) 66·9 (12·7), non-responders: 67·5 (9·2)). Therefore, it was obesity itself, rather than total weight, which seemed to influence response.

Stepwise multiple regression analysis was also applied to the group of patients with cirrhosis (n=24) without finding significantly predictive variables, although obesity, fatty degeneration of hepatocytes, and high γ globulin values tended to correlate with non- response. None of the responders were obese while 20% of the non-responders were obese; fatty degeneration was present in 33% of the responders and in 53% of the non-responders; mean ((SD), g/l) γ globulin values in responders were: 15·1 (4·9), compared with 17·3 (4·8) in non-responders. Among patients without cirrhosis (n=76) a low γGT value (p<0·001), the absence of obesity (p=0·008), and a high platelet count (p=0·02) were independently associated with response to interferon alfa.

Discussion

It has not been clearly established if pretreatment variables that might predict the response of chronic hepatitis C to interferon alfa do exist.[12] Some studies did not find predictive variables,[1 2 5 7] while others gave quite disparate results.[8-12 17] These discrepancies may be attributed to the small number of patients included in some studies, to different criteria for defining response, and to shortcomings in the statistical analyses. Some of these analyses were only univariate. Moreover, some studies have compared patients treated with different doses of interferon alfa and it has been established that different schedules of interferon alfa may achieve different response rates.[3] In this study, we clearly show, in a large series of patients consecutively treated with the same schedule of interferon alfa, that some pretreatment variables may independently predict response to this treatment. The criteria we have used for the definition of response were intended to assess the ability of interferon alfa to achieve a clear cut biochemical response, which is usually associated with a transient or sustained clearance of serum HCV RNA.[18] Spontaneous normalisations of ALT activities have only been seen in about 1% of controls in randomised trials.[6] Relapses during treatment, which were seen in 15 patients when receiving the lower doses of interferon alfa (1·5 MU, three times per week), and relapses occurring after interferon alfa was stopped were not taken into account.

The finding that most young patients (<45 years) without cirrhosis will respond to treatment (95%), while only one third (36%) of the patients >45 years and with established cirrhosis will benefit from treatment is clinically relevant. This finding emphasises the need for early treatment in chronic hepatitis C. In addition, this might explain the discrepancies in the response rates among different clinical trials, which could be because of differences in the characteristics of the populations studied. The reason why γGT values influence response to interferon alfa is not known. It could be speculated that a rise in γGT values might merely reflect an as yet undefined change in liver function, which might somehow impair response to treatment. In fact some preliminary studies have suggested that ursodeoxycolic acid, which decreases γGT and alkaline phosphatase values in some cholestatic liver diseases, might enhance interferon alfa activity in chronic hepatitis C.[19] A preliminary report has also found γGT values to be the best predictor of response to interferon alfa.[20] Unfortunately, most studies have not evaluated the influence of this variable.

The reason why patients with cirrhosis do not respond as well to interferon alfa as patients without cirrhosis is unknown. It can be speculated, however, that host and virological factors could both influence response to interferon alfa. Cirrhosis has been associated with immune changes[21 22] and it is usually seen after many years of infection. Immune selected HCV mutants resistant to interferon alfa treatment might appear in cases with a long evolution of the disease.[23 24] It has been shown that HCV, like most RNA viruses, circulates as a population

of closely related but none the less distinct genomes, a finding that implies a high capacity to generate escape mutants.[25] In addition, cirrhosis may change the pharmacokinetics of some drugs and that could be the case with interferon.

The mechanism by which obesity may adversely influence response also remains unknown. One study found significant differences in the mean weight between responders and non-responders.[26] We found no differences, however, when assessing the total body weight. Interferon alfa bioavailability could be diminished in obese patients because of a decrease in the absorption of the drug when the subcutaneous route is used, as in this study. Other studies have shown that the skinfold thickness inversely correlates with the subcutaneous absorption of some drugs.[27] Pharmacokinetic studies should solve this issue. Meanwhile, we would favour the intramuscular route in obese patients.

Neutralising anti-lymphoblastoid interferon alfa antibodies do not seem to play a significant part in non-response to treatment, as they were detected in only one of 15 non-responders tested. This low frequency of detection is in accordance with a recent study, which detected neutralising antibodies in one of 78 treated patients, and contrasts with the higher frequencies seen with recombinant interferon alfa preparations.[28]

We thank Dr Ignacio Monreal and Carmen de Carlos for performing the PIIIP RIA, and the Department of Pathology (Clínica Universitaria, Pamplona, Spain) for examining the liver biopsy specimens. We are also grateful to Dr Juan I Esteban for helpful comments on the manuscript. This work was supported in part by the Fundación Ramón Areces, Spain, and by a grant from the Comisión Interministerial de Ciencia y Tecnología (SAL90-0625), Spain.

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