Interferon responsiveness in patients infected with hepatitis C virus 1b differs depending on viral subtype

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Abstract

***Background*—Genotype 1b of hepatitis C virus (HCV) comprises mainly three subtypes, each named for its geographic prevalence (worldwide, W; Japan, J; and not in Japan, NJ).**

***Aim*—To characterise the newly identified subtypes of genotype 1b and to review factors associated with response to interferon (IFN) for each subtype.**

***Patients*—Chronic hepatitis patients (80 men and 41 women; mean age 48.5 years, range 20.7–69.3) with HCV genotype 1b (W type, n=41; J type, n=38) or genotype 2a (n=42) were treated according to the same IFN protocol. Forty four patients (36.4%) negative for serum HCV RNA six months after cessation of treatment were considered complete responders.**

***Methods*—Factors associated with complete response were investigated.**

***Results*—Genotype 2a patients had lower viral loads (odds ratio 0.11 (95% confidence intervals (CI) 0.049–0.256)) and a better IFN response (odds ratio 0.25 (95% CI 0.117–0.552)) than genotype 1b patients whereas W type and J type patients had similar viral loads and responses to IFN. IFN response in W type patients was associated with female sex (odds ratio 0.23 (95% CI 0.055–0.983)) and low viral load (odds ratio 84.00 (95% CI 14.04–502.6)) whereas response in J type patients was related to transfusion history (odds ratio 7.20 (95% CI 1.443–35.91)), low viral load (odds ratio 117.0 (95% CI 17.82–768.3)), and genetic mutation in the interferon sensitivity determining region of the virus (odds ratio 0.08 (95% CI 0.013–0.553)). Multivariate analysis found low viral load (odds ratio 64.19 (95% CI 14.66–281.06)) to be the only significant independent factor associated with IFN response.**

***Conclusions*—Factors associated with IFN responsiveness in HCV infection differ with viral subtype.**

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Keywords: hepatitis C virus; genotype 1b; chronic hepatitis; interferon therapy; interferon sensitivity determining region

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Hepatitis C virus (HCV), a major cause of chronic liver disease worldwide, is presently classified into six major types and at least 52 variants based on nucleotide sequences.[1] The geographic distribution of HCV genotypes varies; genotype 1b is found worldwide and in certain areas it is predominant. Although interferon (IFN) is the most successful therapeutic agent in chronic hepatitis C, less than half of patients treated with IFN show sustained response with eradication of the virus. Several pretreatment characteristics, including younger age,[2] female sex,[3] low pretreatment HCV RNA level,[4] absence of fibrosis or cirrhosis,[5] higher or longer doses of IFN,[6] and non-type 1b viral genotype[7] have been associated with an increased response to IFN. Some of these associations are controversial but the most consistently identified independent predictors of response are pretreatment viral levels and viral genotype. IFN treatment is expensive and causes severe side effects; HCV genotype 1b is the most resistant to therapy, with rates as low as 10–30% for successful response (eradication of virus). Thus prediction of response to IFN is especially meaningful for patients with HCV genotype 1b.

A portion of the amino acid sequence of the non-structural (NS) 5A segment in HCV genotype 1b, termed the interferon sensitivity determining region (ISDR), was initially reported in Japan to correlate with responsiveness to IFN.[8] Subsequent studies in Europe failed to confirm this association[9 10] however and the usefulness of ISDR type as a predictor of IFN responsiveness has remained questionable. In our previously reported study,[11] phylogenetic comparisons between HCV 1b isolates in GenBank revealed genotype 1b to consist of three main subpopulations, each showing a particular geographic prevalence: J type, found particularly in Japan; NJ type, not often found in Japan; and W type, distributed worldwide. HCV 1b isolates from Japanese hepatitis patients are predominantly of the W (approximately 60%) and J (approximately 40%) types. A comparison of IFN responsiveness between these two subtypes revealed differences in the ISDR region which could explain the controversial differences in response to IFN reported in Japan and Europe. Thus genotype 1b is likely to be heterogeneous not only geographically but also pathogenetically. The aim of the present study was to characterise these newly identified subtypes and to investigate the predictive factors for IFN response, taking these subtypes into consideration.

Abbreviations used in this paper: HCV, hepatitis C virus; IFN, interferon; NS, non-structural; ISDR, interferon sensitivity determining region; RT-PCR, reverse transcriptase-polymerase chain reaction; HAI, histological activity index.

Materials and methods

PATIENTS

Over a five year period, 139 chronic hepatitis C patients were started on IFN therapy. Patients were treated intramuscularly with 9 MU of IFN-alpha daily for two weeks and then three times a week for 22 weeks. We considered "complete responders" to therapy as those patients who had sustained normal alanine transaminase levels and tested negative for HCV-RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) for six months after cessation of therapy; all others were considered "non-responders". Patients were excluded from the study for the following reasons: infection with HCV subtypes rarely seen in Japan (four patients with genotype 2b, three with subtype NJ) or if the subtype could not be determined (two patients); coinfection with human immunodeficiency virus or hepatitis B virus; evidence of drug induced or autoimmune hepatitis; current alcohol intake more than 40 g daily; evidence of cirrhosis; inability to complete treatment due to severe side effects; or if the final response to IFN could not be determined. Thus 80 men and 41 women with a mean age of 48.5 years (range 20.7–69.3) were enrolled in the study. Seventy nine of these patients had HCV genotype 1b (W type, n=41; J type, n=38) and 42 had HCV genotype 2a. Forty three patients (35.5%) had a history of blood transfusion. The ISDR sequence in genotype 1b patients was determined to be the wild-type in 40 patients and the mutant-type in 39.

METHODS

Serum HCV RNA was detected by RT-PCR using a commercial kit (Amplicor HCV; Roche Diagnostics, Branchburg, New Jersey, USA), and RNA was quantified by branched DNA probe assay (Quantiplex HCV-RNA, version 2.0; Chiron, Emeryville, California, USA) according to the manufacturer's instructions. HCV genotyping was carried out with a second generation reverse hybridisation, line probe assay (Inno-LiPA HCV II; Innogenetics, Ghent, Belgium) according to the manufacturer's instructions. Genotype 1b subtyping was performed as reported previously.[11] Briefly, after extraction of RNA from patient sera, the partial envelope 1 region of HCV was amplified by nested RT-PCR with Smith's primer sets.[12] PCR products were directly sequenced by an autosequencer. The obtained sequences (nucleotides 982–1237) were utilised for subtype determination by calculating the subtype score as described below. Phylogenetic tree analysis among HCV 1b isolates in GenBank had already revealed eight nucleotide residues that differed by subtype. The subtype score of the obtained sequences was determined by counting the nucleotide numbers identical to those specific for each subtype, and the isolate was classified accordingly. This method is consistent with phylogenetic tree analysis.[11] The ISDR type was determined according to ISDR sequences obtained as described previously.[13] A portion of NS5A including ISDR was amplified by nested RT-PCR using Enomoto's primer sets,[13] and the ISDR amino acid sequence identical to the prototype HCV-J strain was considered to be the wild-type. Liver biopsy was performed in all patients just before treatment. Histological assessment was made based on the method of Desmet and colleagues[14] and involved the independent grading and staging categories. Components 1, 2, and 3 of Knodell's histological activity index (HAI)[15] were totalled to assess grade of inflammation (grading score), and the stage of fibrosis was also determined based on the Desmet system (staging score).[14]

STATISTICAL ANALYSIS

Statistical analysis for categorical group comparisons was performed by χ^2 test without Yates' correction and by Fisher's exact test. Multivariate logistic regression was performed to determine the independent association of each factor with response to IFN. Data are expressed as mean (range) or odds ratio (95% confidence intervals (CI)); p values less than 0.05 were considered statistically significant. A statistical software package, Statview 5.0 (SAS Institute Inc, Cary, North Carolina), was used.

Results

PATIENT CHARACTERISTICS AND VIRAL SUBTYPES (TABLE 1)

There were no significant differences in sex or age between patients infected with the three types (W type, J type, and genotype 2a). The grading score for genotype 1b patients was significantly higher than that for genotype 2a patients (p=0.014) due to the apparently higher score for W type patients than that for J type patients (p=0.010). No difference in staging score was detected between genotype 2a and 1b patients, or between subtype W and J

Table 1 Clinical, histological, and virological factors, and interferon responsiveness according to hepatitis C virus genotype and subtype

	Genotype 1b			*W type v J type*			*Genotype 1b v 2a*		
	W type (n=41)	*J type (n=38)*	*Genotype 2a (n=42)*	*Odds ratio*	*95% CI*	*p Value*	*Odds ratio*	*95% CI*	*p Value*
Sex (M/F)	27/14	25/13	28/14	1.00	0.395–2.543	0.995	0.96	0.436–2.127	0.926
Age (SD) (y)	46.05 (13.18)	50.00 (9.33)	49.43 (8.46)			0.122			0.423
Transfusion history (+/−)	20/21	18/20	5/37	1.06	0.437–2.560	0.900	6.86	2.645–17.787	0.0001
Grading score* (1–8/9–18)	13/28	23/15	29/13	0.30	0.122–0.753	0.010	0.38	0.172–0.818	0.014
Staging score** (0–1/2–4)	20/21	22/16	28/14	0.69	0.285–1.682	0.417	0.57	0.261–1.232	0.152
HCV RNA (<1.0/≥1.0 ($\times 10^6$ eq/ml))	12/29	11/27	33/9	1.02	0.384–2.684	0.975	0.11	0.049–0.256	<0.0001
ISDR (wild/mutant)	23/18	17/21		1.58	0.650–3.830	0.313			
IFN response (CR/NR)	10/31	10/28	24/18	0.90	0.328–2.491	0.844	0.25	0.117–0.552	0.0005

*Grading score is a total of components 1, 2, and 3 of the histological activity index (HAI) score according to Knodel and colleagues.[15]
**Staging score is according to Desmet and colleagues.[14]
HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; IFN, interferon; CR, complete responder; NR, non-responder.

Table 2 Univariate analysis of clinical, histological, and virological factors for responsiveness to interferon

	CR (n=44)	*NR (n=77)*	*Odds ratio*	*95% CI*	*p Value*
Sex (M/F)	27/17	53/24	0.72	0.332–1.559	0.404
Age (<50/≥50 (y))	22/22	34/43	1.26	0.602–2.656	0.535
Transfusion history (+/−)	16/28	27/50	1.06	0.489–2.291	0.886
Grading score* (1–8/9–18)	25/19	40/37	1.22	0.578–2.564	0.605
Staging score** (0–1/2–4)	26/18	44/33	1.08	0.511–2.297	0.835
HCV RNA (<1.0/≥1.0 ($\times 10^6$ eq/ml))	41/3	15/62	56.49	20.556–155.233	<0.0001
HCV subtype (W/J/2a)	10/10/24	31/28/18			0.002
ISDR (wild/mutation) (genotype 1b only, n=79)	5/15	35/24	0.23	0.077–0.680	0.008

*Grading score is a total of components 1, 2, and 3 of the histological activity index (HAI) score according to Knodel and colleagues.[15]
**Staging score is according to Desmet and colleagues.[14]
CR, complete responder; NR, non-responder; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region.

patients. Patients with a blood transfusion history were more closely associated with genotype 1b, irrespective of subtype (W *v* J). Forty four (36.4%) of the total were complete responders (20 (25.3%) of 79 genotype 1b patients and 24 (57.1%) of 42 genotype 2a patients). Genotype 2a patients had lower viral loads (p<0.0001) and responded to IFN better (p=0.0005) than 1b patients. W and J type patients showed similar viral loads and IFN responsiveness. The prevalence of the ISDR mutation was also similar between W and J type patients.

VARIABLES ASSOCIATED WITH IFN RESPONSIVENESS (TABLE 2)

Sex, age, history of transfusion, and histological scores did not correlate with response to IFN. High levels of HCV RNA (p<0.0001), wild-type ISDR (p=0.0080), and genotype 1b (p=0.0005) (table 1) were closely associated with poor response to IFN.

IFN RESPONSIVENESS AND VIRAL SUBTYPES (TABLE 3)

Women with W type viruses had a better IFN response than men with W type viruses, and W type patients had a better IFN response if low levels of serum HCV RNA were present. In contrast, J type patients had better responses when there was a history of blood transfusion, low levels of HCV RNA, or mutant ISDR. Although W and J type viruses are of the same genotype, the two subtypes clearly showed different responses to IFN that were dependent on sex, transfusion history, or ISDR type. Genotype 2a patients responded to IFN only if they had low levels of HCV RNA.

MULTIVARIATE ANALYSIS FOR FACTORS ASSOCIATED WITH IFN RESPONSIVENESS (TABLE 4)

In genotype 1b patients, viral load was the only factor independently and significantly associated with IFN responsiveness (odds ratio 126.4 (95% CI 15.3–1043)). Neither ISDR mutation

Table 3 Interferon responsiveness (represented by the case number of complete responders/non-responders) in three different viral types (W, J, and genotype 2a) by univariate analysis according to clinical, histological, and virological variants

Sex	*Male (n=80)*	*Female (n=41)*	*Odds ratio*	*95% CI*	*p Value*
W type	4/23	6/8	0.23	0.055–0.983	0.047
J type	6/19	4/9	0.71	0.160–3.152	0.468
2a	17/11	7/7	1.55	0.425–5.614	0.508
Age (y)	*<50 (n=56)*	*≥50 (n=65)*	*Odds ratio*	*95% CI*	*p Value*
W type	6/17	4/14	1.24	0.290–5.257	0.775
J type	6/11	4/17	2.32	0.540–9.954	0.258
2a	10/6	14/12	1.43	0.401–5.088	0.582
Transfusion history	*Yes (n=43)*	*No (n=78)*	*Odds ratio*	*95% CI*	*p Value*
W type	5/15	5/16	1.07	0.256–4.438	0.929
J type	8/10	2/18	7.20	1.443–35.914	0.016
2a	3/2	21/16	1.14	0.170–7.662	0.891
*Grading score**	*1–8 (n=65)*	*9–18 (n=56)*	*Odds ratio*	*95% CI*	*p Value*
W type	4/9	6/22	1.63	0.372–7.137	0.517
J type	4/19	6/9	0.32	0.073–1.360	0.122
2a	17/12	7/6	1.21	0.326–4.530	0.773
*Staging score***	*0–1 (n=70)*	*2–4 (n=51)*	*Odds ratio*	*95% CI*	*p Value*
W type	5/15	5/16	1.07	0.256–4.438	0.929
J type	5/17	5/11	0.65	0.152–2.754	0.556
2a	16/12	8/6	1.00		1.000
HCV RNA ($\times 10^6$ eq/ml)	*<1.0 (n=56)*	*≥1.0 (n=65)*	*Odds ratio*	*95% CI*	*p Value*
W type	9/3	1/28	84.00	14.039–502.613	<0.0001
J type	9/2	1/26	117.00	17.817–768.332	<0.0001
2a	23/10	1/8	18.40	3.002–112.789	0.0016
ISDR	*Wild-type (n=40)*	*Mutant-type (n=39)*	*Odds ratio*	*95% CI*	*p Value*
W type	4/19	6/12	0.42	0.100–1.772	0.238
J type	1/16	9/12	0.08	0.013–0.553	0.010

*Grading score is a total of components 1, 2, and 3 of the histological activity index (HAI) score according to Knodel and colleagues.[15]
**Staging score is according to Desmet and colleagues.[14]
HCV, hepatitis C virus; ISDR, interferon sensitivity determining region.

Table 4 Multivariate analysis of clinical, histological, and virological factors in relation to poor interferon response

	W and J type HCV patients (n=79)			*Genotype 1b and 2a HCV patients (n=121)*		
	Odds ratio	*95% CI*	*p Value*	*Odds ratio*	*95% CI*	*p Value*
Sex (male)	2.186	0.326–14.666	0.421	1.229	0.389–3.882	0.725
Age (y)	0.999	0.920–1.085	0.806	1.019	0.966–1.076	0.570
Transfusion history (positive)	0.526	0.074–3.766	0.522	0.794	0.224–2.812	0.720
Grading score*	1.212	0.020–2.307	0.203	0.519	0.129–2.079	0.354
Staging score**	3.142	0.287–34.403	0.348	1.401	0.363–5.409	0.625
HCV RNA ($\geq 1.0\times10^6$ eq/ml)	126.423	15.322–1043.138	<0.0001	64.289	14.660–281.064	<0.0001
ISDR mutant (positive)	0.452	0.070–2.914	0.403			
W/J subtype (W type)	1.415	0.234–8.543	0.705			
Genotype (2a)				0.971	0.257–3.658	0.965

*Grading score is a total of components 1, 2, and 3 of the histological activity index (HAI) score according to Knodel and colleagues.[15]
**Staging score is according to Desmet and colleagues.[14]
HCV, hepatitis C virus; ISDR, interferon sensitivity determining region.

nor W/J type was a significant independent factor for IFN responsiveness. HCV RNA level was the only significant predictor of IFN responsiveness (odds ratio 64.3 (95% CI 14.7–281.1)) in all patients; genotype (1b or 2a) was not associated with response.

Discussion

In the present study, 36.4% of all patients (25.3% for genotype 1b, 57.1% for genotype 2a) showed complete response to IFN therapy. In two previously reported large scale studies in Japan, in which similar high doses of IFN therapy were used, 38% (29% for genotype 1b, 76% for genotype 2)[16] and 28.4% (18.7% for serotype 1, 60.6% for serotype 2)[17] of patients were considered to be complete responders. Our data were similar. Our IFN regimen (9 MU daily for the initial two weeks and then three times a week for 22 weeks) is a typical protocol in Japan. A lower dose regimen (3 MU three times a week for six months) is more typical worldwide. A large scale meta-analysis of randomised IFN trials by Poynard and colleagues[18] revealed significant effects of dose and duration on sustained response. In their series, 18% of patients treated with 3 MU IFN three times a week for six months but 28% of patients treated with 6 MU three times a week for six months were sustained responders. As our regimen involved still higher doses of IFN, a slightly higher rate of complete responses was obtained.

In comparison with genotype 2a patients, our genotype 1b patients had greater viral loads, a lower response to IFN, and were more likely to have a transfusion history (table 1). This is consistent with previously reported findings.[19–21] In our series, the response to IFN was significantly correlated with low HCV RNA load, genotype 2a, and ISDR mutation (table 2). Low viral load and genotype 2a were also correlated in other studies[4 7 22 23] but data for ISDR type were not necessarily consistent with ours. Association between ISDR type and IFN responsiveness is observed only in Japan; the disparity may result from the geographic heterogeneity of HCV genotype 1b subtypes, as we noted in our previous report.[11] In Europe, where genotype 1b is as predominant as in Japan, the isolates consist mainly of the W (approximately 50%) and NJ (approximately 50%) types. As the correlation between ISDR type and IFN response is likely to be seen only in patients with Japan specific J type isolates, rare occurrence of this subtype in Europe explains the lack of usefulness of ISDR as a predictive marker of IFN response. Thus different characteristics of the 1b subtypes may explain the previous discrepant observations concerning HCV genotype 1b.

Patients with W and J type HCV were similar in their clinical backgrounds—that is, sex, age, history of blood transfusion, viral load, ISDR mutation rate, and IFN responsiveness. They differed in the histological grading severity of hepatitis however (table 1). As the W viral subtype is associated with the highest grading score among the three viral types in the present study, type W patients may progress more rapidly to cirrhosis or earlier to hepatocellular carcinoma. An association with rapid progression of liver disease has already been reported for genotype 1b in many countries[24–27] although this is still controversial.

Whether female sex is a positive factor for complete response to IFN is also arguable. Several reports from France,[28] Greece,[29] Sweden,[30] and the USA[31] have suggested female sex to be a predictive factor for complete response, but other studies, including several in Japan,[32–34] have failed to find an association. In the present study of Japanese patients, sex was not related to overall treatment response. Once we focused on the W type virus however which is prevalent worldwide, female sex became a weak but significant predictor of successful IFN response (table 3). Therefore, factors independently associated with IFN responsiveness may vary based on virus genotype or subtype, and this may explain the discrepant results between studies in Japan and elsewhere.

Whether the mode of transmission is related to IFN responsiveness has been intensely debated. Most reports comparing IFN responsiveness against mode of transmission (transfusion acquired, by intravenous drug use, or community acquired) have failed to find a significant association.[33 34] One report from Israel noted that the transfusion acquired virus was closely associated with increased rates of remission in cases of IFN treatment.[35] Another report implicated transfusion with increased viral load which could correlate with decreased response to IFN.[36] We observed no association between transfusion history and IFN response in our group as a whole. With J type HCV 1b infected patients however a strong correlation

existed. We cannot account for this based on our current findings.

Viral load was highly correlated with IFN responsiveness for all three viral types (table 3), and viral load is the best predictive marker for IFN responsiveness. Multivariate analysis confirmed this (table 4). On the other hand, transfusion history of J type patients and ISDR mutation of the J type virus were closely correlated with IFN response in a univariate analysis (p=0.016 and p=0.0101, respectively) whereas those of the W type were not. Thus in patients with the J type virus, these two factors as well as viral load may be the most useful predictive markers of IFN response. Combined analysis with several factors that were associated with IFN response may provide for more precise prediction of IFN response. Our observation suggests that factors appropriate for each viral type should be considered for prediction of IFN response

1 Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. *Semin Liver Dis* 1995;**1**:41–63.
2 Schlaak JF, Trippler M, Ernst I, *et al.* Chronic hepatitis C: the viral load per liver cell before treatment as a new marker to predict long-term response to IFN-alpha therapy. *J Hepatol* 1997;**27**:917–21.
3 Chemello L, Cavalletto L, Noventa F, *et al.* Predictors of sustained response, relapse and no response in patients with chronic hepatitis C treated with interferon-alpha. *J Viral Hepatitis* 1995;**2**:91–6.
4 Takagi H, Takehara K, Shimoda R, *et al.* Prediction of effect of interferon on chronic hepatitis C. *Dig Dis Sci* 1997;**42**:2270–6.
5 Kaseres K, Fiedler R, Steindl P, *et al.* Liver biopsy is a useful predictor of response to interferon therapy in chronic hepatitis C. *Histopathology* 1998;**32**:454–61.
6 Bellobuono A, Mondazzi L, Tempini S, *et al.* Prospective comparison of four lymphoblastoid interferon alpha schedules for chronic hepatitis C. A multivariate analysis of factors predictive of sustained response to treatment. *Eur J Gastroenterol Hepatol* 1997;**9**:1169–77.
7 Manesis EK, Papaioannou C, Gioustozi A, *et al.* Biochemical and virological outcome of patients with chronic hepatitis C treated with interferon alpha-2b for 6 or 12 months: a 4-year follow-up of 211 patients. *Hepatology* 1997;**26**:734–9.
8 Enomoto N, Sakuma I, Asahina Y, *et al.* Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b-sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. *J Clin Invest* 1995;**96**:224–30.
9 Khorsi H, Castelain S, Wyseur A, *et al.* Mutations of hepatitis C virus 1b NS5A 2209–2248 amino acid sequence do not predict the response to recombinant interferon-alfa therapy in French patients. *J Hepatology* 1997;**27**:72–7.
10 Zeuzem S, Lee JH, Roth WK. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alpha. *Hepatology* 1997;**25**:740–4.
11 Nakano I, Fukuda Y, Katano Y, *et al.* Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? *J Hepatol* 1999;**30**:1014–22.
12 Smith DB, Pathirana S, Davidson F, *et al.* The origin of hepatitis C virus genotypes. *J Gen Virol* 1997;**78**:321–8.
13 Enomoto N, Sakuma I, Asahina Y, *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996;**334**:77–81.
14 Desmet VJ, Gerber M, Hoofnagle JH, *et al.* Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994;**19**:1513–20.
15 Knodel RG, Ishak KG, Black WC, *et al.* Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;**1**:431–5.
16 Shiratori Y, Kato N, Yokosuka O, *et al.* Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. *Gastroenterology* 1997;**113**:558–66.
17 Kasahara A, Hayashi N, Mochizuki K, *et al.* Risk factors for hepatocellular carcinoma and its incidence after treatment in patients with chronic hepatitis C. *Hepatology* 1998;**27**:1394–402.
18 Poynard T, Leroy V, Cohard M, *et al.* Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effects of dose and duration. *Hepatology* 1996;**24**:778–89.
19 Kleter B, Brouwer JT, Nevens F, *et al.* Hepatitis C virus genotypes: epidemiological and clinical associations. Benelux study group on treatment of chronic hepatitis C. *Liver* 1998;**18**:32–8.
20 Dubois F, Desenclos JC, Mariotte N, *et al.* Hepatitis C in a French population-based survey, 1994: seroprevalence, frequency of viremia, genotype distribution, and risk factors. The collaborative study group. *Hepatology* 1997;**25**:1490–6.
21 Watson JP, Brind AM, Chapman CE, *et al.* Hepatitis C virus: epidemiology and genotypes in the north of England. *Gut* 1996;**38**:269–76.
22 Zein NN, Rakela J, Krawitt EL, *et al.* Hepaittis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. *Ann Intern Med* 1996;**125**:634–9.
23 Liang TJ, Rehermann B, Seeff LB, *et al.* Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann Intern Med* 2000;**132**:296–305.
24 Kobayashi M, Tanaka E, Sodeyama T, *et al.* The natural course of chronic hepatitis C: a comparison between patients with genotype 1 and 2 hepatitis C viruses. *Hepatology* 1996;**23**:695–9.
25 Poynard T, Bedossa P, Opolon P, *et al.* Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997;**349**:825–32.
26 Nousbaum JB, Pol S, Nalpas B, *et al.* Hepaittis C virus type 1b (II) infection in France and Italy. *Ann Intern Med* 1995;**122**:161–8.
27 Lopez-Labrador FX, Ampurdanes S, Forns X, *et al.* Hepatitis C virus (HCV) genotypes in Spanish patients with HCV infection: relationship between HCV genotype 1b, cirrhosis and hepatocellular carcinoma. *J Hepatol* 1997;**27**:959–65.
28 Izopet J, Payen JL, Alric L, *et al.* Baseline level and early suppression of serum HCV RNA for predicting sustained complete response to alpha-interferon therapy. *J Med Virol* 1998;**54**:86–91.
29 Tassopoulos NC, Karvountzis G, Touloumi G, *et al.* Comparative efficacy of a high or low dose of interferon alpha 2b in chronic hepatitis C: a randomized controlled trial. *Am J Gastroenterol* 1996;**91**:1734–8.
30 Weiland O. Interferon therapy in chronic hepatitis C virus infection. *FEMS Microbiol Rev* 1994;**14**:279–88.
31 Alvarez F. Therapy for chronic viral hepatitis. *Clin Inv Med* 1996;**19**:381–8.
32 Akiyoshi F, Seta M, Uchimura Y, *et al.* Hepatic iron strainings in chronic hepatitis C patients with low HCV RNA levels: a predictive marker for IFN therapy. *Am J Gastroenterol* 1997;**92**:1463–6.
33 Hayashi J, Kishihara Y, Tani Y, *et al.* Effectiveness of relative low-dose interferon treatment for patients with chronic hepatitis C. *Fukuoka Acta Medica* 1994;**85**:211–18.
34 Mochida S, Ohnishi K, Matsuo S, *et al.* Effect of alcohol intake on the efficacy of interferon therapy in patients with chronic hepatitis C as evaluated by multivariate logistic regression analysis. *Alcoholism: Clin Exp Res* 1996;**20**(suppl 9):371–7A.
35 Halpern Z, Arber N, Hallak A, *et al.* Post-transfusion etiology and non-cirrhotic histology improve the remission rate of chronic hepatitis C during interferon treatment. *Isr J Med Sci* 1995;**31**:341–4.
36 Lau JY, Davis GL, Kniffen J, *et al.* Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. *Lancet* 1993;**342**:504.