# The natural course of hepatitis C virus infection after 22 years in a unique homogenous cohort: spontaneous viral clearance and chronic HCV infection

S Barrett, J Goh, B Coughlan, E Ryan, S Stewart, A Cockram, J C O'Keane, J Crowe

**Abstract**

***Background/aims*—The cohort of Irish women infected with hepatitis C virus (HCV) genotype 1b via contaminated anti-D immunoglobulin in 1977 represent a unique homogenous group to investigate the natural course of HCV infection.**

***Methods*—The clinical status of 87 polymerase chain reaction (PCR) positive and 68 PCR negative women was investigated at diagnosis (1994/95) and after 4–5 years of follow up (21/22 years after inoculation). Other features investigated included: histological status/progression, psychosocial impact of HCV infection, extrahepatic manifestations, and HLA class II associations.**

***Results*—The most common symptoms reported were fatigue and arthralgia. Furthermore, 77% of women fell within the clinical range for psychological distress. A history of icteric hepatitis was reported in 20.6% of PCR negative and 3.4% of PCR positive women after inoculation (p=0.002). The mean histological activity index/fibrosis scores of PCR positive and negative women were 4.1 (1.4)/1.1 (1.3) and 2.1 (1.5)/0.15 (0.36) at diagnosis and 4.1 (1.2)/1.0 (1.0) in 44 PCR positive women after five years of follow up. Cirrhosis or hepatocellular carcinoma was not observed. The DRB1*01 allele was present in 28.8% of PCR negative and 8.7% of PCR positive women (p=0.004). The prevalence rates of mixed cryoglobulinaemia, sicca complex, positive thyroid autoantibodies, antinuclear antibody, rheumatoid factor, and antimitochondrial antibody in PCR positive women were 12.7%, 7.6%, 13.9%, 5.1%, 3.8%, and 3.8%.**

***Conclusions*—A benign course of HCV infection with lack of disease progression was observed in women with chronic HCV, 22 years after inoculation. Acute icteric hepatitis and the HLA DRB1*01 allele were associated with viral clearance. Despite this favourable outcome, high levels of psychological distress and poor quality of life were present.**

(*Gut* 2001;**49**:423–430)

Keywords: hepatitis C virus; natural history; anti-D spontaneous viral clearance

**Centre for Liver Diseases, Mater Misericordiae Hospital, Eccles St, Dublin 7, Ireland**
S Barrett†
J Goh†
B Coughlan
E Ryan
S Stewart
A Cockram
J Crowe

**Department of Pathology, Mater Misericordiae Hospital, Eccles St, Dublin 7, Ireland**
J C O'Keane

†Joint first authors.

Correspondence to:
Dr J Crowe. liver@mater.ie

Accepted for publication
16 February 2001

Ten years after the discovery of the hepatitis C virus (HCV) as a leading cause of chronic liver disease worldwide, knowledge of the natural history of the disease is still limited.[1] It is believed that up to 85% of infected individuals will develop chronic HCV infection which, depending on a number of factors and patient groups studied, may result in a variety of outcomes, ranging from asymptomatic to severe hepatitis, cirrhosis, and hepatocellular carcinoma.[2] It has also been estimated that approximately 15% of individuals have a spontaneous self limited infection but the exact rate of resolution is not known because of the difficulty in identifying individuals who have recovered from infection. There is now emerging evidence to suggest however that more individuals may spontaneously clear HCV than previously thought.[1–4] In a recent review on the natural history of infection, Alberti *et al* suggested other areas which require further investigation include the effects of chronic HCV on quality of life and the incidence of extrahepatic manifestations caused by HCV.[1]

Since patient groups are often of mixed sex, ethnic origin, genotype, mode of acquisition, and ill defined duration of disease, conflicting reports relating to outcome of infection may relate to the heterogeneity of the patient populations investigated. Seeff (1997) outlined the criteria for a truly informative natural history study but acknowledged it would be difficult, if not impossible, to adhere to all of these requirements.[2] The author suggested that rare events such as examination of outbreaks of past infection could provide valuable information on the natural history of HCV infection. Identification of a large cohort of women (almost half of whom have a spontaneous self limited infection) who received anti-D immunoglobulin contaminated with HCV in 1977 from a single infected donor provides a unique homogenous cohort to investigate the natural history of infection. Some clinical details of the women with chronic HCV infection have previously been presented.[5 6] The homogenous features of this unique cohort included: ethnic

**Abbreviations used in this paper**: HCV, hepatitis C virus; PCR, polymerase chain reaction; ALT, alanine aminotransferase; RIBA, recombinant immunoblot assay; HAI, histological activity index; GHQ, general health questionnaire; HADS, hospital anxiety and depression scale.

origin, sex, demographic characteristics, duration of disease, mode of acquisition, viral genotype (1b), and absence of other possible causes of liver disease. The confounding factor of treatment modifying natural history is also minimised as a minority of these patients received treatment.

The aim of this study was to investigate some of the unresolved issues regarding the natural history of HCV infection in a representative subgroup of women (with chronic infection or spontaneous self limited infection) derived from the Irish cohort. The clinical and histological status of these women was investigated at the time of diagnosis in 1994/95 and after 4–5 years of follow up (21/22 years after inoculation). Other features investigated included: symptomalogy, psychosocial impact of HCV infection, extrahepatic manifestations, and HLA class II associations.

## Methods

### PATIENTS

The discovery in 1994 that anti-D immunoglobulin contaminated with HCV had been administered to Rhesus negative women in 1977 (genotype 1b) and to a smaller group of women between 1991 and 94 (genotype 3) led to a national screening programme conducted by the Irish Blood Transfusion Service Board.[5] As a result of this programme, a large group of polymerase chain reaction (PCR) positive (n=413) and antibody positive PCR negative individuals (n=382) were identified as having received infected anti-D in 1977. All of these individuals were offered a referral to six designated liver centres for further evaluation; PCR positive patients were referred in 1994 and PCR negative patients in 1995.[5]

Women attending this liver centre were randomly referred from across Ireland and constitute a representative cross section of the total infected cohort. Only individuals who received HCV contaminated anti-D in 1977 were investigated in this study. These included 87 consecutive (PCR positive) women with chronic HCV infection and 68 (PCR negative) women considered to have a spontaneous self limited infection who were regularly reviewed in specially designated anti-D clinics. For the purpose of this study, PCR positive and negative patients were followed up for five and four years, respectively.

Thirteen of 87 patients with chronic infection met the criteria for treatment. The preliminary results of interferon monotherapy and combination therapy (interferon and ribavirin) among the Irish anti-D (genotype 1b) cohort using detectable HCV RNA, elevated alanine aminotransferase (ALT), moderate/severe necroinflammation, and/or fibrosis as the basis for treatment have been very disappointing.[7] Therefore, until more effective therapies become available, the consensus at this liver centre has been to treat only patients with moderate hepatitis and bridging fibrosis. Of the 13 patients who met these criteria, 11 were treated with interferon and ribavirin. None of these patients had a sustained response to treatment.

Serological testing for hepatitis B surface antigen and antibodies to hepatitis A were negative in all cases. Demographic data of both groups are summarised in table 1. Approval for all research studies was sought and obtained from the ethics committee at the Mater Misericordiae Hospital.

### VIROLOGICAL TESTING

The serological markers anti-hepatitis B surface antigen and antibodies to hepatitis A were determined by ELISA (Abbot Diagnostics, Germany). Anti-HCV was determined by third generation ELISA (Ortho HCV 3.0; Ortho Diagnostics Systems, UK) and confirmed using four antigen recombinant immunoblot assay (RIBA) (RIBA-3; Chiron Corporation, Emeryville, California, USA). The presence or absence of HCV RNA in serum was determined using a standardised qualitative PCR assay (Amplicor; Roche Diagnostics Systems, UK) and an inhouse nested PCR assay at the Virus Reference Laboratory (University College, Dublin, Ireland).

*Table 1 Demographic, clinical, and psychological data of PCR positive and negative patients*

| *Characteristic* | *PCR positive (n=87) (%)* | *PCR negative (n=68) (%)* | *Statistical analysis (t test or $\chi^2$)* |
|---|---|---|---|
| Age at diagnosis (mean (SD)) (y) | 45.7 (6.0) | 45.8 (4.9) | NS |
| Source of infection (HCV contaminated anti-D) | 87 (100) | 68 (100) | NS |
| Duration of infection (y) | 22–23 | 22–23 | NS |
| Alcohol >14 IU/week (%) | 5 (5.7) | 3 (4.4) | NS |
| No of dependents (mean; range) | 4.1 (1–10) | 4.4 (1–11) | NS |
| Marital status (%) | | | |
| Married | 78 (89.7) | 59 (86.8) | NS |
| Single | 2 (2.3) | 0 | NS |
| Separated | 4 (4.6) | 8 (11.8) | NS |
| Widowed | 3 (3.4) | 1 (1.5) | NS |
| Geographical region (%) | | | |
| Urban | 44 (50.6) | 28 (41.2) | NS |
| Rural | 43 (49.4) | 40 (58.8) | NS |
| ALT (IU/l) at diagnosis (mean (SD)) | 50.6 (31) | 25.1 (15.4) | p=0.000 |
| ALT (IU/l) after 4/5 years follow up (mean (SD)) | 51 (37.5) | 22.7 (6.5) | p=0.000 |
| History of icteric hepatitis (%) | 3 (3.4) | 14 (20.6) | p=0.002 |
| Psychological well being (GHQ30) (mean (SD)) | 15 (9.55) (n=43) | 13.5 (9.84) (n=23) | NS |
| Anxiety (HADS) (mean (SD)) | 9.8 (3.97) (n=43) | 9.1 (6.05) (n=23) | NS |
| Depression (HADS) (mean (SD)) | 7.6 (4.1) (n=43) | 7.1 (5.37) (n=23) | NS |

HCV, hepatitis C virus; PCR, polymerase chain reaction; ALT, alanine aminotransferase; GHQ, general health questionnaire; HADS, hospital anxiety and depression scale.

A nested PCR assay with an internal human albumin control was also used to assess intrahepatic HCV RNA status in 27 serum PCR negative individuals who had undergone liver biopsy.[8] The ability of the technique to amplify extracted HCV RNA from liver biopsy specimens was confirmed using liver biopsies from serum HCV RNA positive patients (n=10). Negative controls (liver biopsies from iron depleted haemochromatosis individuals or patients with autoimmune hepatitis (n=8), no template, no AMV transcriptase) were also included in test runs.[8]

HCV genotyping in those individuals with detectable viral RNA was also determined by means of PCR at the Virus Reference Laboratory. All women for whom viral typing or subtyping was available had a genotype of 1 or 1b.

### SYMPTOMATOLOGY

A symptom review was carried out on 62 (71%) PCR positive and 52 (76%) PCR negative patients. These symptoms included fatigue, arthralgia, myalgia, weight loss, rashes, eye problems, and bruising that were documented during clinical assessment at the first and subsequent follow up visits. Fatigue and arthralgia were the main symptoms reported.

### PSYCHOLOGICAL ASSESSMENT

Psychological well being, mental health, and quality of life were assessed in 43 (49%) PCR positive and 23 (34%) PCR negative women using Goldberg's general health questionnaire (GHQ30), Zigmond and Snaith's hospital anxiety and depression scale (HADS), and Jenkinson and Coulter's short form-36 health survey, respectively.[9–11]

### LIVER HISTOLOGY

Percutaneous liver biopsy was performed on all 87 (100%) PCR positive and 27 (40%) PCR negative patients at the time of initial diagnosis using the Trucut biopsy technique (Sterylab, Italy) following informed consent. A portion of each biopsy specimen was immediately snap frozen in liquid nitrogen and stored at −70°C. The remainder of the biopsy specimens were fixed in 10% neutral formalin and paraffin embedded for histological studies. Sections were cut to 4 μm thickness and stained with haematoxylin-eosin, Masson trichrome, reticulin, and Perl's stains. Histological evaluation was performed by an experienced histopathologist. As recommended by the International Association for the Study of the Liver, inflammation was graded using the modified histological activity index (HAI) on an 18 point scale and fibrosis was staged separately as 0–6.[12] The PCR positive scoring was validated by the use of study sets and joint reporting of every fifth biopsy by two pathologists as previously reported.[6]

As the current study population exhibited low HAI scores with a median score of 4, the necroinflammatory scores were arbitrarily divided into (1) mild activity (HAI $\leq 4$) and (2) moderate to severe activity (HAI $\geq 5$) for statistical analysis. Histological deterioration in the necroinflammatory scores was defined as progression from mild hepatitis to moderate/severe hepatitis on follow up liver biopsy. This was deemed to be a more accurate reflection of histological progression than for example a single point increase in the follow up HAI score from 2 to 3. The rate of fibrosis progression per year was estimated by the ratio between the fibrosis score and duration of infection in years.[13] In addition to the necroinflammatory and fibrosis scores, other features such as lymphocytic infiltration, steatosis, and iron deposition were assessed.

### HLA TYPING

HLA DRB1* typing was performed on 69 (79%) PCR positive and 52 (76%) PCR negative women. DRB1* typing (14 alleles were tested) was performed on genomic DNA isolated from whole blood or serum using a reverse hybridisation assay (Amplicor HLA DRB test; Roche Diagnostics, UK) as described previously.[14]

### EXTRAHEPATIC MANIFESTATIONS

Clinical and laboratory evidence of mixed cryoglobulinaemia, the sicca complex, membranoproliferative glomerulonephritis, autoimmune thyroid disorders, and a general "autoimmune screen" was sought in 79 (91%) PCR positive and 68 (79%) PCR negative women, as described previously.[15] The Wickham survey which observed a prevalence rate of 25% for thyroid autoantibodies among 805 healthy British women (mean age 55.9 years, range 35–75) provided a suitable sex matched control group for thyroid disorders.[16 17]

### STATISTICAL ANALYSIS

Unless otherwise specified, p values were derived from: Student's $t$ test for continuous variables; $\chi^2$ analysis and Fisher's exact test where appropriate for categorical variables; and multivariate statistical techniques including both logistic and multiple regression. All analyses were carried out on SPSS for Windows (version 8.0) and $p<0.05$ was considered significant.

## Results

### CLINICAL FINDINGS

At the time of diagnosis in 1994/95 (17/18 years after inoculation), there was no significant difference in age, source/duration of infection, or alcohol consumption between PCR positive and PCR negative women (table 1). All PCR positive women were RIBA positive while 32 (47.1%) PCR negative women were RIBA positive; 31 (45.6%) were RIBA indeterminate and five (7.4%) were RIBA negative. Mean serum ALT level at diagnosis and after follow up was significantly higher in PCR positive women (table 1) compared with PCR negative women. Furthermore, 66% of the PCR positive group had elevated ALT levels (>40 IU/l) at diagnosis compared with only 1.5% of the PCR negative group.

Three (3.4%) of the 87 PCR positive women admitted to a history of icteric hepatitis shortly following their receipt of anti-D in 1977

*Table 2 Predictors of mental health and psychological adjustment to a diagnosis of HCV infection in a group of previously healthy females (n=66): results of three stepwise multiple regression analyses*

| *Dependent variable* | *No steps* | *Independent variable* | *Beta* | *Multiple r* | *Adjusted $r^2$* |
|---|---|---|---|---|---|
| GHQ30 psychological well being | 3 | Social functioning | −0.44 | 0.68 | 0.46 |
| | | Role limitation emotional problems | −0.39 | 0.81 | 0.64 |
| | | General health perception | −0.23 | 0.83 | 0.67 |
| Depression (HAD) | 3 | Social functioning | −0.55 | 0.76 | 0.58 |
| | | Role limitation emotional problems | −0.32 | 0.84 | 0.69 |
| | | Energy/vitality | −0.21 | 0.86 | 0.72 |
| Anxiety (HAD) | 2 | Social functioning | −0.55 | 0.63 | 0.39 |
| | | Role limitation emotional problems | −0.30 | 0.70 | 0.46 |

Beta is the regression coefficient which expresses the change in the dependent variable that would be produced by a positive increment of one standard deviation in the independent variable concerned. Multiple *r* is the relationship between the dependent and all independent variables. Adjusted $r^2$ is a modified measure of the coefficient of determination taking into account the number of predictor variables included in the regression equation.
HCV, hepatitis C virus; GHQ, general health questionnaire; HADS, hospital anxiety and depression scale.

whereas 14 (20.6%) of the PCR negative women had an acute icteric episode with a mean incubation period of 4.4 (2.0) weeks in 1977 (p=0.002).

### INTRAHEPATIC HCV RNA STATUS

HCV RNA was not detectable by nested PCR in liver biopsy tissue from any of the 27 serum PCR negative patients tested or from any of the negative controls. However, HCV RNA was detectable at all times in liver tissue from all serum PCR positive controls. The human albumin RNA was detectable in all liver biopsies tested, indicating that the negative result obtained in the serum PCR negative biopsies was not due to inefficient methodologies.

### SYMPTOMATOLOGY

Fatigue and arthralgia were the main symptoms reported at diagnosis and during subsequent follow up. Surprisingly, PCR positive patients were found to have fewer complaints of fatigue and arthralgia than PCR negative patients at diagnosis—that is, 15 (24%) versus 23 (44%) (p=0.02) and six (10%) versus 20 (39%) (p=0.001), respectively. It should be noted however that the majority of PCR positive women presented to this liver centre in 1994 while the majority of PCR negative women presented in 1995. The observed differences in reporting of symptoms might be due in part to confounding with calendar year. During 1994–1995 this highly publicised public health issue may have changed awareness of and attitudes to hepatitis C which may have influenced a woman's tendency to report symptoms.[5 6]

In order to adjust for the effect of calendar year, logistic regression analysis was used which assessed, where possible, the independent effect of PCR status on symptom complaints (it was not possible to assess the effect of the confounding year on the symptoms of arthralgia due to inadequate data). This revealed that the odds of a report of fatigue (adjusted for PCR status) was six times higher in 1995 than in 1994 (odds ratio 5.9; p=0.03). When adjustment was made for this calendar year effect, no significant difference was detected in the prevalence of fatigue at diagnosis in PCR positive and negative women (odds ratio 1.43; p=0.63).

### PSYCHOLOGICAL ASSESSMENT

Seventy seven percent (51/66) of women fell within the clinical range for psychological distress on the GHQ30.[9] The percentage of cases reaching the criteria for depression and anxiety on the HADS was 23% and 43%, respectively.[10] A comparison between the PCR positive and PCR negative group indicated no difference in psychological well being, anxiety, or depression (table 1). To assess which quality of life modality best predicted mental health and psychological well being, a series of multiple regression analyses were carried out.[11] It was found that social functioning explained the greatest percentage of variance in the group, accounting for between 39% and 58% of variance for mental health and psychological well being, respectively (table 2).

### HISTOLOGICAL FINDINGS

Overall, necroinflammatory lesions and fibrosis were mild in the majority of PCR positive patients biospied (tables 3, 4). Mild (HAI ⩽4)

*Table 3 Histological data of PCR positive and negative patients*

| | *PCR positive (n=87)* | *PCR negative (n=27)* | *Statistical analysis t test or $\chi^2$* |
|---|---|---|---|
| HAI at diagnosis | 4.1 (1.4) (n=85) | 2.1 (1.5) | p=0.000 |
| Fibrosis score at diagnosis | 1.1 (1.3) (n=80) | 0.15 (0.36) | p=0.000 |
| HAI after 5 years follow up | 4.1 (1.2) (n=44) | N/A | N/A |
| Unchanged (n (%)) | n=19 (43.2%) | | |
| Increased (n (%); mean (SD) score change) | n=15 (34.1%; 1.4 (0.6)) | | |
| Decreased (n (%); mean (SD) score change) | n=10 (22.7%; 1.8 (0.9)) | | |
| Fibrosis score after 5 years follow up | 1.0 (1.0) (n=44) | N/A | N/A |
| Steatosis | 9 (10.3%) (n=87) | 13 (48%) | p=0.000 |
| Mild | 44% | 69% | |
| Moderate/severe | 56% | 31% | |
| Iron deposition | 1+ deposition in 1 patient with HHC* | 0 | NS |

Values are mean (SD) or number (%).
*HHC, genetic haemochromatosis which was incidentally identified in one PCR positive patient.
N/A, not applicable; PCR, polymerase chain reaction; HAI, histological activity index.

*Table 4 Fibrosis related to the histological activity index (HAI) score of PCR positive patients (n=80)*

| *Fibrosis score* | *HAI score (0–4)* | *HAI score (5–9)* |
|---|---|---|
| 0 | 32 (89%) | 4 (11%) |
| 1 | 14 (67%) | 7 (33%) |
| 2 | 2 (22%) | 7 (78%) |
| 3–6 | 1 (7%) | 13 (93%) |
| Total | 49 | 31 |

Data expressed as n (%).
Statistical analysis $\chi^2$; p=0.000.
PCR, polmerase chain reaction.

inflammation was observed in 54/85 (63.5%) biopsies and moderate to severe (HAI ⩾5) inflammation in 31/85 (36.5%) biopsies. Fibrosis was present in 44 (55%) biopsies. The mean HAI and fibrosis scores of these women were 4.1 (1.4) (range 1–9) and 1.1 (1.3) (range 0–5), respectively, at presentation. The principal necroinflammatory lesions were portal inflammation, lobular inflammation, and interface hepatitis. Interestingly, no confluent necrosis was observed. Steatosis was observed in nine (10.3%) PCR positive biopsies. Apart from one individual with iron deposition on liver biopsy (this individual was subsequently found to be homozygous for the C282Y mutation in the HFE gene, the candidate gene for haemochromatosis), excess iron, cirrhosis, or hepatocellular carcinoma was not observed.

Follow up liver biopsy was performed in 44 (50.6%) PCR positive patients who had not received antiviral therapy. The mean follow up HAI and fibrosis scores were 4.1 (1.2) and 1.0 (1.0), respectively (table 3). Over a five year period, 19 (43.2%) patients showed no change in their HAI score, 15 (34.1%) showed a mean increase of 1.4 (0.6) while 10 (22.7%) untreated patients showed a mean decrease of 1.8 (0.9). Neither progression from mild to moderate/severe hepatitis nor deterioration in fibrosis scores was observed over the five year period. The mean rate of fibrosis per year was estimated at 0.06 (0.08). At the time of diagnosis, most patients with mild inflammation (HAI ⩽ 4) had little or no fibrosis. Conversely, most patients with higher fibrosis scores also had higher HAI scores (p=0.000) (table 4).

Liver biopsy was also performed on 27 (40%) PCR negative patients because of elevated ALTs, strongly positive RIBA-3 tests, symptoms dating from the time of inoculation, or patient request. Minimal inflammatory lesions such as mild inflammation and minimal fibrosis were noted. Four (14.8%) individuals had normal liver histology (HAI score of 0). Twenty (74%) had mild (HAI ⩽4) inflammation and three (11.1%) had moderate to severe (HAI ⩾5) liver disease. Minimal fibrosis was present in 4/27 (14.8%) biopsies. The mean HAI and fibrosis scores of this group were 2.1 (1.5) (range 0–6) and 0.15 (0.36) (range 0–1), respectively (table 3). Steatosis was observed in 13 (48%) biopsies. No bile duct damage, lymphoid follicles, or aggregates were observed in liver biopsies of serum PCR negative women.

*Table 5 Frequency (%) of HLA DRB1* alleles in PCR positive and negative patients†*

| *DRB1* allele* | *PCR negative (n=52)* | *PCR positive (n=69)* | *Statistical analysis $\chi^2$* |
|---|---|---|---|
| DRB1*01 | 15 (28.8) | 6 (8.7) | p=0.004 (pc=0.036)* |
| DRB1*0103 | 3 (5.8) | 3 (4.3) | NS |
| DRB1*03 | 16 (30.8) | 27 (39.1) | NS |
| DRB1*04 | 15 (28.8) | 22 (31.9) | NS |
| DRB1*07 | 13 (25) | 19 (27.7) | NS |
| DRB1*08 | 1 (1.9) | 1 (1.4) | NS |
| DRB1*09 | 1 (1.9) | 0 | NS |
| DRB1*10 | 1 (1.9) | 0 | NS |
| DRB1*11 | 5 (9.6) | 5 (7.2) | NS |
| DRB1*12 | 1 (1.9) | 0 | NS |
| DRB1*13 | 3 (5.8) | 14 (20.3) | p=0.02 (pc=NS) |
| DRB1*14 | 1 (1.9) | 3 (4.3) | NS |
| DRB1*15 | 21 (40.4) | 24 (34.8) | NS |
| DRB1*16 | 0 | 0 | NS |

†The frequencies of HLA DRB1* alleles were compared among PCR positive and negative women. To minimise the possibility that the observed probability (p) values might be because of chance, corrections (pc) were made for the number of alleles tested (only alleles present at >3% were stastically analysed; (n=9)).
PCR, polmerase chain reaction.

### HLA DRB1* STATUS

Comparison of HLA DRB1* allele status in PCR positive and negative women revealed an association between the DRB1*01 allele and loss of circulating HCV RNA—that is, viral clearance (table 5). Among 52 PCR negative women, 15 (28.8%) had the DRB1*01 allele compared with only six (8.7%) of 69 PCR positive women (p=0.004). After correction for the number of alleles tested, this association still remained significant (pc=0.04).[18] No other significant DRB1* associations were apparent.

### EXTRAHEPATIC MANIFESTATIONS

The commonest extrahepatic autoimmune condition detected among the PCR positive women was mixed cryoglobulinaemia and 12.7% of the 79 PCR positive patients tested had type 2 (30%) or type 3 (70%) cryoglobulinaemia. Fifty per cent were symptomatic with fatigue, arthralgia, and purpura. Three patients were also diagnosed with the sicca complex and antinuclear antibodies were present in four. These patients were managed symptomatically with analgesics and did not receive interferon therapy.

Thyroid autoantibodies were detected in 11 (13.9%) patients and all were clinically and biochemically euthyroid. The thyroid autoantibody profile was as follows: TM and TG antibodies (n=4) and TM antibody only (n=7). This observed prevalence rate of 13.9% was significantly lower than that observed in the Wickham community (p=0.007).[16 17] The prevalence of other autoantibodies was low and the findings are summarised in table 6. Vasculitis, glomerulonephritis, and dermopathy were not features of this population.

The findings among the PCR negative patients are summarised in table 6. Although the prevalence of thyroid autoantibodies was higher in PCR negative than in PCR positive women, this did not reach statistical significance (23.5% *v* 13.9%). Furthermore, there was no significant difference between the prevalence rate in PCR negative women and that observed in the Wickham community (23.5% *v* 25%).[16 17] In contrast with the PCR positive group, cryoglobulinaemia and sicca complex were not observed in PCR negative patients. No other extrahepatic manifestations were detected.

*Table 6 Extrahepatic manifestations of PCR positive and negative patients*

| *Extrahepatic manifestation* | *PCR positive (n=79)* | *PCR negative (n=68)* |
|---|---|---|
| Mixed essential cryoglobulinaemia | | |
| Total | 10 (12.7% of total) | 0* |
| Type 2 | 3 | |
| Type 3 | 7 | |
| Symptomatic | 5 (50% of cryoglobulin) | |
| Sicca complex | 3 | |
| Sicca complex | | |
| Total | 6 (7.6% of total) | 0* |
| ANA | 2 | |
| RF | 1 | |
| ENA, RNP | 0 | |
| Cryoglobulinaemia | 3 | |
| Thyroid autoantibodies (thyroglobulin and microsomal antibodies) | | |
| Total | 11 (13.9%) | 12 (23.5%) (n= 51) |
| Normal thyroid function | 100% | 83.3% |
| Antinuclear antibody | 4 (5.1%) | 13 (19.1%) |
| Rheumatoid factor | 3 (3.8%) | 0 |
| Antimitochondrial antibody | | |
| Total | 3 (3.8%) | 0 |
| Histological features of primary biliary cirrhosis | 0 | |
| Antismooth muscle antibody | 1 (1.2%) | 0 |
| Glomerulonephritis | 0 | 0 |
| Dermopathy | | 0 |
| Porphyria cutanea tarda | 0 | 0 |
| Lichen planus | 0 | 0 |
| Purpura | 7 (8.8%) | 0 |
| Purpura with cryoglobulinaemia | 5 (6.3%) | 0 |

*Denotes statistical difference.
PCR, polmerase chain reaction.

## Discussion

This study investigated the natural course of HCV genotype 1b infection in a representative subgroup of a larger homogenous cohort (n=795) who were infected with HCV via contaminated anti-D immunoglobulin in 1977.[5] The 87 women with chronic HCV infection and 68 with spontaneous viral clearance described in this study shared similar ethnic origin, sex, demographic characteristics, duration of disease, mode of acquisition, viral genotype, and lacked other possible causes of liver disease. A benign course of HCV infection, lack of disease progression, and low incidence of extrahepatic manifestations were observed in women with chronic HCV infection after a 22 year period.

This benign and non-progressive course of chronic HCV infection was reflected clinically in the mildly elevated serum ALT activity at presentation (17 years after inoculation) (mean 50.6 (31) IU/l) and after five years of follow up (mean 51 (37.5) IU/l). Similarly, the mild histological activity observed at presentation was reflected in the mean HAI and fibrosis scores of 4.1 (1.4) and 1.1 (1.3), respectively. Histological deterioration was not observed after a five year follow up period as the mean follow up HAI and fibrosis scores were 4.1 (1.2) and 1.0 (1.0), and 22.7% (10/44) of biopsies even showed a spontaneous reduction in HAI score.[19] Cirrhosis or hepatocellular carcinoma was not observed on the first or subsequent liver biopsy. These results confirm the findings of fellow investigators on Irish anti-D patients showing lack of disease progression over similar time spans but conflict with other studies observing significant fibrosis, cirrhosis, and advanced liver disease within 20 years of disease duration.[6 19–26] Several variables have been suggested to influence the progression of HCV, including older age at infection and diagnosis, male sex, excess iron, increased alcohol consumption, and coinfection with hepatitis B virus and human immunodeficiency virus.[27–33] Mode of transmission is also thought to be important as patients infected via transfusion receive a more infectious inoculum while those who acquire HCV via anti-D immunoglobulin receive a partially attenuated virus.[34] The relatively young age (late 20s) of the women in this study at infection, together with the mode of transmission, and absence of excess alcohol consumption, iron on liver biopsy, and coinfection with other viruses probably contributes to the favourable outcome observed in this unique cohort.

Sixty eight females considered to have spontaneous viral clearance were also investigated in this study. Of these, 32 (47.1%) were still antibody positive 18 years after inoculation. Debate exists as to whether these individuals have truly resolved infection or actually have current infection undetectable in serum but detectable in liver (as viral levels have been demonstrated to be $10^4$-fold higher in the liver than in serum). Furthermore, it has been demonstrated in two separate studies that antibody positive serum PCR patients have similar clinical, histological, and virological profiles as serum PCR positive individuals.[35–39] In this study, the mean serum ALT was significantly lower in PCR negative women compared with PCR positive women (p=0.000) (table 3). Similarly, the mean HAI and fibrosis scores of the 27 (40%) PCR negative women who underwent liver biopsy were also significantly lower than those of PCR positive women (p=0.000) (table 3). Furthermore, HCV RNA was not detectable in liver biopsies from serum PCR negative women but was detectable in biopsies from all serum PCR positive women, thus providing evidence that the negative serum PCR status is indeed a true reflection of cleared past infection. Steatosis was however observed at a higher frequency in PCR negative liver biopsies (48% *v* 10.3% in PCR positive biopsies; p=0.000). In a recent study of a larger group of serum PCR negative individuals, we have demonstrated that 63.6% (21/33) were overweight or obese, as determined by calculation of body mass index, and the histological findings of these women were more suggestive of non-specific reactive changes, steatosis, or non-alcoholic steatohepatitis rather than chronic HCV.[40]

The 68 women with viral clearance described in this study represent a subgroup of a much larger group with spontaneous viral clearance. The findings from the total infected cohort from 1977 (n=704) may therefore indicate that more individuals spontaneously clear HCV than previously reported, as 314 (45%) of the original cohort were antibody positive but PCR negative for HCV RNA when tested in 1994.[4 5] Moreover, an additional 74 recipients of 1977 contaminated anti-D volunteered a history of jaundice shortly post partum and are now known to test both antibody and PCR negative.[5] In this (table 1) and in another Irish study, a history of acute icteric hepatitis after

inoculation has been demonstrated to be associated with spontaneous viral clearance.[41] Why such a large proportion of women spontaneously cleared infection while the remainder went on to develop chronic HCV is unclear as the difference in disease outcome cannot be accounted for by differences in age, sex, source/duration of infection, size of inoculum, or alcohol consumption. These findings may therefore suggest that immune-host factors play an important role in the spontaneous clearance of HCV, and in this study the class II DRB1*01 allele was significantly increased in women with spontaneous viral clearance compared with those with chronic HCV infection. These results confirm the findings of a previous study on a larger cohort of these patients and also the findings of two other studies of separate groups of women from the same homogenous cohort.[14 42 43]

A wide spectrum of extrahepatic manifestations has been traditionally ascribed to chronic HCV infection, with varying strengths of association. The best described of these include cryoglobulinaemia, sicca complex, autoimmune thyroiditis, membranoproliferative glomerulonephritis, and porphyria cutanea tarda.[44–46] In our experience, cryoglobulinaemia is the most common of these HCV associated autoimmune disorders. Our observed prevalence rate of 12.7% among PCR positive patients and 0% among PCR negative patients further supports the role of the HCV virus in the pathogenesis of cryoglobulinaemia. In keeping with observations made in Britain, type 3 cryoglobulinaemia was the predominant type.[47] However, we have previously demonstrated that Irish anti-D/HCV patients without cryoglobulinaemia were as likely to experience significant fatigue as those with cryoglobulinaemia.[15] Tran *et al* reported a high prevalence of thyroid autoantibodies among patients with chronic HCV infection but did not include the known prevalence rate in healthy controls for comparison.[48] The well conducted Wickham survey designed 20 years ago and recently updated provided an ideal sex matched population in the community for this purpose.[16 17] Our surprising finding of significantly less autoimmune thyroid disease among the PCR positive population compared with that observed in the Wickham survey does not support the earlier investigators' claims. The observed prevalence rate of thyroid autoantibodies among our PCR negative group may mirror the prevalence among healthy women in the community. Indeed, current studies have failed to establish an association between autoimmune thyroid disease and hepatitis C.[49 50] It is likely that the only significance of thyroid autoantibodies in patients with HCV lies in their positive predictive value for interferon associated thyroid dysfunction.[51]

Glomerulonephritis and HCV dermopathy, including porphyria cutanea tarda and lichen planus, and antibodies to liver-kidney microsome 1 were not detected in this study group.

Initially the symptom profile of the PCR positive and negative women presented in this study (PCR positive patients were found to have fewer complaints of fatigue and arthralgia than PCR negative patients at presentation) was contrary to what might be expected, but when calendar year was taken into account there were no differences in complaints of fatigue between the two groups. This observation, together with the psychological profile of 66 of these women, suggested that when suddenly diagnosed with chronic HCV the identity of this illness as chronic, infectious, and associated with intravenous drug misuse may have caused great concern to the infected women. The impact of such a diagnosis on psychological well being may have been even further compounded by the high legal profile related to this patient group.[5] While accepting the uniqueness of this patient cohort, recent studies in other patient cohorts have also documented problems in quality of life and mental health.[52–54]

In conclusion, this study investigated the natural course of HCV infection in a homogenous cohort almost half of whom now have spontaneous viral clearance. A benign course of HCV genotype 1b infection, lack of disease progression, and low incidence of extrahepatic manifestations was observed in women with chronic HCV after a 22 year period. The host HLA class II DRB1*01 allele was found to be associated with spontaneous viral clearance which supports the notion that host factors may play an important role in disease outcome. Finally, although the outcome of HCV infection was favourable, this study also demonstrated that the diagnosis of HCV was a very stressful event for these women who reported high levels of psychological distress and poor quality of life. It is hoped that longer term follow up of this unique cohort may further provide valuable information regarding the natural course of HCV infection.


1 Alberti A, Chemello L, Benvegnu L. Natural history of hepatitis C. *J Hepatol* 1999;**31**(suppl 1):17–24.
2 Seeff LB. Natural history of hepatitis C. *Hepatology* 1997;**26**(suppl 1):21–8S.
3 Van der Poel CL, Cuypers HT, Reesink HW, *et al*. Confirmation of hepatitis C virus infection by second generation four-antigen recombinant immunoblot assay and polymerase chain reaction. *Lancet* 1991;**337**:317–19.
4 Gerlach JT, Diepolder HM, Gruener NH, *et al*. Natural course of symptomatic acute hepatitis C. *J Hepatol* 1999;**30**(suppl 1):120.
5 *Report of the tribunal of inquiry into the Blood Transfusion Service Board*. Ireland: Government Publications, 1996.
6 Kenny-Walsh E, for the Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Engl J Med* 1999;**340**: 1228–33.
7 Clarke G, Stewart S, Mc Kiernan S, *et al*. Response to interferon and ribavirin or interferon alone in chronic hepatitis C genotype 1b infection. *Hepatology* 1998;**28(4)**:Pt 2 , 210A:191.
8 Barrett S, O'Keane JC, Crowe J. Intrahepatic viral RNA status of serum PCR negative individuals with histological evidence of hepatitis C infection. *Gastroenterology* 2000, **118(4)**:Pt 1, A1012.
9 Goldberg D, Williams P. *A users guide to the GHQ*. Windsor, UK: NFER-Nelson, 1988.
10 Zigmond AS, Snaith RP. Hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;**67**:361–70.
11 Jenkinson C, Wright L, Coulter A. *Quality of life measurement in health care. A review of measures and population norms for the UK SF-36*. Oxford: Health Services Research Unit, 1993.
12 Ishak K, Baptista A, Bianchi L, *et al*. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;**22**:696–9.
13 Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997;**349**:825–32.
14 Barrett S, Ryan E, Crowe J. Association of the HLA-DRB1*01 allele with spontaneous viral clearance in an

Irish cohort infected with HCV via contaminated anti-D immunoglobulin. *J Hepatol* 1999;**30**:979–83.
15 Goh J, Coughlan B, Quinn J, *et al*. Fatigue does not correlate with the degree of hepatitis or the presence of autoimmune disorders in hepatitis C. *Eur J Gastroenterol Hepatol* 1999;**11**:833–8.
16 Tunbridge WM, Evered DC, Hall R, *et al*. The spectrum of thyroid disease in a community: the Wickham survey. *Clin Endocrinol* 1977;**7**:481–93.
17 Vanderpump MP, Tunbridge WM, French JM, *et al*. The incidence of thyroid disease in the community: a twenty-year follow-up of the Wickham survey. *Clin Endocrinol (Oxf)* 1995;**43**:55–68.
18 Svejgaard A, Ryder LP. HLA and disease association; detecting the strongest association. *Tissue Antigens* 1994;**43**: 18–27.
19 Levine, RA, Goulding C, O'Keane JC, *et al*. Spontaneous reduction in histological activity index in untreated, non-cirrhotic and persistently viremic Irish women with chronic hepatitis C. *Gastroenterology* 2000, **118(4)**:Pt 1, A944.
20 Alboushi SS, Murray FE, Callagy G, *et al*. Changes in liver histopathology in women infected with hepatitis C through contaminated anti-D immunoglobulin injections in Ireland. *Eur J Gastroenterol Hepatol* 1998;**10**:69–73.
21 Fanning L, Kenny E, Sheehan M, *et al*. Viral load and clinicopathological features of chronic hepatitis C (1b) in a homogeneous patient population. *Hepatology* 1999;**29**:904–7.
22 Niederau C, Lange S, Heitges T, *et al*. Prognosis of chronic hepatitis C: results of a large prospective cohort study. *Hepatology* 1998;**28**:1687–95.
23 Dusheiko GM. The natural course of chronic hepatitis C: implications for clinical practice. *J Viral Hepat* 1998; **5**(suppl 1):9–12.
24 Tong MJ, El-Farra NS, Reikes AR, *et al*. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995;**332**:1463–6.
25 Seeff LB, Hollinger FB, Alter HJ, *et al*. Long-term morbidity of transfusion-associated hepatitis (TAH) C. *Hepatology* 1998;**28**:407A.
26 Lee DH, Jamal H, Regenstein FG, *et al*. Morbidity of chronic hepatitis C as seen in a tertiary care medical center. *Dig Dis Sci* 1997;**42**:186–91.
27 Wiley TE, McCarthy M, Breidi L, *et al*. Impact of alcohol on the histological and clinical progression of hepatitis C infection. *Hepatology* 1998;**28**:805–9.
28 Piperno A, Vergani A, Malosio I, *et al*. Hepatic iron overload in patients with chronic viral hepatitis: role of HFE mutations. *Hepatology* 1998;**28**:1105–9.
29 Shedlofsky SI. Role of iron in the natural history and clinical course of hepatitis C disease. *Hepatogastroenterology* 1998;**45**:349–55.
30 Soto B, Sanchez-Quijano A, Rodrigo L, *et al*. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *J Hepatol* 1997;**26**: 1–5.
31 Roudot-Thoraval F, Bastie A, Pawlotski JM, *et al*. Epidemiological factors affecting the severity of hepatitis C virus-related liver disease: a French survey of 6664 patients. *Hepatology* 1997;**26**:491–4.
32 Alter MJ. Transmission of hepatitis C virus—route, dose and titre. *N Engl J Med* 1994;**330**:784–6.
33 Kobayashi M, Tanaka E, Sodeyama T, *et al*. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. *Hepatology* 1996:**23**:695–9.
34 Gordon SC, Elloway RS, Long JC, *et al*. The pathology of hepatitis C as a function of mode of transmission: blood transfusion vs. intravenous drug use. *Hepatology* 1993;**18**: 1338–43.
35 Bonacini M, Redeker AG. Intrahepatic HCV levels in chronic HCV infection. *Gut* 1999:**45**:630–1.
36 McGuiness PH, Bishop GA, Painter DM, *et al*. Intrahepatic hepatitis C RNA levels do not correlate with degree of liver injury in patients with chronic hepatitis C. *Hepatology* 1996:**23**:676–87.
37 Fong TL, Lee SR, Briggs WK, *et al*. Clinical significance of hepatitis C viral RNA status and its correlation to antibodies to structural HCV antigens in anti-HCV reactive patients with normal liver tests. *J Med Virol* 1996;**49**:253–8.
38 Dries V, Von Both I, Muller M, *et al*. Detection of hepatitis C virus in paraffin-embedded liver biopsies of patients negative for viral RNA in serum. *Hepatology* 1999;**29**:223–9.
39 Haydon GH, Jarvis LM, Blair CS, *et al*. Clinical significance of intrahepatic hepatitis C virus levels in patients with chronic HCV infection. *Gut* 1998:**42**:570–5.
40 Barrett S, Kieran N, Ryan E, *et al*. Intrahepatic hepatitis C viral RNA status of serum polymerase chain reaction-negative individuals with histological changes on liver biopsy. *Hepatology* 2001;**33**:1496–502.
41 Sachithanandan S, Fielding JF. Jaundice at onset signifies a good prognosis in anti-D-associated HCV infection. *Eur J Gastroenterol Hepatol* 1997;**9**:77–9.
42 Fanning LJ, Levis J, Kenny-Walsh E, *et al*. Viral clearance in hepatitis C (1b) infection: relationship with human leukocyte antigen class II in a homogenous population. *Hepatology* 2000;**6**:1334–7.
43 McKiernan SM, Hagan R, Curry M, *et al*. The MHC is a major determinant of viral status, but not fibrotic stage, in individuals infected with hepatitis C. *Gastroenterology* 2000;**118**:1124–30.
44 Gumber SC, Chopra S. Hepatitis C: A multifaceted disease: Review of extrahepatic manifestations. *Ann Intern Med* 1995;**123**:615–20.
45 McMurray RW. Hepatitis C-associated autoimmune disorders. *Rheum Dis Clin North Am* 1998;**24**:353–74.
46 Pawlotsky J, Roudot-Thoraval F, Simmonds P, *et al*. Extrahepatic immunologic manifestations in chronic hepatitis C and hepatitis C virus serotypes. *Ann Intern Med* 1995;**122**:169–73.
47 Wong VS, Egner W, Elsey T, *et al*. Incidence, character and clinical relevance of mixed cryoglobulinaemia in patients with chronic hepatitis C virus infection. *Clin Exp Immunol* 1996;**104**:25–31.
48 Tran A, Quaranta J-F, Benzaken S, *et al*. High prevalence of thyroid autoantibodies in a prospective series of patients with chronic hepatitis C before interferon therapy. *Hepatology* 1993;**18**:253–7.
49 Metcalfe RA, Ball G, Kudesia G, *et al*. Failure to find an association between hepatitis C virus and thyroid autoimmunity. *Thyroid* 1997;7:421–4.
50 Gorez E, Nakib I, Larbre H, *et al*. Lack of epidemiological evidence for a link between thyroid auto-immune disease and hepatitis C virus infection. *J Hepatol* 1997;**26**:738–9.
51 Deutsch M, Dourakis S, Manesis EK, *et al*. Thyroid abnormalities in chronic viral hepatitis and their relationship to interferon alfa therapy. *Hepatology* 1997;**26**:206–10.
52 Foster GR, Goldin DR, Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. *Hepatology* 1998;**27**:209–12.
53 Bayliss M, Gandek B, Bungay K, *et al*. A questionnaire to assess the generic and disease-specific health outcomes of patients with chronic hepatitis C. *Qual Life Res* 1998;**7**:39–55.
54 Zdilar D, Franco-Bronson K, Buchler N, *et al*. Hepatitis C, interferon alfa, and depression. *Hepatology* 2000;**31**:1207–11.