Hepatitis C virus

Hepatitis C virus: epidemiology and genotypes in the north east of England

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Abstract

The epidemiology of hepatitis C virus (HCV) infection was studied in an English teaching hospital over an 18 month period. A total of 104 HCV antibody positive patients were referred for further investigation. They were divided into those diagnosed through screening (blood donors and intravenous drug abusers) and those diagnosed for other reasons, and their mean ages, known risk factors for HCV transmission, genotypes, and liver biopsy histology were analysed. Screened patients were significantly younger than the others. No significant difference in age was found between genotypes. Most patients genotyped (69%) were genotype 1. Intravenous drug abusers had a higher proportion of subtype 1a, and patients who acquired HCV through blood transfusion had a higher proportion of subtype 1b. Liver biopsy specimens were scored using a histological activity index for liver inflammation and fibrosis. Patients with subtype 1b had significantly more severe liver disease than other genotypes when the histological activity index scores for fibrosis were analysed ($p<0.05$). Liver disease worsened significantly with age according to all three histological activity index scores (portal activity: $p<0.01$, acinar activity: $p<0.001$, fibrosis: $p<0.0001$). Liver disease worsened with increased duration of infection ($p<0.002$), and patients who also abused alcohol presented at a significantly younger age (cirrhosis, $p<0.05$, hepatocellular carcinoma, $p<0.02$).

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Hepatitis C virus (HCV) was cloned and characterised in 1989, and it is now clear that it is the major cause of post transfusion hepatitis.[1] HCV infection commonly results in chronic disease: at least 50% of patients develop chronic hepatitis, 20% of whom develop cirrhosis.[1 2] There is also a strong association between HCV and hepatocellular carcinoma.[3 4] The virus is parenterally transmitted and routes of infection include transfusions of blood and blood products, abuse of intravenous drugs, tattoos, and sexual or household transmission.[5–9] Nucleic acid sequence analysis has shown that HCV is a highly variable virus, and a system for the nomenclature of HCV genotypes has been proposed that divides the virus into six main genotypes.[10] It may be that some genotypes are associated with more severe liver disease.[11]

Many patients who become infected with HCV are asymptomatic for decades, and remain undiagnosed until they present with symptomatic advanced liver disease. Early diagnosis is important as treatment with alpha interferon is more likely to be successful in the asymptomatic stage before progression to cirrhosis.[12 13] Thus, a knowledge of the prevalence and natural history of asymptomatic HCV related to disease duration and HCV genotypes has profound health care implications for resource allocation, as treatment of chronic HCV with alpha interferon or liver transplantation is very expensive, and is now a major factor in any health care budget. It has been estimated that the cost per year of life saved in non-cirrhotic patients with chronic HCV infection treated with interferon is $71 950.[14] Another illustration of the scale of the problem is that 97 of 426 (23%) of patients who had liver transplants performed at the University of California, San Francisco between 1988 and 1993 had chronic HCV.[15] In the same study, 11 of 59 patients (19%) who underwent liver transplant for seronegative cryptogenic cirrhosis also developed de novo HCV infection after transplantation.

Serological diagnosis of HCV is made by screening populations such as blood donors, intravenous drug abusers, and patients who present with liver disease of unknown aetiology. The initial diagnostic test, using an enzyme linked immunosorbent assay (ELISA) to detect antibody to the C-100 antigen, was found to generate a high frequency of false positives and false negatives.[16] This obviously presented serious diagnostic problems in a disease that could remain asymptomatic for decades. Second generation ELISAs and confirmatory RIBA tests incorporating antigens from other regions of the HCV genome have reduced this problem.[16]

We previously studied the prevalence of antibodies to HCV in different populations of patients in north east England using the first generation ELISA and RIBA tests: anti-HCV was detected in 21 of 1120 blood donors (0·18%).[17] Because of the problems discussed above with first generation anti-HCV testing, the aim of this study was to assess the population in the north east of England further by studying the epidemiology, genotypes, and liver histology of patients diagnosed as HCV positive by second and third generation assays at the Freeman Hospital, Newcastle upon

Tyne, over the 18 months from August 1992 to January 1994.

Methods

All patients who were found to be HCV antibody positive at the Freeman Hospital between August 1992 and January 1994 were included in the study. HCV antibody results originated from the Microbiology Department at the Freeman Hospital, the Northern Regional Public Health Laboratories, and the National Blood Service, Newcastle. Anti-HCV was determined in the three laboratories using second generation ELISAs supplied by Ortho Diagnostic systems (Raritan, NJ), Abbott Laboratories (North Chicago, IL), and United Biomedical (Hauppage, NY). Confirmatory immunoblots used were supplied by Ortho Diagnostic Systems (RIBA-2, RIBA-3) and Abbott Laboratories (Matrix test). Units of blood tested at the National Blood Service that were found to be initially reactive by ELISA-2 were screened again by ELISA-2 in triplicate. Repeatedly reactive units proceeded to confirmatory immunoblot testing. Those found to be indeterminate or positive on confirmatory testing were referred as described below.

Patients were grouped into those identified through screening and those who presented for other reasons, including possible liver disease. Patient details were obtained from case notes.

SCREENED PATIENTS

Patients identified through screening were either blood donors from the National Blood Service, Newcastle or known intravenous drug abusers referred from the Northern Regional Drug Dependency Unit. Blood donors diagnosed HCV positive or indeterminate by confirmatory immunoblot were notified and advised to consult their general practitioners, who referred them to the specialist liver clinic at the Freeman Hospital for counselling and assessment. Intravenous drug abusers were referred directly from the Drug Dependency Unit to the liver clinic after counselling and HCV antibody testing. All patients were then seen three times in the clinic at monthly intervals, for repeat HCV ELISA and immunoblot serology, together with three sets of liver function tests. If one or more of the liver function tests showed a raised alanine transaminase (ALT>45IU/l) or a raised alkaline phosphatase (ALP>115IU/l), or both, a liver biopsy was advised.

OTHER PATIENTS

Other patients were tested for HCV for a variety of reasons, including those with abnormal liver function tests, patients presenting with hepatocellular carcinoma, patients referred to the Freeman Hospital liver transplant programme, and patients who had received multiple transfusions of blood or blood products. Most were diagnosed in the liver clinic, but some were identified from the records of HCV tests kept in the Microbiology Department at the Freeman Hospital. These included patients from several different hospital departments such as cardiothoracic surgery and nephrology, and in these cases patient details were obtained from their medical records. All patients seen in the liver clinic were seen three times at monthly intervals, for repeat HCV ELISA and immunoblot serology, together with three sets of liver function tests. If one or more of the liver function tests showed a raised ALT (>45IU/l) a raised ALP (>115IU/l), or both, a liver biopsy was advised.

GENOTYPING

Plasma samples from HCV antibody positive patients were collected in EDTA and stored at −80°C until analysis. RNA was extracted using the method of Chomczynski and Saachi.[18] Genotypes were determined either by reverse transcription of RNA and polymerase chain reaction (RT-PCR) followed by restriction fragment length polymorphism as previously described,[19] or by RT-PCR and reverse hybridisation to specific oligonucleotide probes using the INNO-LIPA genotyping kit (Innogenetics, Belgium).[20]

LIVER BIOPSY SPECIMEN ANALYSIS

Seventy five patients included in the study were biopsed. Liver biopsy specimens were available for pathological review from 68 patients (four patients who had been biopsied were HBsAg positive and were excluded from the liver biopsy study, three specimens were not available for review). The liver biopsy specimens were assessed independently by two observers (SJJ, ADB) blinded to the clinical and serological data. Cases in which there was a discrepancy in the independent scores were reviewed by the two pathologists together and a final grade agreed. The biopsy specimens were scored according to Scheuer[21] for features of chronic hepatitis:

- Portal/periportal activity (0 – none or minimal, 1 – portal inflammation, 2 – mild piecemeal necrosis, 3– moderate piecemeal necrosis, 4 – severe piecemeal necrosis;
- Acinar activity (0 – none, 1 – inflammation but no necrosis, 2 – focal necrosis or acidophil bodies, 3 – severe focal cell damage, 4 – damage includes bridging necrosis; and
- Fibrosis (0 – none, 1 – enlarged fibrotic portal tracts, 2 – periportal or portal-portal septa but intact architecture, 3 – fibrosis with architectural distortion but no obvious cirrhosis, 4 – probable or definite cirrhosis).

Results

Number of patients presenting, risk factors, mean ages, genotypes, liver biopsy histology, and associations with other hepatic and non-hepatic diseases were analysed.

NUMBERS OF PATIENTS PRESENTING AND RISK FACTORS

The distribution of the 104 patients diagnosed HCV RIBA positive during the 18 month

TABLE I *Numbers, risk factors, associations, and liver biopsy results of patients confirmed as hepatitis C virus antibody positive by RIBA at the Freeman Hospital from August 1992 to January 1994*

		Risk factors							Associations		Liver biopsy		
Presentation		*None*	*Blood transfusion*	*Household/sexual transmission*	*Intravenous drugs*	*Middle East or Indian subcontinent*	*Tattoo or acupuncture*	*Health care worker*	*HBsAg+ve*	*HBcAb+ve HBsAb+ve*	*Chronic hepatitis*	*Cirrhosis*	*HCC*
Asymptomatic screening													
Blood donors:													
RIBA positive, normal LFT	7	3	2		1		1						
RIBA positive, abnormal LFT	23	6	5	2	6		1	3		2	23		
Total blood donors	30	9	7	2	7	0	2	3	0	2	23	0	0
Intravenous drug abusers:													
RIBA positive, normal LFT	5				5					1			
RIBA positive, abnormal LFT	22				22				3	2	16	1	
Total IV drug abusers	27	0	0	0	27	0	0	0	3	3	16	1	0
Others													
Abnormal LFT	32	13	5	1	4	5	3	1	1	7	11	15	
Liver cancer	3	1	1				1			1			3
Referral for liver transplant	4	1				3			1	1		4	
Dialysis	4		4										
Previous organ transplant	4		4								1	1	
Total others	47	15	14	1	4	8	4	1	2	9	12	20	3
Total	104	24	21	3	38	8	6	4	5	14	51	21	3

LFT=liver function test; HCC=hepatocellular carcinoma.

study is shown in Table I, together with identifiable risk factors and associations. Altogether 94 of 104 (90%) were British born white people. The remainder included an Italian, two patients from the United Arab Emirates referred for transplantation, an Israeli born Hasidic Jew, and six migrants from Pakistan and Bangladesh. All patients except two of the three transplant referrals are now resident in the north east. Only the Italian (drug use) and the Israeli (blood transfusion) had recognised risk factors. The male:female ratio was 61:43 (1.4:1).

SCREENED PATIENTS

Blood donors

A total of 215 291 units were screened between August 1992 and January 1994 by the Blood Transfusion Service. Of these, 283 of 215 291 (0·13%) were repeatedly reactive by ELISA, and 144 of 215 291 (0·07%) were confirmed positive or indeterminate by immunoblot. Altogether 105 of 144 patients were subsequently referred by general practitioners during the 18 months. Thirty of 105 were positive by confirmatory immunoblot on repeat testing in the liver clinic. Twenty one of 30 had identifiable risk factors: seven had previously had blood transfusions, seven had abused intravenous drugs without disclosing this before donating blood, three were health care workers, one had been tattooed, one had received acupuncture treatment, and two had a possible history of sexual or household transmission. Nine of 30 had no identifiable risk factors.

Intravenous drug abusers

Twenty seven patients were tested and referred because of previous or current intravenous drug abuse. All were confirmed RIBA positive.

OTHER PATIENTS

A variety of presentations were seen in the other 47 HCV RIBA positive patients. Thirty two presented with abnormal liver function tests, one of whom subsequently developed hepatocellular carcinoma. Three presented with hepatocellular carcinoma, and four presented for assessment on the liver transplant programme. Of the four patients referred for liver transplantation, one was white and had lived in the north east of England all his life. A second was an immigrant from Pakistan who had lived in England for over 20 years. The other two patients were referred from the United Arab Emirates for transplantation. Four were patients on dialysis, and four had received orthotopic transplants (two kidney, one heart, and one liver). Thirty two had identifiable risk factors: 14 had previously had blood transfusions, four had abused intravenous drugs, one was a health care worker, four had been tattooed, one had a possible history of sexual or household transmission, and eight originated from the Middle East or Indian subcontinent. Fifteen had no identifiable risk factors.

MEAN AGES

Blood donors (mean (SD) age 35·6 (6·9) years) and intravenous drug abusers (mean age 36·0 (6·4) years) were both significantly younger than patients presenting for other reasons (mean age 55·3 (16·0) years, Student's t test $p<0·05$). The mean (SD) age of patients with chronic hepatitis was 38·9 (9·5) years, the mean age of patients with cirrhosis was 60·7 (12·5) years, and the mean age of patients with hepatocellular carcinoma was 67·3 (5·5) years.

GENOTYPES

Stored plasma samples were available from 73 of 104 RIBA positive patients. Fifty eight of

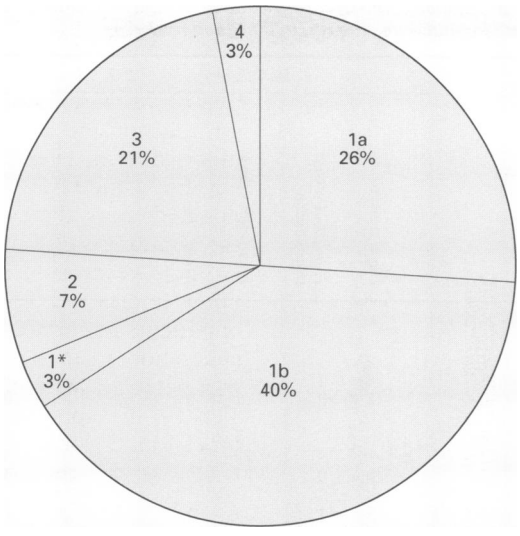

Figure 1: Number and percentages of patients with hepatitis C virus genotypes 1–4 (unclassified subtypes shown as).*

the 73 were HCV RNA positive by PCR. The distribution of genotypes and subtypes is shown in Fig 1. The commonest genotype was genotype 1 (69%), followed by genotype 3 (21%). Subtype analysis revealed 23 patients with HCV type 1b and 15 with type 1a. The association of genotypes with the main identifiable risk factors is shown in Fig 2. Intravenous drug abusers were most likely to have HCV subtype 1a, and patients acquiring HCV through blood transfusion had a high proportion of subtype 1b. Of the three patients of Middle/Far East origin for whom genotypes were obtained, one was type 1a, and two were type 3. Two patients with HCV type 4 were identified – one Israeli born Hasidic Jew with Crohn's disease who had previously received blood transfusions and one white British health care worker. No significant difference in age was found between genotypes. The mean age of all patients genotyped was 45·7 (15·5) years and the mean age of patients with genotype 1b was 51·9 (18·5) years (t=1·61, p>0·05).

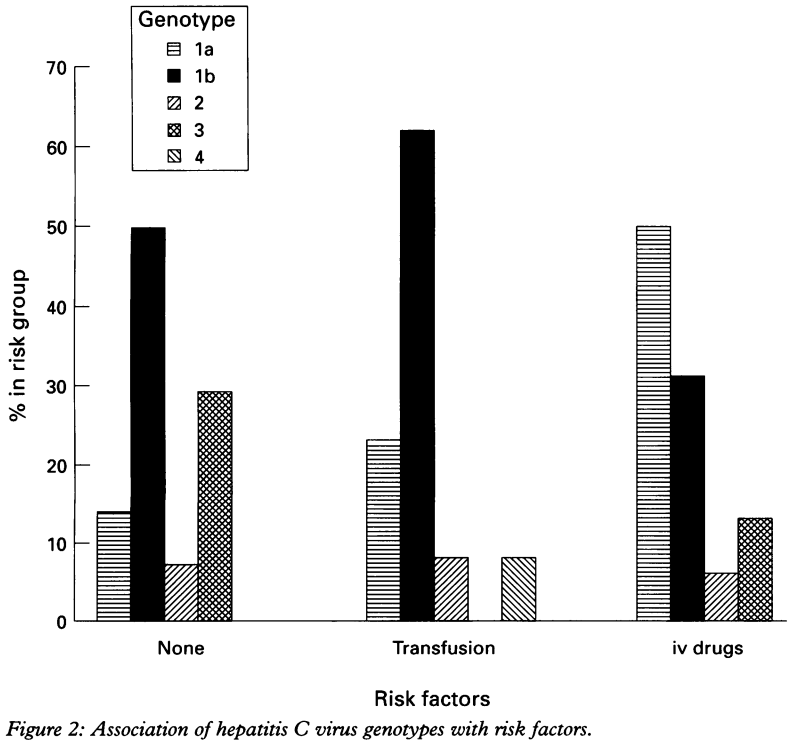

Figure 2: Association of hepatitis C virus genotypes with risk factors.

LIVER BIOPSY HISTOLOGY

Inflammatory changes were present in all biopsy specimens and ranged widely in severity from minimal acinar inflammation only to severe piecemeal necrosis with bridging acinar necrosis and cirrhosis. There was portal inflammation in 63 biopsy specimens, 32 of which showed piecemeal necrosis (17 – mild, 13 – moderate, and two – severe). Six specimens lacked acinar inflammation and 42 showed acinar inflammation but no necrosis. Acinar inflammation with hepatocyte necrosis was present in 20 specimens (14 – mild focal necrosis, two – severe focal damage, and four – bridging necrosis). Most specimens showed either no fibrosis (32) or portal tract enlargement only (11); three specimens showed periportal septa, three severe fibrosis falling short of cirrhosis, and 19 showed established cirrhosis. Portal lymphoid follicles were noted in 24 specimens; the lymphoid follicles varied in size and occasionally contained germinal centres. Steatosis was present in 35 specimens (26 – minimal or mild and nine – moderate or severe). In occasional biopsy specimens there was lymphocyte influx into bile duct epithelium. Scoring by the two observers independently was found to be broadly in agreement; most discrepancies were encountered between the grades of 1 and 2 for portal and acinar activity, better correlation was obtained for the higher grades of inflammation and for the fibrosis scores.

Patients were divided into two groups for each histological activity index score: those scoring 0–2 and those scoring 3–4 (moderate to severe chronic active hepatitis with severe focal cell damage or bridging necrosis, and architectural distortion or cirrhosis). HCV subtype 1b was associated with the highest percentage of patients scoring 3–4 in all three index scores (Fig 3). The index scores comparing HCV subtype 1b with other subtypes were tested using Wilcoxon-Mann-Whitney rank sum test, ranking index scores from 0–4 for 1b patients and comparing ranks with index scores for all other genotypes. A value for z of −1·93 was generated for the fibrosis index (p<0·05). Z values for portal activity and acinar activity were −1·61 and −0·79 respectively (both not significant). Index scores were also compared with age. All three histological activity scores showed a significant correlation with age (Fig 4A, B, C); the most significant correlation was seen with the fibrosis score (=0·659, p<0·0001). Comparison of ALT activities with histological activity scores showed a significant difference between acinar activity scores 0–4 (ANOVA p<0·05), but no significant differences between portal and fibrosis scores 0–4 (ANOVA p>0·05 for both).

Biopsy results were also grouped into three disease states from histology reports issued: chronic hepatitis, cirrhosis, and hepatocellular

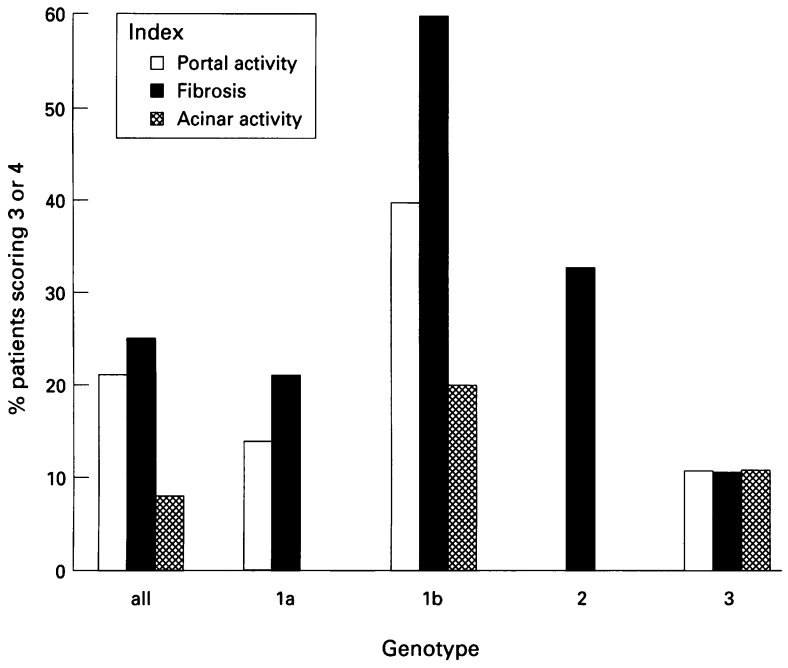

Figure 3: Liver biopsy histological activity index scores in relation to hepatitis C virus genotype.

carcinoma. Disease state was compared with duration of infection, risk factors, alcohol consumption, and ALT (IU/l, the highest score of three measured in the liver clinic). The mean duration of infection increased as the disease state worsened (Fig 5, ANOVA $p<0.002$). Risk factor analysis showed that a high proportion of intravenous drug users (who were in the younger, screened group) had chronic hepatitis (Fig 6). The mean age of patients who drank excess alcohol (defined as >21 U/wk for men >14 U/wk for women) was younger for each disease state than patients who did not drink to excess (Table II, chronic hepatitis: $t=1.82$, NS, cirrhosis: $t=2.28$, $p<0.05$, hepatocellular carcinoma, $t=2.70$, $p<0.02$). All 13 biopsy specimens in those patients who drank excess alcohol reported the presence of steatosis, five of 13 also reported Mallory's hyaline. A wide range of ALT activities was seen with no significant differences in ALT between disease states, due to the high SD values (ANOVA $p>0.05$).

DISEASE ASSOCIATIONS

Six of 27 intravenous drug abusers had evidence of past or current infection with hepatitis B. Eleven of 47 of the patients presenting for other reasons had evidence of past or current hepatitis B infection and one of 47 was also co-infected with delta virus. Two blood donors were HBcAb and HBsAb positive. Non-hepatic disease associations seen were three patients with fibrosing alveolitis, two with porphyria cutanea tarda, one with non-Hodgkin's lymphoma, and one with polycythaemia rubra vera.

Discussion

The prevalence of HCV infection in units of blood donated in north east England using the second generation anti-HCV ELISA is 0·13% (283 of 215 291). A lower prevalence of 0·014% (30 of 215 291) was confirmed positive by immunoblot. This may be an underestimate of the prevalence of HCV positive blood donors over the same period of time because some HCV negative donors may have donated more than one unit of blood during the 18 months of the study.

We have previously studied the prevalence of antibodies to HCV in different populations of patients in north east England using the first generation ELISA and RIBA tests; anti-HCV was detected in 0·18% blood donors (two of 1120).[17] This prevalence is low compared with other blood donor studies using first generation assays: reports were 0·61% United Kingdom,[16] 0·9% in Japan,[5] and 1·3% in Italy.[24] Our more recent blood study using second generation testing also shows a lower prevalence than other recently reported series in the United Kingdom. We find 0·014% positive on confirmatory immunoblot, compared with 1 in 1400 (0·07%) in north London,[25] and 1 in 2000 (0·05%) in a multicentre UK study.[26] Six of 10 633 (0·06%) blood donations from the study reported by Garson[16] were viraemic by HCV-RNA PCR assay and RIBA-2 positive or indeterminate, compared with 65 of 10 633 (0·6%) positive by first generation assays.

Despite the reduction in false positive results since the introduction of second generation tests, problems are still recognised. In this study, 283 blood donors were repeatedly reactive by ELISA-2 but only 30 of the 283 were subsequently confirmed positive by second generation immunoblot. Such discrepancies have been noted before,[27] and interpretation of the significance of RIBA negative and indeterminate results in ELISA-2 positive blood donors remains a problem. In one study, 100% of patients with indeterminate RIBA results and persistently normal liver function tests were PCR negative and had no liver disease on biopsy,[28] suggesting that these patients are false positives. Current and future developments in HCV supplementary test kits may help to improve the specificity of diagnosis. HCV RNA testing can also help to resolve these problems, and is now becoming widely available with the advent of commercially available HCV PCR kits. However, the problems are still not entirely resolved with PCR, as false positive (due to contamination) and false negatives (due to inadequate storage of serum specimens before testing,[29] and the problem of intermittent viraemia which can be seen in chronic HCV infection[30]) are well recognised with diagnostic PCR.[31]

The other asymptomatic group screened was intravenous drug abusers, a group previously noted to have a high frequency of HCV infection,[17] often with hepatitis B co-infection.[24] During this 18 month study, 27 asymptomatic intravenous drug addicts were seen and most were found to have HCV related liver disease on further investigation. Some 22% (six of 27) had evidence of current or previous hepatitis B virus infection. This association

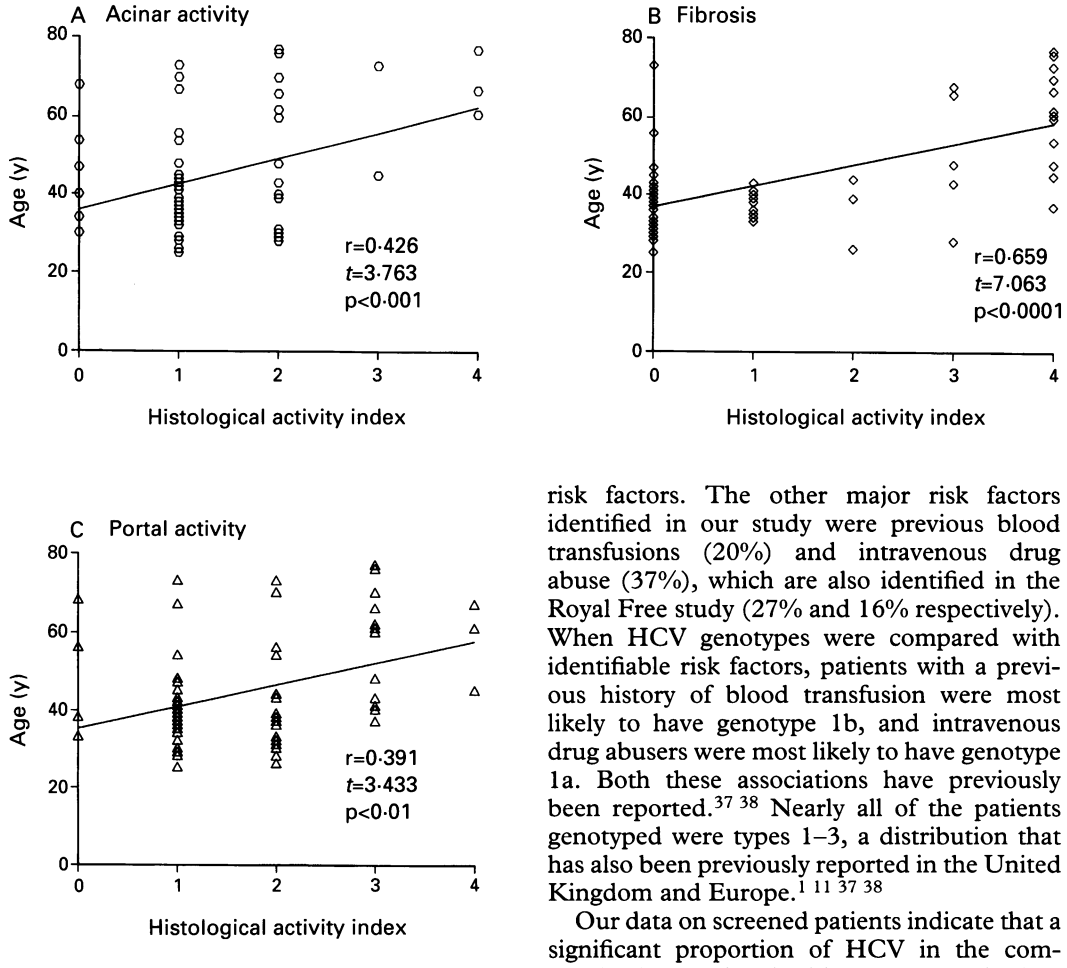

Figure 4: Liver biopsy histological activity index scores compared with age: A acinar activity; B fibrosis; and C portal activity.

was also seen in other HCV infected patients in the group studied: a total of 19 of 104 (18%) had evidence of current or previous hepatitis B virus infection, in contrast with estimates of approximately 0·1% in the UK population as a whole.[32] Other previously recognised non-hepatic disease associations with HCV such as fibrosing alveolitis,[33] porphyria cutaenea tarda,[34] and non-Hodgkin's lymphoma[35] were also seen in this study.

Analysis of identifiable risk factors showed a high proportion (24 of 104, 23%) with no obvious risks. This is similar to a recently reported study from the Royal Free hospital,[36] in which 20% (20 of 102) had no identifiable risk factors. The other major risk factors identified in our study were previous blood transfusions (20%) and intravenous drug abuse (37%), which are also identified in the Royal Free study (27% and 16% respectively). When HCV genotypes were compared with identifiable risk factors, patients with a previous history of blood transfusion were most likely to have genotype 1b, and intravenous drug abusers were most likely to have genotype 1a. Both these associations have previously been reported.[37 38] Nearly all of the patients genotyped were types 1–3, a distribution that has also been previously reported in the United Kingdom and Europe.[1 11 37 38]

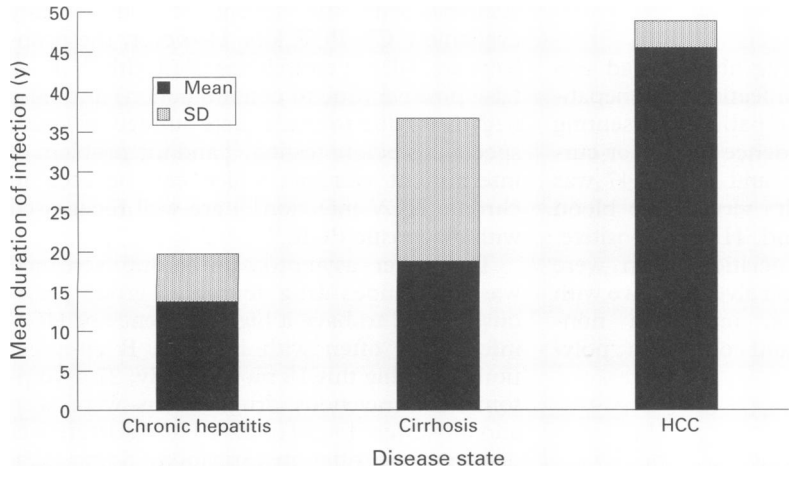

Figure 5: Mean duration of hepatitis C virus infection compared with liver disease HCC=hepatocellular carcinoma).

Our data on screened patients indicate that a significant proportion of HCV in the community is associated with asymptomatic liver disease. Currently, there is no way of diagnosing those asymptomatic individuals who do not donate blood, but the natural history of HCV related liver disease suggests that a proportion will develop symptoms in later life, having progressed to cirrhosis or hepatocellular carcinoma. This prolonged delay between HCV infection and symptomatic liver disease is reflected in our study in the comparison between the screened asymptomatic group and those hospital patients presenting with symptomatic disease. Blood donors and intravenous drug abusers were both significantly younger than patients diagnosed for other reasons, with the mean age of diagnosis differing by nearly 20 years between the screened and the symptomatic group. This is also reflected in the liver biopsy histology, with a difference in mean age of over 20 years in those diagnosed as having chronic hepatitis, compared with those with cirrhosis and hepatocellular carcinoma. Liver disease worsened significantly with age according to all three histological activity index scores. It could be that this is because older patients with mild disease are not symptomatic and have not been screened, and therefore do not present in this study. However, as well as the simpler measure of mean age, the mean duration of infection also rises as the disease state worsens. Although it would help our understanding of the natural history of HCV to screen large populations of asymptomatic elderly patients, it may not be justifiable in health economic terms with the prolonged course of HCV infection indicating that is is unlikely to progress and

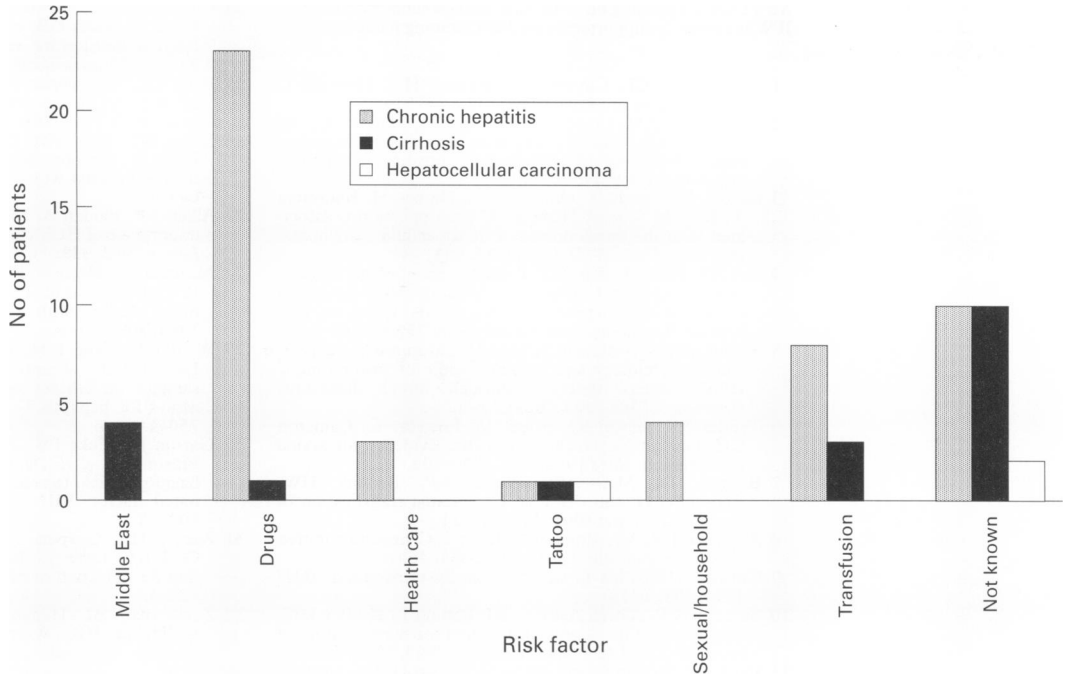

Figure 6: Association of liver disease with risk factors.

cause problems within the lifespan that remains to these patients. Excessive alcohol consumption is also seen to accelerate disease progression. Comparison of mean ALT activities with liver histology shows no significant association except with the acinar activity score, supporting previous reports that the absolute ALT activity is a poor indicator of the degree of liver disease seen on biopsy specimen.[26 39]

The effect of genotype on liver disease has also been studied. It has been reported that patients with genotype 1b have more severe liver disease.[1] The association remains controversial, and it has been postulated that patients with genotype 1b tend to be older and therefore could have developed more severe liver disease due to chronicity of infection rather than genotype.[11 40] In our study, liver disease was more severe with genotype 1b when the fibrosis scores from the histological activity index were analysed, and the effect was independent of age in the population studied as no significant difference in age was found between genotypes. Another possible explanation for the association of HCV genotype 1b with more severe liver disease is the route of transmission. In this study, post transfusion hepatitis was associated with genotype 1b, and intravenous drug abusers were more likely to have genotype 1a. A high proportion of drug abusers also had mild liver disease (chronic hepatitis). It could be postulated that the infecting dose in post transfusion hepatitis is much higher than in intravenous drug abuse and this could be responsible for the more severe disease associated with genotype 1b. There could of course also be an alternative pathogenetic mechanism related to HCV genotype, which is as yet undefined. The severe liver disease associated with genotype 1, in particular type 1b, has previously been documented, together with a reduced response rate to alpha interferon.[1 11 14] The response to alpha interferon was not analysed in this study because the number of patients within the group who have completed alpha interferon treatment was too small for statistical purposes.

In summary, our study of a predominantly British born white patient group indicates that a significant proportion of asymptomatic HCV positive individuals diagnosed by screening have liver disease. The severity of disease may be influenced by HCV genotype. Our results suggest that chronic infection of at least 20 years is required before presentation with symptomatic end stage liver disease, emphasising the insidious course of HCV infection and the need for effective screening of asymptomatic younger individuals. The issue of whether antiviral treatment should be offered to all asymptomatic individuals in an attempt to prevent progressive disease remains unresolved, particularly in view of results suggesting that patients with little inflammatory activity (previously termed 'chronic persistent hepatitis') may have a good prognosis.[42 43] The role of HCV genotypes in disease severity also needs further investigation. Future trials of therapy should be stratified for histological activity scores and HCV genotype.

TABLE II *Mean ages and liver biopsy results of hepatitis C virus infected patients who did and did not drink to excess (>21 u/wk for men, > 14 u/wk for women, hcc: hepatocellular carcinoma)*

	No excess alcohol		*Excess alcohol*	
Histology	*Mean (SD) age*	*No of patients*	*Mean (SD) age*	*No of patients*
Chronic hepatits	39·5 (9·6)	43	34·3 (6·0)	6
Cirrhosis	65·4 (7·8)	13	50·5 (15·1)	6
Hepatocellular carcinoma	70·0 (4·2)	2	62·0 (0·0)	1

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