A STUDY OF THE NATURAL HISTORY OF HEPATITIS C INFECTION WITHIN A GEOGRAPHICALLY DETERMINED POPULATION (TRENT HCV STUDY)

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PREFACE

Why study the natural history of Hepatitis C?

A thorough understanding of the natural history of Hepatitis C is needed to instruct patients and clinicians on the clinical course and prognosis of this infection. Only then can both parties make an informed decision on management. It also informs cost-benefit analyses, on which important decisions on the funding of therapy is made.

Since its discovery in 1989, much has already been written on the epidemiology and natural history of Hepatitis C. The patient population studied has an important bearing, however, on the results of epidemiological studies of disease progression in Hepatitis C. The majority of studies have focused on patients attending tertiary referral centres and exclude patients with co-morbidity such as hazardous alcohol consumption. The principle aim of the collection of studies that form this thesis is to enhance our understanding of the natural history of Hepatitis C by examining a geographically determined population that typifies those patients currently attending our hepatitis clinics.

SUMMARY

The epidemiology and natural history of Hepatitis C has been studied in a large geographically determined population (Trent HCV study).

It has previously been suggested that patients with Hepatitis C and a persistently normal Alanine aminotransferase (PNALT) represent a group of patients with mild disease and at low risk of disease progression. Patients with PNALT were, therefore, compared to those with an elevated ALT. The majority of patients initially fulfilling the definition of a PNALT had an abnormal ALT within 3 years of follow-up. They also demonstrated similar rates of fibrosis progression as a sub-group of HCV infected patients with an elevated ALT who were re-biopsied prior to any institution of therapy. They, therefore, warrant the same consideration with regard to treatment.

The morbidity and mortality associated with Hepatitis C with severe fibrosis was assessed in a group of patients with a liver biopsy demonstrating Ishak fibrosis stage ≥ 4. A worse prognosis than previously reported was observed for this patient population. Once decompensation develops, HCV infection is associated with a high mortality rate. Indicators of poor synthetic liver function and hypergammaglobulinaemia were important prognostic factors for mortality, while combination antiviral therapy was associated with improved survival.

The majority of HCV infected patients (75%) diagnosed with hepatocellular carcinoma (HCC) were known to have cirrhosis at least 6 months prior to

diagnosis of HCC and were, therefore, amenable to surveillance. There was a variable application of surveillance, however, and no significant improvement in survival was demonstrated. Age, duration of infection and immunoglobulin G levels were associated with an increased risk of HCC in cirrhotic patients in the univariate analysis. Achieving an SVR was associated with a reduced risk. No variable in cirrhotic patients was shown to be independently associated with HCC in the multivariate analysis.

A comparison of disease progression and treatment outcome in White and Asian (Indian subcontinent) patients was made. Asian patients generally presented at an older age and with more severe disease on biopsy. The patient's ethnic group was not associated with the likelihood of either an SVR or completion of therapy. Instead cirrhosis and a raised GGT were associated with a failure to achieve SVR in the multivariate analysis.

The platelet count is a surrogate marker for the severity of liver fibrosis and correlates with the Ishak fibrosis stage. An analysis of factors associated with an SVR was performed. In the multivariate model, age at start of treatment was the only independent predictor of SVR in Genotype 1, while estimated duration of infection and Ishak stage were predictors in genotype 2/3 patients. The platelet count was not an independent predictor of SVR or completion of therapy.

Keywords: Hepatitis C, Sustained virological response (SVR), Hepatocellular carcinoma (HCC), Ishak stage

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LIST OF ABREVIATIONS

AFP - Alphafetoprotein

AIDS – Acquired Immunodeficiency Syndrome

ALT – Alanine aminotransferase

AUC – Area Under the Receiver operator curve

BMI – Body Mass Iindex

CDC – Centers for Disease Control + prevention

CD (e.g 4/8) – Cluster of Differentiation

CI – Confidence Interval

CMV - cytomegalovirus

CRS – Cirrhosis Risk Score

DAA – Directly Acting Antiviral

DALY – Disability Adjusted Life Year

DNA – Deoxyribonucleic acid

EOTR – End Of Treatment Response

EVR – Early Virological Response

GGT – Gamma-Glutamyl Transferase

GTP – Guanosine Triphosphate

GUM – Genitourinary Medicine

HAI - Histological Activity Index

HAART – Highly Active Anti-Retroviral Therapy

HBV – Hepatitis B virus

HBcAb – Hepatitis B core antibody

HBsAg – Hepatitis B surface antigen

HCV – Hepatitis C virus

HCC – Hepatocellular Carcinoma

HES – Hospital Episode Statistics

HIV – Human Immunodeficiency Virus

HLA - Human Leucocyte Antigen

HOMA-IR – Homeostasis Model of Insulin Resistance

HR – Hazard Ratio

IDU/ IVDU – Intravenous Drug User

IFN - Interferon

Ig – Immunoglobulin

IL - Interleukin

IMPDH – Inosine Monophosphate Dehydrogenase

ISG – Interferon Stimulated Gene

LDL – Low-Density Lipoprotein

MW – Mann-Whitney

NAT – Nucleic Acid Testing

NBA – National Blood Authority

NHANES – National Health and Nutrition Examination Survey

NHSCR – National Health Service Central Register

NR – Non-Responder

NS – Non-Structural

ONS – Office of National Statistics

OR – Odds Ratio

PCR – Polymerase Chain Reaction

PEG - Pegylated

PNALT – Persistently Normal Alanine Aminotransferase

QALY – Quality Adjusted Life Year

RNA – Ribonucleic acid

RR – Responder-relapser

RVR – Rapid Virological Response

SD – Standard Deviation

SEER – Surveillance Epidemiology and End Results

SHR – Standardised Hospitalisation Ratio

SMR – Standardised Mortality Ratio

SNP – Single Nucleotide Polymorphism

STD – Sexually ransmitted disease

SVR – Sustained Virological Response

UK – United Kingdom

US/ USA – United States of America

UAPMP – Unlinked Anonymous Prevalence Monitoring Programme

VA – Veteran Affairs

YLD – Years of healthy Life lost to Disability

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Chapter 1. Introduction

1.1 The Hepatitis C virus

Hepatitis C (HCV) is a positive sense single strand, enveloped RNA virus, which constitutes a separate genus (hepacivirus) within the *Flaviviridae* family. The HCV genome was defined in 1989 [1]. It contains a highly conserved 5‘ untranslated region, preceding the translation initiation codon. This is the target of diagnostic testing for HCV as because it is highly conserved it is suitable for PCR primers. There is also a 3‘ untranslated region of variable length. The N-terminal quarter of the genome encodes the structural proteins; a non-glycosated nucleic acid binding nucleocapsid protein (core, C) and membrane associated glycoproteins (envelope, E1 and E2/ NS1). The rest of the genome encodes the non-structural proteins NS2-NS5[2-3].

The investigation of the HCV life cycle and pathogenesis has been hampered by the lack of efficient cell culture systems and small animal models. Recent advances, including the development of HCV replicons and infectious viral particles in tissue culture systems [4-5] and the establishment of an in vitro model of HCV virions [6-7] expands the tools available for HCV study. The virus mainly replicates in the hepatocytes, but can also replicate in peripheral blood mononuclear cells. The virus enters cells, through a complex interaction between virions and cell surface molecules, including CD81, SR-BI, claudin-1, occludin, LDL receptor and DC-SIGN [8]. Once in the cell cytoplasm the virus is uncoated and the genome transcribed to form a complementary negative sense RNA molecule, which in turn is translated into the progeny positive

strand RNA molecules. The enzyme capable of performing both steps of RNA synthesis is the virally encoded RNA dependent RNA polymerase NS5B[9]. The virus is not directly cytopathic to hepatocytes and persistent infection appears to rely on rapid production of virus and continuous cell-to-cell spread, along with a lack of vigorous T-cell immune response to HCV antigens.

HCV has a wide variety of genotypes (1-6), defined by the sequence of genetic material within the virus. It mutates rapidly due to rapid viral replication (10^{10} to 10^{12} virions per day) and a high error rate on the part of the NS5B coded RNA dependent RNA polymerase. HCV is, therefore, highly heterogeneous, existing as a collection of virus quasispecies [10]. Mutations are most pronounced in the hypervariable regions of the envelope proteins. The virus, therefore, typically escapes immune surveillance by the host and as a consequence most patients develop chronic infection [2-3]. Moreover such heterogeneity hinders the development of vaccines [11].

1.2 Epidemiology of Hepatitis C

1.2.1 Prevalence and incidence of Hepatitis C

In 1999 the World Health Organisation (WHO) estimated that the worldwide seroprevalence (positive HCV antibody) of Hepatitis C infection was approximately 3%, with the virus infecting 170 million people [12]. There is wide geographic variation with estimates ranging from < 1% in Northern Europe to greater than 5% in Northern Africa. In Europe higher seroprevalence rates > 1% are reported from Italy and France [13-14], with higher levels still in Eastern Europe (median 2%) [15]. In the United States nearly 2% of the population are infected [16]. The United Kingdom is among countries with the lowest seroprevalence, with the Health Protection Agency reporting a seroprevalence of antibodies to HCV (anti-HCV) of 0.44% in individuals aged 15-59 years living in England and Wales [17]. It is estimated that there are approximately 200,000 HCV infected individuals living in the United Kingdom.

As well as geographic variation, there are temporal differences in the pattern of HCV infection. Countries, such as the United States, Australia, Spain, Turkey, Italy and Japan have a similar overall seroprevalence of HCV infection (1-1.9%), but differ in their age specific seroprevalence. In the United States and Australia persons 30-49 years old account for two-thirds of those infected, with lower than average rates among those less than 20 years and greater than 50 years old. In contrast it is those persons greater than 50 years old who in Spain, Turkey, Italy and Japan account for most infections. These patterns indicate

that most HCV transmission occurred in the last 20-40 years in the US and Australia and twenty years earlier to this in Spain, Turkey, Italy and Japan [18]. The highest seroprevalence has been reported from Egypt (13-24%), equating to more than 10 million anti-HCV positive patients and with high rates of infection observed in all age groups [19-20].

Determining the incidence of HCV infection is difficult due to most acute infections being asymptomatic and laboratory assays being unable to distinguish acute from chronic infection. In the United States HCV incidence has been modelled using age-specific incidence from reported cases of acute disease (identified by detectable HCV RNA while HCV antibody negative) [21] and age-specific seroprevalence from a cross-sectional national survey (National Health and Nutrition Examination Survey – NHANES) [22]. The model demonstrates that the estimated annual incidence of HCV infection was low (18 per 100,000) prior to 1965, rose steadily through to 1980 and then remained high (130 per 100,000) through to 1989 [23]. Because chronic liver disease may develop many years after infection, the past incidence is a major determinant of the future burden of HCV associated complications [24]. Since 1989 the incidence of reported cases of HCV in the United States has declined by 80% [25]. According to the Centers for Disease Control and Prevention (CDC) HCV infection in the United States has fallen from approximately 230,000 per year in the 1980s to 19,000 per year in 2006 [26]. In a complex Markhov model, Davis et al estimated that the seroprevalence of HCV infection peaked in the United States in 2001 at 3.6 million and will fall to half that number by 2030 [27].

High incidence rates persist in African countries and in Egypt in particular, where the incidence rate ranges from 80 to 680 per 100,000 depending on the background seroprevalence [28]. More than 60% of acute infections are in persons below the age of 15 years and high incidence rates (1410 per 100,000) have been detected in Egyptian children younger than 10 years of age living in households with an anti-HCV positive parent [28-29].

In the UK a back calculation method based on knowledge of age-specific HCV progression and using information on deaths from HCV-related hepatocellular carcinoma from the Office of National Statistics (ONS), together with age specific prevalence of end stage liver disease from Hospital Episode Statistics (HES) records allows estimates of incidence in the distant past. By this approach it is suggested that again there was an increase in incidence until the late 1980s, peaking at around 14,900 (95 % CI: 8,000 – 28,100) new infections per year in 1988 [17]. Estimates of current HCV incidence in the UK are based on studies of intravenous drug users (IDUs). Mathematical modelling using seroprevalence data in those attending a range of specialist services for drug users in England and Wales estimated the incidence in susceptible IDUs between 1999 and 2003 [30]. The incidence of infection was 16% in the first year of injecting declining to 6% thereafter. Other studies have estimated the incidence by identifying individuals with recent infection, as evidenced by the presence of HCV RNA in IDUs who are anti-HCV negative. A London-based study[31] using stored serum from anti-HCV negative current IDUs (1999-2001) and a surveillance study[32] (2002-3) involving IDUs having HCV tests

in five English regions estimated the incidence at 14.3 and 12.9 per 100 person years respectively.

As well as reporting the seroprevalence of HCV infection in the United States, the NHANES study has provided a picture of the HCV infected population. In the context of an overall seroprevalence of 1.8%, seroprevalence was higher in men (2.5% vs 1.2%, $P<0.05$) and in non-hispanic blacks (3.2% vs 1.5%, $P<0.05$) as compared to non-hispanic whites. 65% of all those with HCV infection were aged 30-49 years. There was also a higher seroprevalence in those living below the poverty level (3.2% vs 1.6% $P<0.05$) and in those who had completed 12 or fewer years of education (2.8% vs 1.3%, $P<0.05$). Neither sex nor ethnic group were associated with HCV infection independently of socio-demographic and behavioural risk factors. On the other hand living below the poverty level (OR 2.37, 95% CI 1.5-3.75) and 12 or fewer years of education (OR 1.92, 95% CI 1.01-3.67) were independently associated with HCV infection [22]. In a study of the epidemiology of HCV in a UK regional population of approximately 5 million, the male to female ratio was 2:1 and 95% were Caucasion [33]. Since 1990 the Public Health and NHS Laboratories in England/ Wales have reported confirmed cases of HCV infection. Between 1996-2007 69% of reported infections were in men and 51% were in individuals aged 25-39. These figures were similar for IDUs and those where no risk factor was identified [34]. In 2008 61 blood donors tested positive for HCV (31 per 100,000 donations). The ethnic origin was known in 56/61. 43(77%) were white and 8 (14%) were South Asian (Pakistan, India, Bangladesh). The proportion of donations from new white donors that tested

positive for HCV was 26.5 per 100,000 donations (0.03%) compared to 185.3 per 100,000 donations (0.19%) in South Asian Individuals[34].

1.2.2 Risk factors for transmission of Hepatitis C

Hepatitis C is most likely to be transmitted through large volume or repeated direct percutaneous exposure to blood, for example through blood transfusion from infected donors, unsafe therapeutic injections or injecting drug use. Three characteristics – an abnormal alanine aminotransferase (ALT), history of IDU and a history of transfusion prior to 1992 identified 85% of HCV RNA positive individuals aged 20-59 years within the NHANES study [16]. Table 1.1 details the main risk factors for HCV transmission. Historically blood product transfusion has been a major mode of transmission. The introduction of HCV screening in the United States in 1990 reduced post transfusion HCV from 3.84% to 0.57% per patient (0.03% per unit of blood)[35]. In England estimates for the frequency of HCV infected donations dropped from 1:520,000 (1993-98) to 1 in 30 million (1999-2001) when all donations were tested for HCV RNA[36]. Nucleic acid testing (NAT) has the advantage over anti-HCV testing in that the potential "window period" between infection and a positive test is shortened. Individuals who received multiple transfusions, including those with thalassemia or haemophilia, have been at particularly high risk of developing hepatitis C. The seroprevalence of anti-HCV in haemophiliacs who regularly received concentrates of clotting factors before adequate procedures were used to inactivate viruses was 76-99% [37-38]. The risk of transfusion associated HCV remains an important risk factor in other areas of the world that lack the resources to implement adequate donor

screening and continue to use commercial donors to supplement their blood supplies [39-41].

Intravenous drug use is an efficient means of HCV transmission and the predominant mode of transmission in the United States and Europe. In a study of 716 IDUs from Baltimore, USA, the seroprevalence among those who had injected for one year or less in 1998-9 was 64.7% [42]. Cumulative seroprevalence rates in IDUs living in New York City during the first 2-3 years of injecting have declined from 80% during the late 1980s to 30% in the late 1990s[43], most likely due to heightened awareness and needle exchanges motivated in principle by concern for HIV risk. In 2007, 42% of current and former IDUs from England who participated in the Unlinked Anonymous Prevalence Monitoring Programme (UAPMP) survey had positive salivary HCV antibodies. There were regional variations in seroprevalence from 21% in North East England to 58% in London and 60% in the North West. One in four injectors reported sharing needles and syringes[44]. Another UK (London) study found that 44% of IDUs below the age of 30 were anti-HCV positive compared to 4% for HIV[45]. The seroprevalence of anti-HCV among people who acquired HIV through IDU is approximately 90%[46]. Of those laboratory confirmed HCV infections in England between 1996 and 2008, in which risk factor for acquisition was reported, 92.5% were as a consequence of IDU[17].

There is a high seroprevalence of HCV in prison inmates. In Rhode Island, United States, 23.1% of inmates in an adult correctional facility were HCV positive, compared to 1.8% for HIV [47]. The higher seroprevalence is also a

consequence of IDU, with a study of four Scottish prisons in 1994-6 demonstrating positive salivary HCV antibodies in 49% of injecting inmates compared to 3% of non-injectors[48]. In a follow-up study of the incidence of HCV infection in prison inmates conducted between 1999-2000, the incidence per 100 person-years of incarceration risk for inmates who reported never having injected drugs, ever having injected drugs, having injected drugs during follow-up, and having shared needles/syringes during follow-up were 1, 12, 19, and 27, respectively[49].

In many developing countries unsafe therapeutic injections performed by both professionals and non-professionals remains an important route of HCV transmission. It is estimated that 2 million HCV infections are acquired annually as a consequence of contaminated health care injections and that this may account for 40% of all HCV infection worldwide[50]. The re-use of glass syringes during a mass treatment campaign of parenteral Schistosomiasis therapy may have played a major role in the spread of HCV throughout Egypt [51]. In the District Buner study in Pakistan (1998-2002), which demonstrated a seroprevalence of HCV infection of 4.6%, all 751 anti-HCV positive individuals had a history of injection[52]. With the adoption of universal precautions, nosocomial infection had declined in developed countries as a mode of HCV transmission, though in the 1990s seroprevalence rates of 10-50% were reported among haemodialysis patients [53-55]. Health care workers are also judged to be among those at risk of HCV infection, though the reported seroprevalence is no greater than found in blood donors [56]. A

contaminated needlestick injury is associated with an anti-HCV seroconversion in 1.2% of cases [57].

One of the most controversial areas of Hepatitis C epidemiology is the extent to which HCV is transmitted by sexual activity. Phylogenetic analysis enables the genetic relatedness of viral isolates in sexual partners to be determined, but even then studies on the sexual transmission of HCV are limited by the potential of the confounding variable of IDU or shared items such as razors between sexual partners. In the NHANES study, the number of sexual partners (OR 2.54 for 2-49 partners) and age at first sexual intercourse (OR 2.94) had a significant correlation with anti-HCV [22]. The evidence for sexual transmission is also supported by a study of non IDU women with an injecting partner, in which 15% of the women were positive for HCV[58]. On the other-hand a 10 year prospective study evaluated transmission in 895 monogamous spouses (providing a follow-up period of 8060 person-years) and showed no evidence of heterosexual sexual transmission [59]. There is also a lack of evidence for significant sexual transmission among men who have sex with men [60], except in those with HIV co-infection [61]. Overall sexual transmission of HCV is believed to be rare, though may be less so in higher risk populations. In a review of the subject the incidence of HCV seroconversion in long-term monogamous couples was reported as 0-0.6%/ year and for individuals with multiple partners or a history of sexual transmitted diseases 0.4-1.8%/ year. Studies in monogamous, heterosexual partners of HCV-infected, HIV-negative persons, demonstrate a frequency of antibody-positive and genotype-concordant couples of 2.8% to 11% in

Southeast Asia, 0% to 6.3% in Northern Europe, and 2.7% in the United States[62]. The risk of sexual transmission appears to be higher when the index case is co-infected with HIV, with anti-HCV seroprevalence 2.2 times higher compared to partners of those with HCV only [63]. The increased risk of transmission in HIV co-infection probably relates to the enhanced HCV viraemia associated with co-infection.

Perinatal transmission of HCV has been the subject of a systematic review which included studies published between 1992-2000. The perinatal transmission rate was 1.7% in infants of anti-HCV positive mothers irrespective of HCV RNA, 4.3% when the mother was HCV RNA positive and 19.4% if the mother was co-infected with HIV [64].

There are a wide variety of other activities that may pose a risk for HCV transmission. These include tattooing, body piercing, acupuncture, intranasal drug use and religious or cultural practices. There is insufficient data, however, with which to determine their contribution to overall HCV transmission, though it is likely to be small.

1.2.3 Molecular epidemiology

In addition to the geographic variation in seroprevalence of HCV, there are also region-specific patterns in terms of the virus' genome, which have arisen during the evolutionary history of HCV in different human populations. Variants are classified into six major viral genotypes, each with a number of closely related subtypes. Some HCV genotypes (1, 2 and 3) are distributed

worldwide, while others are confined to more restricted geographical areas. Type 1a is most frequently found in Northern Europe [65-66] and North America [69], while 1b is the most common genotype in Japan[70], Southern and Eastern Europe [65-66]. Type 2 is less frequent than type 1, though type 2c is common in Italy [71]. Type 3 is endemic in South-East Asia [66] and the Indian Sub-continent [72]. It is also particularly prevalent in IDUs in the United States and Europe [73-74]. A study of 567 individuals, including IDUs, Haemophilia patients, blood donors, antenatal patients and those attending genitourinary (GUM) and liver clinics showed that genotype 1 (1a 32%, 1b 15%) and 3a (37%) were the most prevalent genotype in England and Wales. The genotype distribution was similar in the different groups, except for Haemophilia patients in whom there was a lower frequency of genotype 3a (21%)[75].

Of the rarer HCV genotypes, type 4 is principally found in the Middle East, Egypt and Central Africa[76], type 5 is almost exclusively found in South Africa[77] while type 6 is present in Hong Kong and Vietnam[78]. The geographic distribution of HCV genotypes may provide clues to the evolution of HCV. The presence in some regions of numerous subtypes may suggest HCV has been endemic there for a long time, whereas limited diversity of genotype could be related to recent introduction of the virus to a region[79].

Table 1.1: Risk factors for the transmission of Hepatitis C

Factor	Risk	Reference
Blood transfusion	**Risk of post transfusion HCV:** USA: Pre 1990 – 3.84%, Post 1990 (anti HCV testing) – 0.57% Japan: Pre 1990 – 4.9%, Post 1990 (anti-HCV testing) – 1.9% Haemophiliacs – seroprevalence of anti-HCV 76-99% pre viral inactivation procedures **Frequency of infected donations:** England: Post HCV RNA testing 1 in 30 million	Donahue et al. [35] Japanese Red Cross [67] Brettler et al. [37] Mauser-Bunschoten et al [38] Soldan et al. [36]
Intravenous drug use	**Seroprevalence among IDUs:** USA (Baltimore) – 1998-9 – 64.7% USA (New York) – 1980s – 80% Late 1990s – 30% UK (IDUs below age of 30) - 2001 – 44% UK (current and former IDU) – 2007 – 42%	Garfein et al. [42] Des Jarlais et al. [43] Judd et al.[45] Health Protection Agency[68]
Prison	**Seroprevalence among prison inmates:** USA (Rhode Island) – 23% Scotland – 1994-6 - IDU inmates – 49%, Non-injectors – 3%	 Macalino et al.[47] Gore et al. [48]
Nosocomial	**Contaminated health care injections:** Estimated may account for 40% of infections worldwide Pakistan (District Buner) – all anti-HCV patients had a history of injection **Risk of seroconversion following contaminated needle stick injury**: Italy – 1992-3 - 1.2%	Hauri et al. [50] Muhammed et al. [52] Puro et al. [57]
Sexual	**Prevalence in Female sex workers:** 6% **STD clinic attendees (IDU excluded):** 1.6-7% **Heterosexual monogamous spouses of IDU:** South East Asia 2.8-11% Northern Europe 6.3% USA 2.7%	 Terrault [62]
Perinatal	**Risk in infants of HCV RNA positive mothers:** 4.3% **Risk in infants of HCV RNA positive / HIV co-infected mothers:** 19.4%	Yeung [64]

1.3 Natural History of Hepatitis C infection

1.3.1 Acute infection and spontaneous clearance of Hepatitis C

Acute HCV infection by definition must precede the development of chronic infection, but understanding of the natural history of the acute stage of HCV infection is limited. Acute HCV is usually defined as the new occurrence of viraemia, at which stage serum from the patient is usually HCV RNA positive and anti-HCV negative[80]. It is infrequently diagnosed as the majority of acute infections are asymptomatic [81-82], but is reported to account for approximately 10% of acute hepatitis in China [83], 8% in Japan [84] and 31% in Egypt [28]. The 25-30% of patients with acute HCV who develop symptoms, do so 3-12 weeks after exposure to the virus and as jaundice is often absent, fatigue may be the only manifestation [85-86]. Based on a study of healthcare workers who acquired HCV through accidental needlestick exposure, HCV RNA is detectable in the majority of patients within 1-2 weeks and a rise in serum transaminases is observed after 2-8 weeks, with ALT capable of reaching levels greater than 10 times the upper limit of normal [85]. Acute HCV is nearly always self-limiting though when symptoms are present they can persist for several weeks. Fulminant liver failure as a consequence of HCV is rare [87]. Anti-HCV seroconversion typically occurs near the onset of symptoms, approximately 3-12 weeks after exposure. Anti-HCV is unreliable, however, in the diagnosis of acute HCV as 30% of patients will test negative at the onset of symptoms [88]. Almost all patients will eventually develop anti-HCV, though titres may be low in the context of immunosuppression.

The majority of patients infected with HCV will go on to develop chronic infection, a fact attributed to the genetic diversity of the virus and its tendency to rapid mutation, thus allowing it to escape immune recognition. A proportion of patients, estimated as being between 20-40% [89], will however, spontaneously clear the virus, becoming HCV RNA negative. It is unusual for this to occur more than 6 months after the onset of acute infection and, therefore, persistence of HCV RNA for 6 months is indicative of chronic HCV infection. There are no reliable predictors of spontaneous resolution, though several factors have been reported to influence chronicity. Table 1.2 gives the details of those factors associated with spontaneous clearance of HCV.

The NHANES study demonstrated a chronicity rate of 30% in subjects aged less than 20 years and 76% for those more than 20 years [22]. Similarly the Dionysos study in Italy showed chronicity rates of 56% in residents aged 12-25 years and 87% in those over the age of 25 years [90]. Further evidence that age at infection influences the likelihood of spontaneous clearance comes from a long-term follow-up study of children with post-transfusion hepatitis, where only 55% remained HCV RNA positive in adulthood [91].

Although the NHANES [22] and Dionysos [90] studies showed similar rates of chronicity in men and women, there is evidence from elsewhere that the rate of chronicity is lower for women than men. Two large follow-up studies of women who received contaminated immune globulin, both showed a chronicity rate of 55% after approximately 20 years [92-93]. A study within an IDU population also showed women to have a significantly higher incidence of

viral clearance than men (age-adjusted hazard ratio, 2.91 [95% CI, 1.68-5.03]) [94]. Bakr *et al*. similarly found clearance rates to be significantly greater in women (44.6%) compared with males (33.7%, adjusted OR 1.77) in an Egyptian community [95]. Overall spontaneous clearance of HCV occurs in approximately 40% of women and 19% of men [89].

African Americans have a higher rate of chronic HCV infection than Caucasians or Hispanic Whites (African American 86% vs Caucasian 68%, P=0.02) [22]. The host immune genetics are important in determining the likelihood of spontaneously clearing HCV. HLA class II alleles DRB1*11 and DQB1*0301 have both been shown to be associated with spontaneous resolution of infection [96]. Recent genome-wide association studies have shed more light on the underlying host factors that may be involved in the ability to spontaneously clear virus and present a possible explanation for racial differences. A single nucleotide polymorphism (rs12979860) at a site close to the IL28B gene has been shown to influence clearance rates of HCV RNA. In a study of 1008 patients the presence of the C/C allele was associated with spontaneous clearance rates of 50-55% as compared to 16-20% for the T/T allele [97]. The C/C allele is more common in individuals of European ancestry compared to African ancestry. In a further study of 284 patients, the C/C allele was present in 73% of the 69 patients who spontaneously cleared HCV RNA, compared to 46% in those with persistent infection [98].

Table 1.2: Factors associated with spontaneous clearance of Hepatitis C infection

Variable		% cleared	RR (95% CI)	P value	Reference
Age	USA (NHANES) < 20 years > 20 years	70% 24%			Alter et al. [22]
	Italy (Dionysos) 12-25 years > 25 years	44% 13%			Bellentani et al. [90]
	Micallef et al. (Review) ≤ 30 years 31-50 years > 50 years	33% 42% 26%	reference 1.14 (0.83-1.60) 0.89 (0.69-1.16)	 0.36 (tested for trend)	Micallef et al. [99]
Sex	Micallef et al. (Review) Female Male	42% 20%	reference 0.43 (0.36-0.53)	 <0.001	Micallef et al. [99]
	Egypt (village residents) Female Male	45% 34%	1.77 (1.27-2.46) reference	0.001	Bakr et al. [95]
	USA (San Francisco IDUs) Female Male	32% 15%	2.91 (1.68-5.03) reference	0.01	Page et al. [94]
Ethnicity	USA (NHANES) a African Americans b Non-Hispanic whites c Mexican Americans	14% 32% 36%		a vs b 0.02 a vs c 0.02	Alter et al. [22]
Genotype	Micallef et al. (Review) 1 Non – 1	34% 31%	reference 0.96 (0.76-1.21)	 0.76	Micallef et al. [99]
	UK HCV national register 1 Non - 1		Reference 0.47 (0.29-0.78)	0.003	Harris et al. [100]
Immune Response	Micallef et al. (Review) Post transfusion Acute clinical hepatitis	18% 31%	reference 1.7 (1.24-2.32)	0.001	Micallef et al. [99]
	USA (IDUs) HCV only HCV/ HIV (CD4 > 500) HCV/HIV (CD4 < 200)	14% 8% 5%	reference 0.58 (0.24-1.42) 0.33 (0.14-0.80)	 0.23 0.01	Thomas et al. [101]

Jaundice during acute hepatitis was more common in patients with the C/C allele as compared to non-C/C patients (C/T and T/T), though interestingly despite the C/C allele being associated with spontaneous clearance, jaundice was not [102].

The host immune response plays a crucial role in determining the likelihood of spontaneous resolution of HCV. Viral clearance is associated with a robust and multispecific CD4+ and CD8+ T cell response in the blood and liver [85, 103]. In situations such as HIV co-infection with a CD4 < 200, where the host's ability to mount an appropriate immune response is compromised, rates of HCV chronicity are higher (adjusted OR for clearance in HIV negative patient, 2.19 [95% CI, 1.26-3.47]) [101]. Jaundice may result from a more robust immune response and there is evidence within IDU patients [104] and in the German contaminated Rhesus immune gobulin cohort [93] of lower rates of chronicity when there is a history of jaundice with acute HCV.

1.3.2 Chronic Hepatitis C and fibrosis progression

Infected individuals who do not achieve viral clearance are at risk of progressive liver disease, characterised by hepatocellular inflammation and fibrosis. Increasing hepatic fibrosis in chronic HCV may ultimately lead to cirrhosis and in time decompensated liver disease and/ or hepatocellular carcinoma. The interval between HCV infection and the subsequent development of significant liver disease is long, often measured in decades rather than years. In a Japanese cohort the mean interval between blood transfusion and diagnosis of HCC was approximately 29 years [105]. Similar observations were made in the United States with a mean interval between

blood transfusion and cirrhosis of 20.6 years and HCC 28.3 years [106]. A systematic review of 111 studies analyzing the natural history of HCV estimated that the prevalence of cirrhosis 20 years after infection was 16 % (95% CI 14-90%) [107]. This will vary, however, depending on the cohort studied. Sweeting et al. evaluated 971 patients with HCV from 3 separate cohorts and showed that the 20 year probability of cirrhosis varied from 6% in a cohort of transfusion recipients to 23% in a cohort from a tertiary referral centre [108]. Fibrosis progression is, therefore, highly variable and is influenced in particular by several host factors, such that some patients are without significant liver disease after many decades, while others rapidly develop cirrhosis.

Given that the natural history of infection may be over decades rather than years, progression of fibrosis on liver biopsy has been extensively used as a surrogate marker for the hard end points of cirrhosis (compensated and decompensated) and hepatocellular carcinoma. Such studies are of two types; cross-sectional and longitudinal. Both rely on scoring fibrosis on biopsy samples, usually either the METAVIR[109] or Ishak[110] Histological Activity Index (HAI) staging systems. Cross-sectional studies use a single liver biopsy and a known date of infection, usually the date of blood transfusion or the first year of intravenous drug use. These studies include large numbers of patients but rely on the accuracy of the estimate of duration of infection and assume that liver fibrosis progresses at a linear rate. The largest study, which included 1157 untreated patients demonstrated a median rate of fibrosis progression of 0.133 METAVIR fibrosis stages/ yr (0.125-0.143), which

equates to a median duration from infection to cirrhosis of 30 years (28-32)[111].

Longitudinal studies require 2 liver biopsies and assess the rate of fibrosis progression between them. With this method there is no assumption that fibrosis progression is linear or a requirement to estimate the duration of disease. The need for two biopsies means the number of patients in these studies is smaller and biased toward patients with mild disease, as it is these untreated patients who often have repeated biopsies to detect worsening. In a study of 123 patients from Maryland, United States with all stages of fibrosis on the initial biopsy and a mean interval between biopsies of 44 months, fibrosis progressed at a mean of 0.12 Ishak fibrosis stages/ year, with 39% of patients demonstrating worsening fibrosis between biopsies [112]. In a UK population of predominantly patients with mild liver disease on initial biopsy; 183/214 (85.5%) with Ishak stage 0 or 1 and a median interval between biopsies of 30 months, a third of patients progressed by at least 1 Ishak stage and 11% by at least 2 stages. This equated to an overall rate of fibrosis progression of 0.17 Ishak fibrosis stages/ yr [113].

Histological scoring systems for fibrosis do by necessity consist of rather arbitrary points on a continuum between normal and cirrhosis. Evaluation of the collagen proportionate area demonstrates that the relationship between the Ishak stage and the amount of fibrous tissue is non-linear. The greatest gains in fibrous tissue are seen between stage 3 and 5, with the higher stages in particular depending more on architectural change than fibrous tissue [114]. Since progression through each

stage does not follow a straight line, there has been justified criticism of studies such as those described above, that apply statistical analysis to the numerical value of the stages in order to calculate an annual fibrosis progression rate [114]. Though fibrosis progression between Ishak stages is non-linear, the stages have been shown using data collected from the HALT-C trial to have prognostic value. Using a composite clinical outcome that included an increase in Child-Pugh score to ≥ 7, ascites, encephalopathy, bleeding varices, hepatocellular carcinoma or death, the 6 year cumulative incidence of first outcome showed a remarkably linear increase through each stage – 2 (5.6%), 3 (16.1%), 4 (19.3%), 5 (37.8%) and 6 (49.3%) [115].

Several factors (Table 1.3) can accelerate fibrosis progression in a given individual. Age is the key factor enhancing fibrosis risk, with a stepwise reduction in the median duration to cirrhosis with advancing age at infection; from 44 years (40-48) in those infected aged 20 years or less, to 30 years (25-36) for those aged 31- 40 years and 12 years in those older than 50 years at the time of infection [111]. Age *per se* also appears to be important during infection with HCV, with the rate of fibrosis progression suggested to increase in those over the age of 50 years whatever the duration of infection [116]. These findings also strongly suggest that fibrosis progression is not linear, the risk increasing with increasing age. The means by which age influences fibrosis progression is uncertain but evidence taken from fibrosis progression in the post liver transplant setting suggests that it is liver factors which are the most important. Patients who received a liver from a donor aged less than 40

years progressed to cirrhosis after an average of 10 years, compared to 6.7 years if the donor was aged 41- 49 years and 2.2 years if 50 years or older [117]. An ageing liver might be more vulnerable to progression of fibrosis via an increased vulnerability to oxidative stress or a reduction in hepatic blood flow or mitochondria capacity.

Fibrosis progression is also greater where another cause of liver injury is present. Alcohol intake >50g/d has been consistently associated with an increased rate of fibrosis progression in HCV [111, 116, 118-120], the rate of fibrosis increasing by 34% in those drinking > 50g/d compared to non-drinkers[111]. In another study of 176 HCV infected patients; those who drank > 40g/d for women and > 60g/d for men had a 58% chance of having cirrhosis by the second decade of infection compared to 10% who drank non significant levels [119]. Lower levels of alcohol intake may still carry an increased risk. In a paired biopsy study of patients who drank < 40g/d, more progressive fibrosis was observed in those with higher levels of alcohol consumption (5.7 vs 2.6g/ day P=0.03) and a higher drinking frequency (34.5 vs 8.2 days/ year P=0.006) [121]. It is suggested that alcohol accelerates fibrosis progression in HCV through its effect on both the virus (increased viral load and increased mutation (quasispecies)) [122] and on the liver through increased apoptosis, steatosis, increased iron stores and through oxidative stress secondary to the increased production of oxygen free radicals [123].

Co-infection with other viruses also plays an important role in fibrosis progression. A meta-analysis of published data in HIV co-infection shows that

fibrosis progression is increased at least 2-fold [124]. Liver fibrosis progresses faster in HIV co-infected patients as compared to those with HCV infection alone, with expected time to cirrhosis of 26 years compared to 34 years [125]. A CD4 cell count of ≤ 200 cells/ μl was an independent factor associated with higher liver fibrosis rates suggesting it may be immunosuppression, as is also seen post liver transplantation [126] and in patients with primary hypogammaglobulinaemia [127] that influences fibrosis progression. Similarly, patients with co-infection with either Hepatitis B (n=19) or B and D (n=17) have been shown to exhibit more severe fibrosis at presentation than with HCV infection alone [128]. Conversely, HCV has been shown to accelerate progression of HIV to Acquired immunodeficiency syndrome (AIDS) [129].

Hepatic Steatosis is a frequent histological finding in chronic HCV infection and HCV core protein causes progressive steatosis in a transgenic mouse model [130]. Cross-sectional studies of HCV infected patients have shown higher grades of steatosis to be associated with an increased body mass index (BMI) and that these patients have higher serum levels of ALT, increased periportal necrosis and increased levels of fibrosis [131-132]. In patients with a known date of infection the estimated annual rate of fibrosis progression was higher in those with steatosis grade 3-4 (> 30% of hepatocytes affected) compared to lower grades [132]. Progression of fibrosis between paired biopsies is also more likely if patients had steatosis evident on the first biopsy[133]. This effect was seen mainly in genotype 3 patients, and it is well recognised that steatosis is more common in this group than with other genotypes [133-134]. Further evidence highlighting the importance steatosis

plays in fibrosis progression comes from a small study where a 3 month weight reduction programme led to reduction in steatosis on the second biopsy in 9/10 patients with 5/9 showing decreased fibrosis [135]. The mechanism is not understood but it is hypothesised that steatosis increases lipid peroxidation which in turn increases stellate cell activation [131]. It is also likely that genotype 3 exerts a direct cytopathic effect involving steatosis with reports of steatosis disappearing after successful antiviral therapy [136].

Steatosis in HCV infection is also intimately associated with insulin resistance, particularly in genotype 1 infection [137]. Increasing insulin resistance (homeostasis model assessment of insulin resistance - HOMA-IR > 2) correlates positively with the stage of fibrosis in HCV [138]. This association is independent of obesity and diabetes [139].

Given that steatosis is much more common in genotype 3 HCV infection it may be expected that this genotype would carry a greater risk of fibrosis but there is little concrete evidence to support this. Data from a UK registry suggests that genotype 1 infection is associated with a histological stage above the median compared to non-genotype 1 types (OR 2.03, 95% CI 1.07-3.83, P = 0.03) [100]. Indeed, there have been several other reports that genotype 1b is associated with more severe disease, but this may simply be a cohort age related effect as this genotype formed the first wave of infected humans in Europe [141-142]. Benvegnù *et al.*[143] examined histopathological severity in 429 HCV positive patients and found no association with genotype. In addition

Table 1.3: Factors associated with fibrosis progression in Hepatitis C infection

Variable		Estimated time (years) to cirrhosis - Median (95% CI)	P Value	Reference
Age at infection:	≤ 20 years 21-30 years 31-40 years 41-50 years > 50 years	44 (40-48) 38 (32-40) 30 (25-36) 20 (17-23) 12 (11-15)	< 0.001	Poynard et al [111]
Sex:	Female Male	36 (32-40) 26 (24-28)	< 0.05	Poynard et al [111]
Ethnicity:	African American Non African American	% cirrhotic by 2nd decade of infection - 9% 4th* - 22% - 28% - 51%	* <0.05	Wiley et al.[140]
Alcohol (daily):	Nil 1-49g ≥ 50g	32 (28-36) 28 (25-34) 24 (23-30)	< 0.05	Poynard et al [111]
	≤ 40g (women) or ≤ 60g (men) > 40g (women) or > 60g (men)	% cirrhotic by 2nd decade of infection - 10% - 58%		Wiley et al.[119]
HIV:	HIV infection Non- HIV infected	26 (22-34) 38 (32-47)	<0.001	Benhamou et al [125]
Hepatic steatosis:	a) Nil b) Grade 1-2 c) Grade 3-4	33 29 17	a v c = <0.001 b v c = 0.03	Adinolfi et al [132]
Genotype :	1a 1b 3	31 (20-44) 44 (36-50) 24 (18-32)	ns	Poynard et al [111]

no correlation has been reported between the level of viraemia and liver fibrosis outside of the transplant setting [144-145].

Hepatic iron overload is known to cause hepatic fibrosis and iron is an essential element in viral replication. Serum iron stores, though not necessarily hepatic iron concentration are frequently increased in chronic HCV [146-147]. The presence of iron staining on liver biopsy was associated with a higher stage of fibrosis in a study of 38 HCV positive patients[148], though there was no correlation between the amount of iron and fibrosis score. It has also been suggested that iron may be released from damaged hepatocytes [149], and the question of whether the relationship between hepatic iron and fibrosis progression in chronic HCV is one of cause or effect is unanswered. In a study of 164 HCV positive patients neither the C282Y or H63D mutation was associated with an increase in the hepatic iron concentration or in the grade or stage of the HCV related liver injury [150].

Male sex has been consistently shown to increase fibrosis progression. Poynard *et al* [111], showed male sex to be an independent risk factor for fibrosis progression with the median duration to cirrhosis reduced from 36 years for women to 26 years for men. A protective role for oestrogen, possibly by inhibiting the proliferation of stellate cells and fibrogenesis is proposed as an explanation for this difference.

The influence of ethnicity on the likelihood of spontaneous viral clearance has been described and may in part relate to IL28B polymorphism. The evidence for racial differences in terms of fibrosis progression is less robust. A retrospective examination of 112 African Americans and 243 non-African Americans suggested that significantly fewer African Americans had cirrhosis in the 3rd (18 vs 31%, $P \leq 0.05$) and 4th (22 vs 51%, $P \leq 0.05$) decades of exposure, though there was no difference in the overall mean fibrosis grade [140]. There were no differences in sex or alcohol consumption to explain the observations, though the study was not controlled for other potential confounders. Host genetic factors will clearly account for some of the variability in disease progression. IL28B polymorphism has been shown to influence biochemical and histological levels of inflammation in the post liver transplant setting, but not fibrosis progression [151]. An initial genome wide scan which included 24,823 candidate single nucleotide polymorphisms (SNPs) in 433 biopsy proven chronic HCV patients, identified 100 potential SNPs associated with an increased risk of advanced fibrosis, though the association only held for two in the validation cohort [152]. Further study by the same group identified a seven gene signature, termed the "cirrhosis risk score" (CRS), which consisted of those markers most predictive for developing cirrhosis. The CRS had an area-under-the-ROC curve (AUC) of 0.75 in the training cohort and 0.73 in the validation cohort [153]. The CRS has been further validated in cohorts of patients from Italy, Belgium and Germany [154-155]. Variations in genes encoding key cytokines involved in human fibrogenesis, in particular inheritance of high TGF-ß1 and angiotensinogen

producing genotypes have been found to be associated with fibrosis progression [156].

The route of transmission of HCV has been proposed as an important factor in disease progression. In a large French study of 5,749 patients a significant increase in the prevalence of cirrhosis was found in transfusion recipients (23.4%) compared to the IDU group (7.0%) [157]. This difference was independent of age at infection and duration of disease. The authors proposed that that this difference could be due to either the HCV genotype or an increased amount of injected viral inoculum associated with blood transfusion.

Necroinflammatory activity at liver biopsy has been shown in some [112, 158-159] but not all studies [111, 113] to be an independent predictor of fibrosis progression. Fontaine et al [158] found that of those patients with a low initial activity (METAVIR A0-A1) 13.3% had an increase in fibrosis compared to 43.8% if there was a high initial activity index (METAVIR A2-3) ($P < 0.01$). Cirrhosis developed in all patients with a high grade of necroinflammation on initial biopsy, when followed-up for at least 10 years [159]. The location of activated hepatic stellate cells has also been shown to correlate with areas of necroinflammatory injury further supporting an association [160].

1.3.3 Symptoms and extra-hepatic manifestations of Hepatitis C infection

In the absence of cirrhosis, the majority of individuals with Chronic HCV are asymptomatic or have mild non specific symptoms[161]. The most frequent complaint is fatigue, with other less common manifestations include nausea, anorexia, myalgia, arthralgia, weakness, and weight loss. Fatigue is a common complaint and in a study of HCV patients without cirrhosis versus healthy blood donors, fatigue was the most commonly reported symptom in both groups (62% vs 70%) [162]. Symptoms are rarely incapacitating and may be difficult to ascribe to liver disease; nevertheless, chronic HCV may lead to a decrease in the quality of life [163-164], which may in part be accounted for by awareness of infection[165], and which can be improved following successful treatment [166].

Extrahepatic manifestations are seen in 1-2% of patients with chronic HCV [167-168] and may be a direct consequence of HCV infection or secondary to the resulting immune stimulation. A mixed cryoglobulinaemia is the most common and was 11 times more frequent in a US Veterans HCV cohort than in a control population [167]. Immune complex deposition in various organs leads to fatigue, rash, arthralgia, Raynaud's phenomenon, vasculitis, renal disease and peripheral neuropathy. Other conditions possibly associated with HCV include a membranoproliferative glomerulonephritis, porphyria cutanea tarda, lichen planus, vitiligo, non-Hodgkins lymphoma, autoimmune thyroiditis, Sjögrens syndrome and a seronegative arthritis.

1.4 The "disease burden" of Hepatitis C

1.4.1 Mortality and morbidity secondary to Hepatitis C

The "disease burden" is the impact of a disease on the health of a population and includes its effect on longevity (mortality and years of life lost because of premature death); morbidity, including impairment of health and quality of life; and finance, including direct healthcare expenditure and indirect costs related to loss of income from premature deaths or disability. In terms of HCV; Morbidity in the advanced stages of the disease may include the development of hepatocellular carcinoma together with complications of portal hypertension and liver failure, including ascites, variceal haemorrhage and hepatic encephalopathy. Experts have estimated that HCV accounts for 20% of cases of acute hepatitis, 70% of cases of chronic hepatitis, 40% of cases of cirrhosis, 60% of cases of hepatocellular carcinoma (HCC) and 30% of liver transplants in industrialised countries [169]. Though it is suggested that the seroprevalence of HCV infection peaked in the United States in 2001 and will fall by half by 2030 [27], the same modelling points to the proportion of patients with cirrhosis reaching 45% in 2030 and the total number with cirrhosis to peak in 2020. Hepatic decompensation and liver cancer will be expected to continue to increase for a further 10 to 13 years after the peak in cirrhosis. Similar modelling for an English population suggests a doubling in the number of patients with HCV related cirrhosis and HCC between 2005 and 2015. The number of HCV infected people living with compensated cirrhosis is predicted to increase from 3705 (95% CI 2820-4975) in 2005 to 7550 (95% CI 5120-11640) in 2015 while those with decompensated cirrhosis or HCC will increase

from 1150 (95% CI 1055-1250) to 2540 (95% CI 2035-3310) [170]. These projections point to an increasing burden associated with HCV over the coming years.

Mortality statistics are often based on death certification and their quality is dependent on the accuracy with which the death certificate is completed. In a study of mortality rates in a UK cohort of HCV infected patients, HCV was mentioned on 23% of death certificates and only on 52% of those dying of liver disease [171]. Matching records from a sentinel surveillance scheme and death records suggested that the true number of deaths in the UK from HCV is 185-257% higher than recorded in mortality statistics [172]. Mortality data based on death certification data alone will, therefore, invariably underestimate mortality associated with HCV, though trends over time may still be of interest. The number of deaths in England due to end stage liver disease or HCC with any mention of HCV on the death certificate increased from 81 in 1996 to 230 in 2008 [34]. Analysis of HCV related mortality rates in the US over a similar period (1995-2004) showed a 123% increase in mortality during the study period (1.09 per 100,000 persons to 2.44 per 100,000) [173]. In the last year of study there were 7427 HCV related deaths (mean age 55 years) corresponding to 148,611 years of potential life lost.

A more accurate estimation of the mortality attributable to HCV comes from linkage studies that match persons within population [174-175] and clinic databases [171, 176-177] with national registries of deaths. The standardised mortality ratio (SMR) was 3 times higher than expected in a cohort of 2285

HCV patients identified from a secondary care database (Trent HCV study). Excess deaths were liver and drug-use related with a pattern between age and cause of death, with murder, suicide, and drug overdose mortality peaking between 36 and 42 years, while deaths from cirrhosis occurred at a median age of 51.4 and liver cancer 63.7. Age, sex, antiviral therapy (protective) and liver fibrosis were predictors of all cause mortality while age, antiviral therapy, liver fibrosis and mean alcohol consumption predicted a liver-related death [171]. The largest population study included 75834 persons with HCV (and 2064 with Hepatitis B co-infection) notified to the New South Wales, Australia health department between 1990 and 2002. 5.3% of HCV infected patients died during the study period (7.1% with co-infection) and the all-cause mortality was again approximately three times that expected (SMR 3.1 [95% CI 3.0-3.2]). The all-cause mortality in Hepatitis B co-infected patients was higher still, with an SMR of 5.6 (95% CI 4.8-6.6). The liver-related SMR was 16.8 (95% CI 15.4-18.3) and 32.9 (95% CI 23.1-46.7), and the drug use-related SMR was 19.3 (95% CI 18.1-20.5) and 24.7 (95% CI 18.2-33.5) in mono-infected and co-infected patients respectively [174]. There was again a bimodal distribution of death with younger people dying of lifestyle related problems and a later peak from liver disease. Both these studies may have underestimated mortality relating to HCV, in that they both excluded deaths in the first 6 months [174] or year [171] after diagnosis without a corresponding adjustment in the time at risk which remained from the time of diagnosis. Authors of the Australian study showed that correction of this methodological flaw slightly increased the observed SMRs [178]. A similar threefold increase in mortality was seen in a study of HCV infected and non-infected US blood

donors, including a significantly increased mortality from liver disease, drug and alcohol use, trauma/ suicide and perhaps unexpectedly cardiovascular causes [179].

A higher mortality was seen for 20163 HCV infected persons and 4637 HIV co-infected persons living in Scotland between 1991 and 2005 [175]. The all cause SMR was 5.5 (95% CI 5.5-5.7) and 33.8 (95% CI 28-36.1), and the mean age at death 45.4 years and 38.4 years for mono-infected and HIV co-infected patients respectively. The majority of deaths were related to drug-use (30%), but liver disease contributed significantly (26%) and was ahead of circulatory (11%) and neoplastic (8%) causes.

Assuming an average life expectancy in Northern European populations of mid-70s for men and mid-80s for women then collectively mortality data suggests a reduction in overall life expectancy in HCV infected individuals of 8-12 years [180].

The morbidity associated with HCV and the burden for people living with the condition can be described in terms of "years of healthy life lost to disability" (YLDs). Disability refers to any departure from a state of perfect health and YLDs are weighted according to the severity of the condition. Using data collected by the WHO[181] it is suggested that across the WHO European region HCV caused 200,104 YLDs in 2002, with 96% due to HCV related cirrhosis. The "disability adjusted life year" (DALY) is a time based measure that combines years of life lost due to premature mortality and years lost due to

time lived in a state of less than full health. 1.2 million DALYs were lost due to HCV in the WHO European region in 2002, which corresponds to 134.54 DALYs per 100,000 residents. HCV related cirrhosis accounted for 81% of lost DALYs and the overall figure for HCV was similar to that for HIV/ AIDS and stomach cancer [182].

Linkage studies of HCV notifications and hospital admission records also demonstrate an increase in hospitalisation associated with HCV. In the New South Wales, Australia cohort described earlier, rates of hospital admission were 42% higher than expected. Standardised Hospitalisation ratios were calculated using rates of hospital admission for the New South Wales population by 5 year age group, sex and calendar year of hospitalisation. The greatest excess was in 15-19 year olds (Standardised Hospitalisation ratio, SHR, 3.8 [95% CI 3.4-4.2]). Lifestyle factors accounted for the highest absolute and relative rates in young adults while liver disease contributed the greatest burden in older adults [183]. In a similar study of a Scottish cohort the any diagnosis SHR when adjusted for age, sex and social deprivation was 3.4 (95% CI 3.3.-3.5) and for liver related admissions 41.3 (95% CI 39.6-43)[184].

1.4.2 Hepatocellular carcinoma in Hepatitis C infection

Hepatocellular cancer (HCC) is the third most common cause of cancer death in the world (approximately 600,000 deaths/ year). The poor prognosis means that the number of deaths is almost the same as the number of cases [185]. Chronic HCV is a major risk factor for HCC and together with Hepatitis B

accounts for 80% of HCC worldwide [186]. Markers of HCV infection are found in 44-66% of HCC cases in Italy [187-189], 27-58% in France [190], 60-75% in Spain [191] and 80-90% in Japan [192]. In a meta-analysis of 21 case control studies HCC was increased 17-fold (95% CI 14-22) in HCV infected patients [193].

HCC is currently one of the fastest growing causes of cancer-related deaths in many developed countries, with age-adjusted incidence rates in the US having increased more than 2 fold from 1985 to 2002 [194-195]. Much of the increase in HCC observed in the developed world can be ascribed to HCV infection [196-197]. In several European countries the age standardised death rate from HCC correlates with the seroprevalence of HCV [198]. In a study of persons ≥ 65 years from the US SEER-medicare database the incidence of HCV related HCC increased by 226% between 1993 and 1999 compared to a 67% increase for HBV related HCC [199]. Incidence rates for HCC in Japan tripled between 1970 and 2000 and the proportion due to HCV increased from 49% of cases in 1982, to 61% in 1990, and 81% in 2003 [200]. Since 1995 the rate of the rise in HCC in Japan has slowed and this may predict future trends in HCC incidence elsewhere given the different temporal trends in HCV transmission between regions described earlier.

The HCV virus unlike HBV is not able to integrate into the genome of the infected cell. It typically, therefore, causes HCC via the indirect route of chronic inflammation, cell death, proliferation and cirrhosis. HCV related HCCs are, therefore, almost exclusively found in patients with cirrhosis and the

majority occur 25-30 years after the onset of infection [106, 201]. The rare cases of HCC in HCV infected patients without cirrhosis raises the possibility of a direct pathway mediated through viral protein expression, with the transforming potential of NS3 and core-protein described [202]. The rate of HCC development in HCV infection is 1-3% after 30 years [203]. Once cirrhosis is present then an annual rate of 1-4% is observed [204], though as high as 7% is reported in Japan [205]. Prospective studies in Europe have shown too few HCC in non-cirrhotic patients to calculate an incidence rate [206-207], but in Japan the incidence per 100 person years rises with incremental increases in liver fibrosis (F0/1: 0.4, F2: 1.5, F3: 5.1 and F4: 6.9 per 100 person years) [208]. The annual incidence of HCC in the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) study [209] was 1.4% in the cirrhotic group and a surprisingly high 0.8% in the non-cirrhotic group, though all patients entered in this study had significant fibrosis (Ishak fibrosis stage 3-6).

There are a number of risk factors and co-factors that influence the likelihood of developing HCC in association with HCV infection. Those factors that accelerate fibrosis progression which were described earlier will shorten the time to cirrhosis and consequently also to HCC. These include age (2-4 fold increase in HCC in those over 55 years [210-211]) and male sex (2-3 fold increase over females [212]). Co-infection with HBV significantly increased the risk of HCC in the Italian Brescia HCC study, with an OR 165 (95% CI 81-374) for HBV/ HCV co-infection compared to 17 for HCV and 23 for HBV alone [213]. Markers of prior HBV infection (anti-HBc and/or anti-HBs) are

also associated with a 2 fold excess risk of HCC in patients with HCV cirrhosis [205]. The Brescia HCC study together with others have also showed a synergy between HCV and alcohol in the pathogenesis of HCC, with a 2-4 fold excess risk in those consuming greater than 60-80g of alcohol/ day [214-215]. Type 2 diabetes is associated with a 1.5 fold increase in the risk of HCC in the presence of other major risk factors for HCC such as HCV, but also alcohol and HBV [216]. The increased risk associated with diabetes may simply reflect the increased prevalence of steatohepatitis in the diabetic population as hepatic steatosis is known to increase the risk of HCC in conjunction with HCV [217]. Type 2 diabetes is also associated with hyperinsulinaemia which may act as a potential hepatocarcinogen [218]. Caucasians are 2-3 times less affected by HCC than African Americans who in turn are 2-3 times less affected than Asians, Pacific Islanders and Native Americans in the United States [194]. While much of the race difference probably relates to prevalence and acquisition times for the major HCC risk factors (HCV/ HBV), these ethnic differences are also observed within the HCV infected population. In a case control study of 464 patients with HCV and cirrhosis (207 cancer patients and 257 controls) the cancer risk was increased significantly among Asians (adjusted OR, 4.3 [95% CI, 2.1-9.0] for men, and 4.6 [95% CI, 1.2-18.5] for women) and somewhat increased among African-American men (adjusted OR, 2.4 [95% CI, 0.9-6.3]) [219]. It is less clear whether hepatic iron is associated with an increased risk of HCC in HCV with studies both in support [220] and against [221] hepatic iron being more frequently increased in HCV patients with HCC compared to those who are HCC free.

1.4.3 Liver transplantation and Hepatitis C

End-stage HCV accounts for more than 40% of liver transplants in Europe and North America [222]. Between 1990 and 2000 there was a 5-fold increase in the number of orthotopic liver transplant recipients with HCV in the US and the proportion of recipients with HCV increased from 12 to 37% [223]. More than a third of patients transplanted for HCV will have HCC [224]. Recurrent HCV post transplantation is universal in patients who are viraemic before transplantation and the natural history follows an accelerated course compared to the pre-transplant setting. Most patients will develop histological features of acute hepatitis C between 4 and 12 weeks post transplant [225], associated with a steep rise in HCV viral load [226]. Within 5 years of transplantation, 10-30% of patients will have progressed to cirrhosis [227] and this increases to more than 40% after 10 years [222]. Once cirrhosis develops the rate of decompensation is also higher at more than 40% at 1 year and 70 % at 3 years [228-229] compared to less than 5% and 10% in the non-transplant setting [211]. Patient survival following the onset of allograft failure is less than 10% at 3 years [229] compared to greater than 60% following decompensation in the non-transplant patient [211]. A small number of patients (2-5%) will develop a severe cholestatic form of recurrent HCV infection with histological features that include intrahepatic cholestasis and biliary ductal proliferation. Most such cases rapidly progress to allograft failure and death within the first year after transplantation.

The long term outcome for HCV positive liver transplant recipients is poorer than for patients transplanted for other indications. There is a decreased graft

and patient survival for HCV positive recipients beyond 5 years post transplantation [230-231]. Patient survival at 3 years in US transplant centres was 78% for HCV positive recipients and 82% for HCV negative recipients (HR 1.14 95% CI 1.05-1.23, P<0.001) between 1991 and 2001. In Europe, 7 year graft and patient survival was 51% and 55% for HCV positive recipients and 67% and 70% for HCV negative recipients [227]. There is some evidence to suggest that fibrosis progression may be linear for the first few years post transplant, but that accelerated progression is observed beyond 5 years [232].

As in the pre-transplant setting there are a number of factors associated with the accelerated progression of fibrosis in recurrent HCV. It is typically donor factors together with the management of immunosupression and not recipient factors that influence the rate of progression. Recipient variables, including age, sex, HLA type and ethnicity do not appear to significantly affect the severity of HCV recurrence [233]. As described earlier the age of the liver infected with HCV and not necessarily the patient's age has most bearing on fibrosis progression. A median rate of fibrosis progression of 0.6 Ishak[110] fibrosis stages/ year in donors younger than 40 years was observed compared to 2.7 fibrosis stages/ year where the donor was older than 50 years. This equated to a reduction in the time to cirrhosis from 10 years to 6.7 years [117]. In another series with a median follow-up of 3 years only 14% of patients who received a donor organ from a donor less than 30 years developed cirrhosis, compared to 45% where the donor was aged 31-59 years and 52% if more than 59 years [234]. A number of viral factors including genotype, and quasispecies emergence have been variably reported to impact on the severity of recurrent

HCV [233], but only a high viral load at the time of transplantation appears to hold a strong association [235-236]. Infection with Cytomegalovirus (CMV) and Human herpesvirus 6 post transplantation has been repeatedly and strongly associated with an increased severity of recurrence [237-238], even after adjusting for covariables such as degree of immunosuppression [239].

Donor factors are to some degree amenable to modification or selection. The treatment of the donor liver following harvesting can influence the post transplant course of recurrent HCV. Both the length of cold (cooling post procurement surgery to implantation) [240] and warm ischaemic times (implantation to reperfusion via portal vein) [241] correlate with the severity of recurrent disease and graft survival, as do poor quality grafts either from non-heart beating donors or as a result of preservation injury leading to biliary complications [242]. On the other hand the use of HCV positive donors does not worsen progression or survival [243]. The choice of immunosuppression is also amenable to modification with bolus steroid therapy [236] and T cell depleting therapies [244-245] both associated with an accelerated disease course when used to treat rejection. Steroid sparing immunosuppression protocols have to date, however, failed to demonstrate a benefit [246-247] and most practitioners will maintain patients on a low dose of steroids with the addition of Azathioprine. Ciclosporin based protocols may also be beneficial as it possesses weak antiviral activity, though this is only against genotype 1b [248], while tacrolimus enhances HCV replication [249].

1.4.4 The economic burden of Hepatitis C

There is also an economic and utilisation of healthcare resources burden associated with HCV. Using computer modelling of the NHANES III HCV cohort, the calculated direct medical costs relating to HCV in the US are estimated to be $6.5-13.6 billion for the period 2010-19. Additional indirect costs relating to premature death and disability from decompensated cirrhosis and HCC were $21.3 and $54.2 billion respectively [250]. Analysis of a US insurance claims database (2002-6), showed HCV patients to have significantly higher healthcare expenditure ($20,961 vs $5451) and hospitalisation (24% vs 7%) compared to age, sex and plan enrolment matched controls [251]. Analysis of hospital admission and prescription data shows an accelerated growth in healthcare resource utilisation due to HCV. Over an 8 year period (1994-2001) there was a 200% increase in hospitalisation associated with a 3-4 fold increase in complications relating to HCV infection [252]. Other studies confirm a high number of hospitalisations and physician visits associated with HCV [253-254].

1.5 The treatment of Hepatitis C

1.5.1 Awareness of Hepatitis C and access to treatment

According to the results of modelling of HCV seroprevalence and complications, treatment of all infected patients in 2010 could reduce risk of cirrhosis, decompensation, cancer, and liver-related deaths by 16%, 42%, 31%, and 36% by 2020 [27]. Treating just 10 or 50% of the HCV population will result in a 6 and 26% reduction in morbidity and a 4 and 20% fall in mortality respectively by 2030 [255]. A significant barrier to this goal is that the majority of HCV infection is undiagnosed. There are an estimated 200,000 HCV infected patients in England, but at the time of the Department of Health's 2004 Hepatitis C Action plan only 38,000 diagnosed cases were reported [256]. By the end of 2008 this number had increased to 69,865 [34]. Similarly in Scotland it is estimated that two-thirds of chronically HCV infected individuals are undiagnosed and that two-thirds of this group are former IDUs [257]. This may in part be due to a lack of awareness and knowledge of HCV among both at risk groups and healthcare professionals. Results of a survey of primary care physicians in New Jersey, United States suggested insufficient knowledge regarding viral hepatitis risk factors [258]. In another survey of 1412 US primary care physicians only 70% reported that they tested for HCV in all patients with risk factors and only slightly more (78%) tested all patients with elevated liver enzymes for HCV [259]. In the United Kingdom the Royal College of General Practitioners have issued guidance on prevention and diagnosis of HCV in primary care [260]. The majority of new HCV infections in industrialised countries are through IDU. In a sample of young (15-30 years)

IDUs from five US cities (HCV seroprevalence of 34.1%), 72% of anti-HCV-positive and 46% of anti-HCV-negative IDUs were not aware of their HCV serostatus. Those IDUs who knew they were anti-HCV-negative were less likely than those unaware of their status to share needles or other injecting paraphernalia, but knowledge of a positive test result was not associated with safer injection practices[261]. Within another sample of street recruited IDUs unaware of their HCV infection status, 61% tested positive [262].

Given the prevalence of undiagnosed HCV infection, there may be a role for population or targeted screening for HCV. France introduced a voluntary, anonymous, cost-free screening programme, supported by a media campaign that led to a 20% increase in testing per year between 1998 and 2002. By the end of 2002 approximately 60% of all new cases of HCV were diagnosed through screening [263]. In Japan there is nationwide testing for anti-HCV at 5 year intervals in those over the age of 40 years [200]. The US preventive service task force concluded, however, that there was insufficient evidence on which to recommend either population or targeted screening of high risk groups [264-265]. Independent review of the evidence supports targeted testing in high prevalence groups, but nationwide population screening is unlikely to be cost effective [266-267].

Targeted screening of 4998 individuals from the Asian community, living in England showed an overall seroprevalence of HCV in this population of 1.6%, but it varied by country of birth being 0.4%, 0.2%, 0.6% and 2.7% in people of this ethnic group born in the UK, India, Bangladesh and Pakistan, respectively

[268]. A study of opportunistic testing and counselling of 30-54 year old attendees at a primary care practice in an area of high HCV seroprevalence and drug use in Scotland identified 584 eligible attendees, of which 117 (28%) accepted testing. The overall seroprevalence of anti-HCV was 13% (15/117) of which the majority (13/15) were current or former IDUs. The authors concluded that targeted testing of current and former IDUs would be a more effective intervention [269]. Dried blood spot and oral fluid testing have both been found to be useful methods with which to conduct public health surveillance for HCV among IDUs [270], a group in whom it can be difficult to obtain blood samples through conventional phlebotomy. The sensitivity and specificity of a commercial dried blood spot test is of the order of 99% in detecting anti-HCV [271]. In a controlled study of HCV testing in 22 specialist drug clinics and 6 prisons there was a 14.5% increase in uptake of testing in the intervention sites offering dried blood spot testing [272].

There remain barriers to treatment even in those with a positive anti-HCV test. Between 2000 and 2002, 256 individuals tested positive for HCV through a single Public Health laboratory. Less than half were subsequently referred to an appropriate specialist clinic and only 10% started treatment. The route of testing influenced whether patients were referred for specialty care with 64.3% of positive tests performed in primary care leading to referral, compared to 42.4% from drug and alcohol units and just 18.4% from prisons. Only 68/125 (54%) of patients referred went on to attend their clinic appointment [273]. Prison outreach clinics have become a popular means of addressing

deficiencies in the testing and treatment of a population with a high HCV seroprevalence. Of 41 HCV RNA positive patients identified by a prison outreach clinic led by specialists from Southampton University Hospitals Trust only 6 were deemed eligible for treatment due in a large part to contraindications, particularly psychiatric disease, but also to logistical reasons such as the movement of prisoners [274]. Interrogation of the department of Veterans database identified 113927 HCV infected patients during the period 1999-2003. The prescription rate for antiviral therapy was 11.8%. Non-treatment was associated with increasing age, non-white race, drug and alcohol abuse/ dependence and co-morbid illnesses [275]. When questioned, the majority (72%) of US primary care physicians would refer an HCV-positive patient with elevated aminotransferases to a specialist, but only 28% would do so if the aminotransferases were normal [259].

1.5.2 Interferon and ribavirin therapy in chronic Hepatitis C

The immediate goal of HCV treatment is to achieve a sustained virological response (SVR), defined by the continued absence of HCV RNA 6 months after completion of therapy. Several studies have demonstrated that this response is durable in more than 99% of patients receiving the current standard of care [276-279]. There were attempts to treat HCV even before identification of the virus. Interferon α was shown to be of benefit in the treatment of chronic non-A, non-B hepatitis in 1986 [280], though trials in the mid 1990s of interferon monotherapy administered by subcutaneous injection three times a week were associated with an overall SVR of only 6-10% [281-282]. The addition of the broad spectrum antiviral agent ribavirin, a guanosine nucleotide

analogue subsequently increased the SVR rate to more than 30% [283-284]. Combination therapy has been further refined by the substitution of standard interferon with pegylated interferon, where polyethylene glycol is covalently attached to interferon in order to increase its half life. Pegylated interferon together with ribavirin forms the current standard of care for chronic HCV and is associated with an SVR in approximately 50% of patients [285-287].

Despite these advances in the treatment of HCV, the mechanisms by which interferon and ribavirin achieve viral clearance are not fully understood. Interferon α has potent antiviral activity but does not act directly on the virus. Instead it binds to cell receptors, which in turn leads to the induction of interferon stimulated genes which confer antiviral activity within the cell [288]. The mechanisms by which the virus can evade the action of interferon include inhibition of the transcription of interferon induced antiviral genes by the HCV core protein [289-290] and inhibition of the interferon amplication loop by HCV NS3/4A protease [289, 291]. Ribavirin monotherapy has no appreciable effect on HCV viral load [292], but augments the antiviral action of interferon and appears to be particularly important in reducing the risk of relapse after completion of treatment. Ribavirin has a small direct effect on HCV RNA replication by binding to the nucleoside binding site of polymerases. It is also able to induce mutation in the virus, possibly pushing it towards a threshold of error catastrophe, appears able to alter T helper cell response, down regulates interferon inhibitory pathways and inhibits the host inosine monophosphate dehydrogenase (IMPDH) leading to depletion of the intracellular GTP pool

necessary for viral RNA synthesis [293]. It is unclear though, whether any or all of these constitute its main mechanism of action in vivo [288].

As well as only achieving success in approximately half of patients, current therapy for HCV is associated with significant side effects that lead 10-20% of patients to prematurely discontinue treatment [294]. Fatigue, fever, headache and myalgia are common and were experienced by more than half of patients treated in the registration trials for pegylated interferon and ribavirin [285-287]. More serious side effects include depression, witnessed in 20-30% of patients and probably precipitated principally by an effect of interferon on serotonin activity [295]. Interferon also induces neutropenia and thrombocytopenia, while ribavirin leads to a dose dependent haemolytic anaemia. Dose reduction is required during treatment in 32-42% of cases and in the majority of instances is explained by laboratory abnormalities [295]. There are also a number of contraindications to the use of interferon and ribavirin, including severe concurrent disease or recipients of renal, heart or lung transplants. Ribavirin is teratogenic and, therefore, contraindicated in pregnancy and in those patients with an inability to practice birth control. Conditions known to be exacerbated by interferon including autoimmune hepatitis, active psychiatric illness and untreated hyperthyroidism also represent contraindications [296].

1.5.3 Factors associated with response to antiviral therapy

There are a number of factors known to influence the response to interferon based therapies and, therefore the likelihood of an SVR. Table 1.4 illustrates some of the more important patient and viral factors associated with an SVR in patients treated with pegylated interferon and ribavirin. Host factors, as described earlier can have a major impact on disease progression while the virus has little bearing. Both host and virological factors help predict treatment response, but here (at least until recently) the virus has been felt to be the major determinant. Though the underlying functional mechanism is unknown, viral genotype is an important predictor of SVR, with rates of 41-52% for Genotype 1 and 76-84% for genotypes 2 and 3 [285-287]. The odds ratio associated with non-1 genotypes was 3.25 (95% CI, 2.09-5.12) in the randomised controlled trial conducted by Fried et al [286]. The degree of viral quasi-species complexity [297-298] and the number of mutations within specific regions of the genome are also associated with the likelihood of an SVR [299-301]. Quasi-species diversity may in part explain why early intervention with interferon monotherapy for acute HCV was associated with an SVR rate of 100% while delaying treatment for a year reduced the SVR to 53% [302], suggesting non-response to interferon is acquired during chronic infection. The baseline viral load is also an independent predictor of SVR, with an odds ratio for ≤400,000 iu/ml vs. >800,000 iu/ml of 3.01 (95% CI, 2.15 - 4.20, $P<0.001$) and for >400,000 to 800,000 iu/ml vs. >800,000 iu/ml of 1.64 (95% CI, 1.10 -2.46, $P=0.02$) in the study by Shiffman and colleagues [303].

Response to treatment is also characterised by the viral kinetic profile with responders typically displaying a biphasic decay of viraemia. A rapid initial decline reflects blocking of viral production and elimination of free virions, while the subsequent slower decline represents clearance of virus infected cells [304-305]. A Rapid Virological Response (RVR) is defined as undetectable HCV RNA at week 4 of treatment and in a retrospective analysis of 1383 patients was shown to correlate with a high probability (88-100%) of SVR [306]. The presence of RVR generated an odds ratio of 5.47 (95% CI, 3.97-7.52) for predicting SVR in the multiple logistic regression analysis. Identification of virological factors associated with improved SVR rates, has allowed the length of treatment to be tailored to reflect the likelihood of a successful outcome. Consideration should be given to shortening the length of treatment in genotype 1 infection where there is the combination of a low baseline viral load (<600-800,000) together with a RVR as more than 75% of patients will achieve an SVR after 24 weeks treatment [307-309]. Shortening treatment to 16 weeks in genotype 2/3 patients with a RVR and a baseline viral load of <800,000 iu/ml also has relatively little impact on the SVR rate achieved [303]. Early Virological Response (EVR) is defined as a viral load decline of ≥ 2 $\log_{10}$ (partial EVR) or undetectable at week 12 (complete EVR). Less than 3% of patients who fail to achieve an EVR will have an SVR [286, 310] and treatment should, therefore, be stopped at week 12 when the requirements for EVR are not met. Genotype 1 patients with a complete EVR have an SVR of 80% compared to 17% in those with only a partial EVR after 48 weeks treatment. Extending treatment to 72 weeks in those patients with a partial EVR increased the SVR rate to 29% suggesting a role for extended

treatment in slow virological responders (partial EVR and undetectable HCV RNA at 24 weeks) [311]. Less than 2% of patients with a partial EVR who still have detectable virus at week 24 will achieve an SVR and this should again lead to the stopping of treatment [310, 312].

A number of host factors are concerned with the response to treatment. The best results from interferon based therapies are seen in those less than 40-45 years, with a normal BMI and who consume minimal alcohol. Younger age correlates significantly with SVR with an odds ratio for ≤ 40years of between 1.4 (95% CI; 1.1-1.9, P=0.005) [313] and 2.6 (95% CI; 1.72-3.95, P<0.001) [286] in clinical trials. A high BMI is inversely correlated with SVR [311, 314] and weight ≤ 75kg was associated with an odds ratio of 1.91 (95% CI; 1.27-2.89, P=0.002) in the study by Fried and colleagues [286]. Shiffman et al [303] likewise found the odds ratio for SVR in those whose weight was ≤ 80kg compared to patients weighing > 80kg was 1.75 (95% CI; 1.37-2.24, P <0.001). These were both trials of pegylated interferon alfa-2a which is administered weekly in a standard dose of 180μg. Trials of pegylated alfa-2b did not find either weight or BMI to be independent predictors of SVR, though here the dose is weight based which may negate any effect of obesity on SVR [315-316].

Table 1.4: Predictors of an SVR with pegylated interferon and ribavirin therapy in HCV

Variable	Genotype	Odds ratio (95% CI) – Multivariate analysis	P Value	Reference
Age: ≤ 40 years	1-6	2.60 (1.72-3.95)	<0.001	Fried et al [286]
≤ 45 vs. > 45 years	2,3	1.5 (1.17-1.93)	0.002	Shiffman et al [303]
Sex:	1-6	Not significant when weight taken into account	ns	Fried et al [286]
Ethnicity: White vs Black	1	1.96 (1.48-2.60)	<0.0001	Conjeevaram et al [317]
Body weight: ≤ 75 kg	1-6	1.91 (1.27-2.89)	0.002	Fried et al [286]
≤ 80 vs. > 80 kg	2,3	1.75 (2.37-2.24)	<0.001	Shiffman et al [303]
Hepatic steatosis: ≤ 5% vs > 5%	2,3	SVR 89% vs 72%	0.012	Zeuzem et al [316]
Fibrosis: Absence of bridging fibrosis or cirrhosis.	2,3	2.15 (1.63-2.81)	<0.001	Shiffman et al [303]
Cirrhosis vs no cirrhosis	1,2,3	0.58 (0.47-0.73)	<0.0001	Jacobson et al [315]
Genotype: Non-1	1-6	3.25 (2.09-5.12)	<0.001	Fried et al [286]
	1-6	5.4 (4.1-7.1)	<0.001	Hadziyannis et al [287]
2 vs 3	2,3	1.88 (1.46-2.43)	<0.001	Shiffman et al [303]
Baseline viral load: ≤ 400,000 vs > 800,000 IU/ml	2,3	3.01 (2.15-4.20)	<0.001	Shiffman et al [303]
< 600,000 IU/ml	1	No data	0.0001	Zeuzem et al [308]
RVR:	1-4	5.47 (3.97-7.52)	<0.0001	Fried et al [306]

In an earlier trial of pegylated interferon alfa-2b weight was an independent predictor of SVR [285], though this predated the introduction of weight based Ribavirin dosing which is now standard practice with both forms of pegylated interferon. The data for an effect of alcohol consumption on SVR is less robust, being based on limited data from relatively few patients; in part due to the fact clinical trials of antiviral therapy generally exclude patients with other potential causes of liver disease such as alcohol. A consumption dependent decrease in response to treatment with standard interferon has been demonstrated [318] and it has also been observed that patients who drink alcohol in the 12 months before treatment are more likely to discontinue treatment as compared to non-drinkers (40% vs 26%, P=0.002), though in this prospective multicentre trial patients who did finish treatment had comparable response rates to non-drinkers [319].

Response rates to interferon based therapy appear to be lower in patients with HIV/ HCV co-infection. SVR rates range from 14-29% in genotype 1 and 44-73% in genotype 2 and 3 infection [320-322]. HIV co-infected patients are more likely to discontinue therapy, in part due to increased susceptibility to the haemolytic effects of ribavirin in those patients taking highly active antiretroviral therapy (HAART) [322-323].

A negative correlation between male sex and SVR was observed in both pegylated interferon registration trials, though the correlation did not remain significant in the multivariate analyses [285-286]. Sex was no longer significant once weight was taken in to account in one study [285]. Ethnicity

also has a significant impact on response to treatment with numerous studies demonstrating that African Americans having a reduced likelihood of SVR [317, 324-331]. Even with the more potent pegylated interferon regimens significant differences in SVR rates remain for African Americans as compared to non-African Americans (19-28% vs 39-52%) [317, 324, 331], an observation that is independent of genotype, socio-demographic characteristics or compliance to treatment. Hispanic Americans also appear to have poorer SVR rates compared to white patients [325, 332], while individuals of south east Asian extraction have in smaller numbers been shown to achieve better SVR rates [333].

Recent reports from genome-wide association studies point to a genetic basis for much of the observed differences in treatment response between ethnic groups. Three published analyses examining US [334], Japanese [335] and Australian [336] populations described single nucleotide polymorphisms (SNPs) in the vicinity of the IL28B gene, which encodes Interferon - λ3 as being highly predictive of response to pegylated interferon and ribavirin in genotype 1 infection. The C allele at polymorphism rs12979860 is positively associated with SVR (OR 5.79 [95% CI; 2.76-12.57, P= 9.0×10^{-6}]) [337] and a subsequent intention to treat analysis confirmed a 2-3 fold increase in SVR for C/C patients of all ethnic groups [338]. The favourable C/C allele is less frequent in African Americans (16%) as compared to Caucasians (39%), but IL28B polymorphism is only felt to explain approximately half the observed difference in SVR rate as African Americans with the C/C allele still have a lower response than Caucasians with the same genotype (53% vs 82%) [334].

SNP rs8099917 was identified in the Japanese and Australian studies as being most significant and this was replicated by Rauch *et al.* who identified it as being independently associated with treatment failure (OR 5.19 [95% CI; 2.90 – 9.30, $P = 3.11 \times 10^{-8}$]) [339]. The original studies of IL28B polymorphism were in genotype 1 patients, but subsequent studies confirm the relevance to genotype 2 and 3 infection. In a study of 213 genotype 2 and 55 genotype 3 patients in which pegylated interferon and ribavirin were given for 12 weeks in those with an RVR and 24 weeks in those without; 82% of patients with the C/C allele had an SVR compared to 75% for C/T and 58% for T/T (P=0.0046 for trend) [340]. The difference between IL28B genotypes was greatest among patients who did not have an SVR. The mechanisms of how IL28B polymorphism affects HCV suppression are unknown, but there is some evidence that IL28B may influence the expression of interferon stimulated genes (ISGs). A high baseline expression of ISGs in liver tissue is known to be associated with a poor response to interferon based therapy [341-343] and non-C/C IL28B genotypes have been shown to exhibit higher levels of ISGs than the C/C genotype [344].

Histological and biochemical factors are also important in predicting the response to treatment. Advanced fibrosis or cirrhosis are major predictors of non-response [345], across all genotypes (cirrhosis vs no cirrhosis: OR 0.58 (95% CI 0.47-0.73, P <0.0001) [315]. The absence of significant fibrosis on the other hand is associated with an increased likelihood of SVR (OR 2.15 (95% CI; 1.62-2.81, P<0.001) [303]. In a study aimed at assessing the efficacy of treatment in cirrhosis, data collated from three randomised controlled trials of

peglylated interferon alfa-2a was examined. SVR rates for genotype 1 and 4 patients with cirrhosis were 33% compared to 60% if there was no bridging fibrosis or cirrhosis, for genotype 2 and 3 infection the figures were 57% and 76% [346]. The absence of steatosis on liver biopsy is also strongly correlated with SVR [134, 316].

Baseline levels of ALT have been shown in one study of genotype 2 and 3 infected patients to be correlated with SVR [303], though this has not been confirmed elsewhere [286, 315]. An association between low pre-treatment levels of gamma-glutamyl transferase (GGT) and SVR has also been demonstrated with an odds ratio (5.7 [95% CI; 3.2-10.0, P <0.0001]) similar to that of genotype [347]. GGT levels have been shown to closely relate to levels of hepatic steatosis and fibrosis, as well as to insulin resistance [348-349]. The latter is associated with a poor treatment response, particularly in genotype 1 patients [138, 317, 350]. Concurrent treatment with Metformin has been shown to improve insulin sensitivity during antiviral therapy, but only female patients saw a statistically significant improvement in SVR [351].

The nature of previous failure to achieve an SVR with antiviral therapy also predicts the likelihood of a response with retreatment. This is an increasingly important area of treatment as by 2015 the number of non-responders to either standard or pegylated interferon with ribavirin seeking retreatment is expected to exceed treatment naive patients [352]. The EPIC-3 study [353] enrolled 2333 chronic HCV infected patients with significant fibrosis (≥ F2) who had previously failed to achieve an SVR with interferon and ribavirin therapy.

Patients were retreated with pegylated interferon and ribavirin for 48 weeks regardless of genotype. There was a higher SVR in previous responder-relapsers as compared to non-responders (38% vs 14%) and in patients whose previous treatment had been with standard interferon rather than pegylated (25% vs 17%). The likelihood of SVR remained dependent on genotype and hepatic fibrosis with SVR rates less than 5% for genotype 1 infected patients with cirrhosis who had been previous non-responders to pegylated interferon based therapy. In contrast a genotype 2/3 infected patient with F2 fibrosis, who had previously been a responder-relapser with peglyated interferon and ribavirin had a chance of SVR as high as 75% with 28 weeks therapy. The REPEAT study [354-355] evaluated higher doses and a longer duration of pegylated interferon alfa-2a and ribavirin in previous non-responders to pegylated interferon alfa-2b therapy, in a predominantly (>90%) genotype 1 infected population. Higher SVR rates were achieved with 72 weeks treatment as compared to 48 weeks (16% vs 8%, P=0.0006). The HALT-C study [356] randomised patients who had previously failed to respond to pegylated interferon to either low dose maintenance interferon or no treatment for 3.5 years. No benefit in terms of disease progression was seen for maintenance interferon.

1.5.4 The impact of treatment on the natural history of Hepatitis C

Achieving an SVR is the immediate target of therapy, but long term efficacy depends on treatment improving wellbeing, halting disease progression and improving outcomes in terms of liver failure, HCC and death. Successful

treatment has been shown to lead to an improvement in fatigue and the presence of cryoglobulin, though many patients still complain of persistent fatigue despite a virological response [357]. A 9 year follow-up study of 455 patients, 384 of whom who received standard interferon based therapy examined the long-term outcome of treatment. The frequency of HCC and liver transplantation or death was similar for both untreated patients and treatment non-responders showing no overall benefit to receiving treatment. Non-responders, however, had an 11 fold increased risk of developing HCC and an 8.8 fold increased risk of death as compared to patients with an SVR. The risk of hepatic decompensation was also much reduced in those patients with an SVR at 10 years (3% vs 35%, $P<0.001$) [358]. A decreased risk of HCC following successful interferon therapy has also been seen in other studies [359-361].

An important question has been whether antiviral therapy still confers long term benefits in patients who already have significant hepatic fibrosis at the time of treatment. There is some evidence to suggest that treatment can be associated with regression of fibrosis [362] and reduction in portal pressure in patients with cirrhosis [363]. A retrospective analysis of 568 patients with cirrhosis who received pegylated interferon and ribavirin demonstrated that those patients with an SVR had a higher survival (98% vs 86%, $P=0.005$) and a higher probability of remaining free of decompensation (97.3% vs 66.7%, $P<0.001$) at 5 years. Fewer patients had developed a HCC at 5 years, though the difference was not statistically significant (4% vs 16%, $P=0.09$) [364]. Another retrospective analysis of 317 patients with bridging fibrosis or

cirrhosis did show failure to achieve an SVR to be an independent predictor of HCC development with a 4.72 fold higher rate of HCC among non-responders [365]. There has been limited study of the benefits of treatment in patients with cirrhosis and a prior history of hepatic decompensation. Iacobellis et al studied 129 consecutive patients with decompensated cirrhosis (mean Child-Pugh score approximately 8) of which 66 agreed to 24 weeks of treatment with pegylated interferon and ribavirin. Those who declined treatment formed the control group. The SVR rate was 43.5% for genotype 2 and 3 patients and 7% for those with genotype 1. Though the SVR rate was low there were no deaths among those patients who achieved an SVR during a median 30 months follow-up compared to 32.2% in the control group and 20.8% of non-responders [366].

In a systematic review of studies examining the long term health outcomes and costs associated with antiviral therapy for HCV, treatment with pegylated interferon and ribavirin was shown to prolong life, improve long term health related quality of life and be cost effective in the treatment of treatment naïve and non-responders/ relapsers with HCV [367]. In a further economic analysis, antiviral therapy for HCV was felt to be comparable in terms of cost effectiveness to other well accepted medical interventions including haemodialysis, screening for colon cancer and treating HIV infection and hypertension [368].

1.5.5 New and future therapies for Hepatitis C

Given that only approximately half of patients achieve an SVR with current therapy, there is a pressing need for new, more efficacious therapies. Ideally new drugs should be more potent, less toxic and allow a shorter duration of therapy. One avenue has been to further refine interferon and ribavirin based therapy. Taribavarin is a pro-drug of ribavirin that appears to be associated with less anaemia, but unfortunately efficacy is only at best equivalent to ribavirin [369-370]. Alternative forms of interferon have also been investigated including daily consensus interferon and albinterferon (interferon alfa fused to human albumin). Neither drug has entered common practice, though albinterferon which can be administered fortnightly due to its extended half life appears to have similar efficacy to pegylated interferon with less impairment of quality of life [371-372]. More recently there has been interest in IFN-λ1 (also known as interleukin-29), which has functional similarities to IFN-α. The expression of the IFN-λ receptor is more restricted than that of IFN-α, however, being only expressed on hepatocytes. This suggests the potential for reduced adverse events and a phase 1b study has evaluated 4 weeks of treatment with IFN-λ1 alone and in combination with ribavirin. Antiviral activity was demonstrated with 29% of treatment naïve patients achieving an RVR and 86% having at least a 2 log10 drop in HCV RNA with minimal constitutional and haematologic effects [373]. Whether this translates to acceptable SVR rates remains to be seen, but with receptors restricted to hepatocytes there will be a risk of relapse as a result of HCV replication in peripheral blood mononuclear cells.

A more productive approach has been the development of new drugs that target steps in the HCV life cycle including entry into the host cell, proteolytic processing, viral replication and the assembly and release of new virions. Designated Directly Acting Antiviral (DAA) drugs, these new compounds potentially represent a significant advance in the treatment of HCV. The most promising targets are two viral enzymes required for viral replication; the NS3/4A protease and NS5B polymerase. NS5B functions as an RNA dependent polymerase and is key to the synthesis of complementary minus-strand RNA. Two types of HCV polymerase inhibitors (nucleoside and non-nucleoside analogues) have been under development. Mericitabine is a nucleoside analogue polymerase inhibitor with high in-vivo potency, a high genetic barrier to resistance and which to date has not been associated with any severe adverse effects. A planned interim analysis of week 12 data from the PROPEL study demonstrates that the addition of Mericitabine 1000mg bd for the first 12 weeks of 48 weeks treatment with pegylated interferon/ ribavirin is associated with an RVR of 62% vs 18% and and cEVR of 80% vs 49% [374]. Preliminary data from a phase IIb study (JUMP-C) of Mericitabine in combination with pegylated interferon and ribavirin demonstrates virological suppression is achieved in 91% of patients at week 24 compared to 62% of patients receiving pegylated interferon and ribavirin alone[375]. Unfortunately a number of other promising agents in this class including Valopictabine (NM283), R-1626 and HCV-796 have been withdrawn because of safety concerns and an unfavourable risk-benefit profile[376].

The NS3/4A serine protease inhibitors are the class of DAA molecules that look set to first reach clinical practice. The NS3 protein and its co-factor NS4A form a serine protease that is required to cleave proteins from the HCV polyprotein before viral replication can occur. The two drugs within this class that are furthest in development are telaprevir and boceprevir. PROVE 1 (US) [377] and PROVE 2 (Europe) [378] were phase 2b studies of telaprevir in treatment naïve genotype 1 infected patients. PROVE 1 had 3 arms in which telaprevir was given for 12 weeks together with 12 (T12PR12), 24 (T12PR24) or 48 (T12PR48) weeks of pegylated interferon and ribavirin. The SVR rate for the T12PR24 and T12PR48 arms were 61% and 67% as compared to 41% in the control arm (pegylated interferon and ribavirin for 48 weeks). The improvement in SVR was despite a higher rate of discontinuation in the telaprevir group, mostly due to rash. PROVE 2 also showed that 12 weeks of telaprevir together with 24 weeks of pegylated interferon was associated with an SVR rate of 69%, but that efficacy was lost in the study arm without ribavirin (SVR 36%). PROVE 3 [379] incorporated similar study arms in the re-treatment of genotype 1 infected patients who had previously failed to achieve an SVR with pegylated interferon and ribavirin. In a difficult to treat population, that included 17-20% of patients in the telaprevir arms with cirrhosis, 51% of patients who received telaprevir for 12 weeks and pegylated interferon and ribavirin for 24 weeks achieved an SVR. The efficacy of boceprevir was assessed in treatment naïve genotype 1 infected patients in the SPRINT 1 study [380]. There were 4 boceprevir study arms, all of which had significantly higher SVR rates than the control arm (pegylated interferon and ribavirin for 48 weeks). Pegylated interferon and ribavirin for 4 weeks

followed by both drugs together with boceprevir for a further 44 weeks was associated with an SVR rate of 75%. SPRINT 2 enrolled 938 non black and 159 black patients and demonstrated an SVR with 4 weeks lead in with pegylated interferon and ribavirin followed by 24 weeks of pegylated interferon, ribavirin and boceprevir (PR4, PRB 24) of 67% in non blacks and 42% in blacks compared to 40% and 23% with standard therapy [381]. RESPOND 2 confirmed efficacy for boceprevir in the treatment of previous treatment failures with significantly higher SVR rates for both boceprevir arms (PR4, PRB 32 = 59%, PR4, PRB 44 = 66%) than controls (PR 48 = 21%) [382].

Both telaprevir and boceprevir significantly improve cure rates in difficult to treat genotype 1 infected patients. The error prone nature of the HCV RNA dependent RNA polymerase means, however, that drug resistance will inevitably occur in patients treated with drugs directed against specific HCV enzymes. The NS3/4A serine protease inhibitors and non-nucleoside polymerase inhibitors have a relatively low genetic barrier to resistance. The dominant mutation seen with telaprevir (A156S) [383-384] is at the same position as the mutation selected most frequently with boceprevir (A156T) [385] and cross resistance has already been confirmed. While extremely promising, enthusiasm for these drugs needs to be tempered by the major threat of drug resistance.

Nucleoside analogue polymerase inhibitors and protease inhibitors have also been studied in combination. The INFORM-1 study examined an IFN sparing

regime combining Mericitabine and Vaniprevir. After 14 days patients with genotype 1 infection had an average reduction in HCV RNA of 5.1 log 10 IU/ml in the higher dose arm [386]. This represented the first proof of concept that IFN sparing regimes may be effective in treating HCV.

There are many other areas of drug development in HCV therapy, including targeting the cellular immune response. Agents such as toll-like receptor agonists can promote an effective host immune response by inducing/ modulating cytokine response [387]. The immunosuppressant agent, cyclosporin A has been shown to suppress HCV replication [388]. This is mediated through specific blockade of cyclophilins, important host factors in RNA replication. Non-immunosuppressive cyclosporin A analogues which inhibit either cylcophilin A (Debio-025) or cyclophilin B (NIM-811) have been shown to suppress HCV replication in vitro. NS5A is a non-structural protein which interacts with a number of cellular proteins, including cyclophilin A. A small molecule inhibitor (BMS-790052) of NS5A has been the subject of a phase 1 study in which a single dose led to a 3.3 $\log_{10}$ reduction in mean viral load at 24 hours[389]. Another target is NS4A, a co-factor for NS3 protease, but unfortunately concerns regarding nephrotoxicity have led to cessation of development of ACH-806, an initial drug in this class [390].

1.6 Aims

The aim of the studies comprising this thesis is to examine the natural history of Hepatitis C (HCV) in a cohort of patients followed up longitudinally as part of the Trent HCV cohort study (established in 1991 with the aim of studying the epidemiology and natural history of HCV infection in a defined and representative area of the UK). The initial three data chapters examine the natural history of HCV infection within groups of patients with differing degrees of liver disease. Starting with patients, perceived to have mild disease (those with a persistently normal ALT), moving onto those with severe fibrosis on liver biopsy and finally to those who have developed Hepatocellular carcinoma as a result HCV infection. The final two data chapters examine the influence of ethnicity and thrombocytopenia on the success of treatment with Pegylated Interferon and Ribavirin.

1.7 The Trent Hepatitis C cohort study

The Trent HCV cohort study was established in 1991, with 3 main research objectives:

- To address the epidemiology of HCV infection in a defined and representative area of the UK (Trent Health Care region: population 5.1 million – See Figure 1.1).

- To study the natural history of HCV infection

- To devise optimal strategies for the management of HCV infected patients

The Trent HCV study group developed following an initiative by the Trent centre of the National Blood Authority (NBA) in response to the introduction of HCV antibody screening of donated blood. They arranged for referral of antibody positive donors to one of seven clinics (merger of centres now means five hospital trusts) which collaborated in collecting detailed epidemiological data on these patients in a central database. Subsequently the study group collected identical data on any antibody positive patient referred to one of the centres. The study has been funded since 1991 variously by the National blood Authority, the Department of Health, and the pharmaceutical industry. The study has approval from a Multi-centre Research Ethics Committee.

The study is currently run by a multidisciplinary group of specialists in hepatology, infectious diseases, pathology, virology, epidemiology and blood transfusion (current membership is listed in Appendix 1). It is the largest cohort of HCV infected patients in the UK, and is felt to be highly representative of HCV infection in the country at large, as none of the participating clinics operate as a tertiary referral centre for areas outside the region. The study population reflects those referred to secondary care and this will not necessarily mirror a primary care population. As described in Chapter 1.5.1 a significant proportion of HCV infected patients remain undiagnosed and there are a number of barriers to those with a diagnosis accessing secondary care. There are no resources within the Trent study for the screening of patients for HCV, even amongst high risk populations, though individual centres may in some instances run outreach clinics in prisons and drug rehabilitation centres from which patients may be recruited. There is also no attempt to recruit patients identified through the central laboratories as having a positive HCV antibody. The population studied does as previously alluded to, have a significant bearing on the natural history of HCV and the Trent study cohort should be though of as reflecting the natural history of HCV infection within a secondary care cohort.

Figure 1.1: Map illustrating the Trent region, from which patients are recruited to the Trent HCV cohort study

All patients referred to one of the participating clinics (Derby, Leicester, Lincoln, Nottingham and Sheffield) with a diagnosis of HCV infection are invited to enrol in the study, with informed consent. Participation in the study is open to all patients referred to these clinics and as none of the centres are tertiary referral centres to which patients are referred from outside the Trent region, it is likely that the majority of patients in the study will reside in the Trent geographical region illustrated in Figure 1.1. The patient's postcode is recorded at the time of enrolment, but entry to the study does not require this to be within the Trent region. A detailed epidemiological questionnaire is administered by interview with trained personnel and the patient derived data is stored on a centralised database. Basic demographic data collected includes place of birth and ethnicity (categorised according to the definitions used in the 2001 national census). Risk factor data are categorised hierarchically as: 1) injecting drug use, 2) receipt of blood (pre-1991) or blood product (pre-1986) transfusion, 3) other risk factor (born abroad, non-professional tattoo, professional tattoo pre-1982), 4) none of the above risk factors, and 5) risk factor data missing. Duration of infection is estimated on the assumption that infection was acquired at the first exposure to risk (e.g first year of IDU or date of blood transfusion). Information is also collected on past alcohol consumption (history of heavy alcohol consumption defined as > 50g per day for most days over a period of 6 months) at enrolment and subsequently on alcohol consumption over the week prior to each clinic attendance.

Liver histology was scored by a single pathologist (Dr J Underwood) and using the Knodell Histological Activity Index (HAI) [391] during the early years of

the study, but in recent years biopsies have being scored by the local pathologist at each centre using the Ishak modified HAI [110]. Hepatic steatosis is scored from 0 (none) to 3 (extensive). Training in the use of the Ishak modified HAI and sharing of cases to assist in standardising scoring has been employed in order to reduce inter-observer error. The scoring of the Ishak Modified HAI is shown in Table 1.5.

The current recommendation for evaluation of chronic viral hepatitis is that liver biopsies should be at least 20mm in length and contain at least 11 portal tracts [392]. Synchronous biopsies with a mean length greater than 2 cm show minimal variation in stage [393], while smaller biopsies, each with only 4-5 portal tracts differ in staging by two or more categories in 20% of cases [394]. Smaller biopsies are likely to underscore disease while biopsy specimens obtained from the subcapsular region are prone to overestimating the stage of disease as increased stroma, including septum formation and perhaps even nodularity, may be within the spectrum of normal [395]. Unfortunately there is no recording of the size or adequacy of the liver biopsy within the study database and, therefore, there is the potential that some biopsies will have been under or over scored.

Patient management was up until 1998 according to an agreed protocol (Interferon therapy recommended to those with a total Knodell score [391] ≥ 6 on liver biopsy), and subsequently has been in line with national guidelines

[396-397]. Initially those patients who declined treatment or had a Knodell score < 6 had a repeat liver biopsy after an interval of 2 years. Laboratory data is captured from the participating centres' own pathology reporting systems and regularly downloaded to the study database. Patients with known human immunodeficiency virus infection or inherited coagulation disorders are identifiable within the cohort but excluded from analysis as the natural history may differ in these groups.

Patients are flagged with the National Health Service Central Register (NHSCR). This system covers nearly all residents of England and Wales and has reciprocal links to the equivalent Scottish register. It identifies deaths, cancer registrations and emigrations and forwards the information monthly to the study group. When a death is identified by the NHSCR, information forwarded includes a copy of the death registration data and coroner's findings where relevant.

Table 1.5: The Ishak modified Histologic Activity Index (HAI) for scoring of necroinflammatory activity and fibrosis in chronic Hepatitis C

Feature	Score
A. Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	0
Mild (focal, few portal areas)	1
Mild/ moderate (focal, most portal areas)	2
Moderate (continuous around < 50% of tracts or septa)	3
Severe (continuous around > 50% of tracts or septa)	4
B. Confluent necrosis	
Absent	0
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis + occasional portal-central bridging	4
Zone 3 necrosis + multiple portal-central bridging	5
C. Focal (spotty) lytic necrosis, apoptosis and focal inflammation	
Absent	0
One focus or less per x 10 objective	1
Two to four foci per x 10 objective	2
Five to ten foci per x 10 objective	3
More than ten foci per x 10 objective	4
D. Portal inflammation	
Absent	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/ marked all portal areas	3
Marked all portal areas	4
Maximum necroinflammatory score	**=18**
Fibrosis	
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas, with occasional portal to portal bridging	3
Fibrous expansion of portal areas, with marked bridging as well as portal - central	4
Marked bridging (portal to portal and/or portal-central) with occasional nodules	5
Cirrhosis, probable or definite	6
Maximum fibrosis score	**=6**

Patients are flagged with the National Health Service Central Register (NHSCR). This system covers nearly all residents of England and Wales and has reciprocal links to the equivalent Scottish register. It identifies deaths, cancer registrations and emigrations and forwards the information monthly to the study group. When a death is identified by the NHSCR, information forwarded includes a copy of the death registration data and coroner's findings where relevant.

The epidemiology of those patients recruited to the Trent HCV study between 1st September 1991 and 1st December 1998 has previously been described [33]. During this period there were a total of 2546 patients who were found to be anti-HCV positive by one of the diagnostic laboratories serving the region, of which 44% had been recruited into the study. There were relatively more females (32 vs 27%, P=0.004) in the study cohort than amongst the total anti-HCV positive population, together with a slight shift in the age distribution towards an older population. The majority of those patients not enrolled in the study had not been referred to or attended an outpatient appointment at one of the participating clinics. No information, however, is recorded on the consent to participate rate of those approached with a view to entering the study. Consistent with the development of the study, 50% of new patients in 1991-1993 were referred by the NBA, while this route of referral had reduced to 2% in 1997. 95 % of the study population were Caucasian and approximately two-thirds of patients had a history of intravenous drug abuse. The infecting genotype was 1 (47%), 2 (10%), 3 (39%), 4 (1%) and 5 (2%). Past markers of

hepatitis B infection (antibody to hepatitis B core antibody) were positive in 37% of patients.

As of August 2011, there were 3612 patients in the study database of which 2041 have had at least one liver biopsy and 1255 have received pegylated interferon based treatment. 850 patients have more than 5 years follow-up since the date HCV infection was discovered, of which 457 patients have more than 10 years. Data from the initial patient questionnaire and subsequently from the hospital notes and IT systems is entered into the study database by data entry clerks, with the number employed having fluctuated over the years depending on the health of the study's funds. Regular data review meetings are held to highlight areas with missing data and to prioritise certain data fields that are relevant to current or planned research. The quality of the data entered into the study database is the subject of periodic validation audits of random sets of casenotes.

Chapter 2

Hepatitis C infected patients with a persistently normal alanine aminotransferase.

Do they exist and is this really a group with mild disease?

2.1 Background

Alanine aminotransferase (ALT) is a frequently used serum marker of liver injury. Evidence from data on volunteer blood donors, however, suggests that 20-40% of Hepatitis C (HCV) RNA positive patients will have a normal ALT level at the time of diagnosis [162, 398-399]. A proportion of these patients will have a persistently normal ALT (PNALT), usually defined as two or three consecutive measurements within the normal range over a 6-month period [296, 400]. There is conflicting evidence, however, on whether these patients represent a subgroup with mild disease and at low risk of disease progression [401-403]. Few longitudinal studies have specifically looked at HCV patients with PNALT [404-406] and of those that have, some estimate fibrosis progression on the basis of a single biopsy [404], or do not include a comparison group of patients with an elevated ALT [405]. There is also controversy on what constitutes a normal ALT, with some proposing that the upper limit of normal is set too high because the cohorts used to establish the normal range included apparently health individuals with unrecognised fatty liver disease. A threshold of 40U/L may, therefore, fail to identify some patients with significant liver disease [407].

2.2 Aims

The aim of this study was to examine the natural history of HCV infected patients with a persistently normal ALT. It examines the likelihood of the ALT remaining normal for two groups of patients with PNALT, defined using either

a threshold of 30U/L or 40U/L. It also examines the risk of disease progression as compared to patients with an elevated ALT.

2.3 Patients and methods

2.3.1 Study population

At the time of this study (2007) the Trent HCV study cohort included 2184 HCV RNA positive patients for which there were over 27,000 separate ALT measurements recorded on the database. All laboratories in the participating centres have an upper limit of normal for ALT of 40IU/L. All treatment-naïve patients were included and divided according to whether they had either an elevated ALT, defined as at least one ALT > 40 in the 6 months following diagnosis (n=1140) or a PNALT. Two definitions of PNALT were used, either an ALT ≤ 30 (n=43) or an ALT ≤ 40 (n=87) on 2 or more occasions (at least one month apart) in the 6 months following diagnosis. Patients with a PNALT ≤ 30 will by definition also be included in the PNALT ≤ 40 group.

2.3.2 Follow-up

Entry into the study was defined as the date on which the patient was diagnosed with HCV. Follow-up for this study was until the date of death or transplantation, last recorded clinic visit or initiation of antiviral therapy. ALT measurement and liver biopsy were performed as part of routine clinical care and the latter was scored by a histopathologist at each participating centre using the Ishak disease severity score [110]. The likelihood of the ALT remaining normal in PNALT patients was assessed by scrutiny of ALT values

taken after the initial 6 month definition period for PNALT. Liver histology was considered for analysis when the first biopsy was within one year of diagnosis (PNALT ≤ 30: 28/43 (65%), PNALT ≤ 40: 59/87 (68%) and Elevated ALT: 628/1140 (55%)). Fibrosis progression in those patients with paired biopsies (PNALT ≤ 30: n=11 (26%), PNALT ≤ 40: n=15 (17%), Elevated ALT: n= 164 (14%)) was expressed as Ishak fibrosis points per year. Patients with cirrhosis (Ishak stage 6) on the initial biopsy, and who could not therefore progress, were excluded from this analysis, as were patients who received antiviral therapy between the two biopsies. Analysis of mortality was restricted to those patients with an Ishak score of 0, 1 or 2 on the initial liver biopsy.

2.3.3 Statistical analysis

Continuous variables were compared using the Mann-Whitney U-test. Categorical variables were compared using the chi-squared test or Fisher's exact test. A two-tailed P value of < 0.05 was considered significant. Statistical analysis using life tables was used to estimate the likelihood that the ALT remained normal at 1, 3, and 5 years and to calculate the survival probability following discovery of HCV in those patients with mild disease (Ishak fibrosis stage 0, 1 or 2). Kaplan-Meier plots were used to express the proportion of patients maintaining a PNALT and to display survival curves for patients with both PNALT and an elevated ALT. Multiple logistic regression analyses was performed with PNALT as the dependent variable and included age at diagnosis, sex and those variables from the univariate analysis with a P value $<$ 0.1.

2.4 Results

One thousand one hundred and forty patients with at least one elevated ALT in the first 6 months from diagnosis were identified, compared to 87 with a PNALT ≤ 40 IU/L and 43 with a PNALT ≤ 30 IU/L. The remaining patients in the cohort had either no ALT measurements available (n=615) or a single normal value (n=342) during the definition period and were not included in the study. Of the 1140 patients with an elevated ALT, 97 subsequently had a 6-month period prior to any treatment in which the ALT was ≤ 40 on 2 or more occasions (at least one month apart) and no elevated ALT. The median follow up was 61, 36 and 21 months in the PNALT ≤ 30, PNALT ≤ 40 and elevated ALT group respectively. Initiation of antiviral treatment marked the end of follow-up and was more common in group with an elevated ALT (49%) than in either the PNALT ≤ 30 (19%) or PNALT ≤ 40 (28%) group (P < 0.001). When initiation of antiviral treatment was removed from the list of potential endpoints, the period of follow-up remained shorter for patients with an elevated ALT as compared to PNALT ≤ 30 patients (61 vs 42 months, P=0.02).

2.4.1 Patient details

Table 1 shows the baseline characteristics of patients in the study at point of diagnosis with HCV. Patients in both PNALT groups were more likely to be female (P < 0.001) and to have a lower body mass index (BMI) (P < 0.001) than patients with an elevated ALT. In the multivariate analysis (Table 2) both female sex and a lower BMI remained independent predictors of having a PNALT. Other parameters including age, estimated duration of disease, route

Table 2.1: Characteristics of patients with persistently normal ALT (PNALT) and with an elevated ALT at the time of diagnosis with HCV

	PNALT ≤ 30 (n=43)	PNALT ≤ 40 (n=87)	Elevated ALT (n=1140)
Age (years)	36 (19-64)	36 (19-64)	36 (15-86)
Sex - Male:Female (% male)	18:25 (42%) [a]	41:46 (47%)[b]	809:329 (71%)[a,b]
Ethnicity (White)	39 (91%)	79 (91%)	993 (89%)
Main risk factor			
IVDU	32 (74%)	65 (77%)	746 (68%)
Blood Transfusion	4 (9%)	11 (13%)	130 (12%)
Estimated duration of infection (years)	14 (3-40)	14 (2-40)	14 (0-63)
Genotype			
1	16 (55%)	26 (45%)	352 (42%)
2	4 (14%)	7 (12%)	70 (8%)
3	9 (31%)	23 (40%)	385 (46%)
Hepatitis B status			
HBsAg positive	0	0	36 (4%)
HBcAb positive	9 (33%)	13 (23%)	216 (33%)
Drinks alcohol	n=37 19 (51%)	n=78 45 (58%)	n=972 553 (57%)
Average alcohol consumption (units/week)	n=33 0.6 (0-155)	n=69 1.5 (0-189)	n=926 2.5 (0-248)
Past or present history of excess alcohol (>50g/d for at least 6 months)	12 (36%)	31 (42%)	461 (50%)
BMI (kg/m^2)	22.0 (17-34)[c]	22.7 (16-35)[d]	25 (15-47)[c,d]
Subsequent antiviral therapy	6/32 (19%)[e]	20/72 (28%)[f]	458/ 932 (49%)[e,f]
Necroinflammatory score on biopsy (Ishak)	n=25 2 (0-5)[g]	n=55 2 (0-7)[h]	n=586 3 (0-15)[g,h]
Fibrosis score on biopsy (Ishak)	n=27 0 (0-3)[i]	n=58 0 (0-6)[j]	n= 620 1 (0-6)[i,j]

Results presented are median (range)
Unless indicated no statistically significant difference between PNALT groups and elevated ALT group
[a,b, e, f] Significant difference between groups with $P < 0.001$ (X^2 test)
[c,d, h, j] Significant difference between groups with $P < 0.001$ (Mann Whitney test)
[e,f] Significant difference between groups with $P < 0.001$ (X^2 test)
[g] Significant difference between groups with $P = 0.004$(Mann Whitney test)
[I] Significant difference between groups with $P = 0.006$ (Mann Whitney test)

Table 2.2: Multivariate analysis of factors associated with a PNALT

Variable	OR (95% CI)	P value
PNALT ≤ 40 IU/L		
Age at diagnosis (per year)	0.98 (0.94 – 1.02)	n.s
Sex - (Female)	3.09 (1.70 – 5.60)	<0.001
BMI (per point)	0.91 (0.85 – 0.98)	0.009
Estimated duration of infection at diagnosis (per year)	1.002 (0.998 – 1.005)	n.s
PNALT ≤ 30 IU/L		
Age at diagnosis (per year)	0.99 (0.94 – 1.05)	n.s
Sex - (Female)	2.92 (1.21 – 7.04)	0.017
BMI (per point)	0.83 (0.74 – 0.94)	0.003
Estimated duration of infection at diagnosis (per year)	1.001 (0.996– 1.006)	n.s

of infection, genotype and a history of heavy alcohol consumption were similar between the groups.

2.4.2 Persistence of a normal ALT

Figures 1 demonstrates the proportion of patients in whom the ALT remains persistently normal over time. In those patients with a PNALT ≤ 30, the proportion in whom the ALT had remained persistently ≤ 30 by 1, 3 and 5 years was 69.8%, 42.2% and 37.5%. For patients with a PNALT ≤ 40 the proportion in whom the ALT had remained persistently ≤ 40 at 1, 3 and 5 years was 68.9%, 41.7% and 35.4%.

Figure 2.1: Proportion of patients maintaining a PNALT ≤ 40 or PNALT ≤ 30

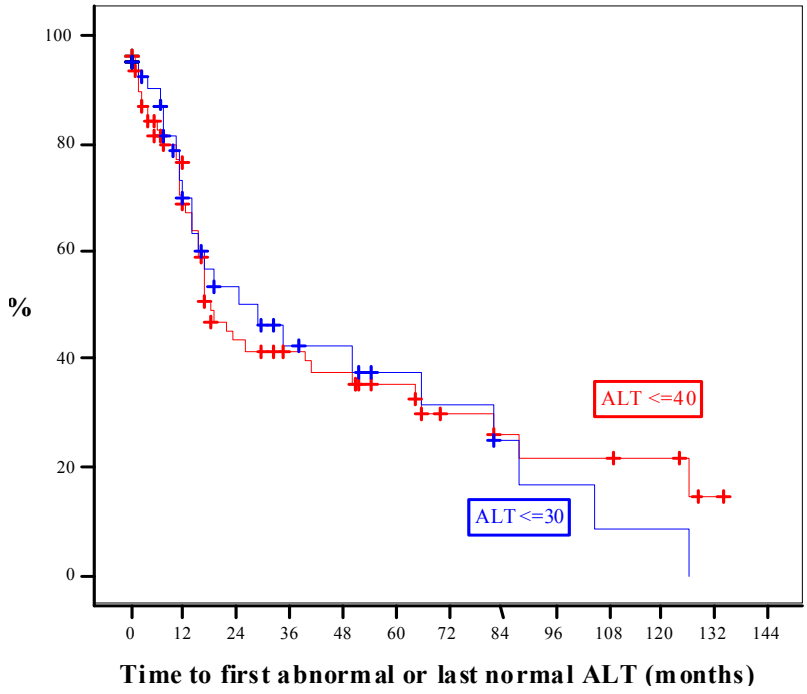


Figure 2.1 demonstrates that the majority of patients who initially fulfilled the criteria for PNALT ultimately have an abnormal ALT. By 3 years less than half of patients could still be described as having a persistently normal ALT

2.4.3 Liver Histology

The proportion of patients having a liver biopsy within one year of diagnosis was higher in the PNALT ≤ 40 group (68%) than those with an elevated ALT (55%) (P=0.02). There was no statistical difference in the frequency of liver biopsy between PNALT ≤ 30 patients (65%) as compared to the elevated ALT group. Figures 2a and 2b illustrate the distribution of the Ishak necroinflammatory and fibrosis score on liver biopsies performed within 1 year of HCV diagnosis. Patients in both PNALT groups had a significantly lower necroinflammatory (PNALT ≤ 30: 2.12 ± 1.27 vs 3.34 ± 2.16, P =0.004; PNALT ≤ 40: 2.05 ± 1.41 vs 3.34 ± 2.16, P <0.001) and fibrosis score (PNALT ≤ 30: 0.63 ± 0.84 vs 1.80 ± 2.05, P =0.006; PNALT ≤ 40: 0.83 ± 1.47 vs 1.80 ± 2.05, P <0.001) on their initial biopsy, than patients with an elevated ALT. Significant levels of fibrosis (Ishak fibrosis score ≥ 3) were seen in 29.6% of patients with an elevated ALT, as compared to 3.7% (P=0.002) and 8.3% (P<0.001) of patients with PNALT ≤ 30 and PNALT ≤ 40 respectively.

Figure 3a and 3b illustrate the change in the necroinflammatory and fibrosis score in those patients with paired biopsies. The median interval between biopsies was 31 months in both PNALT groups and 28 months in the group with an elevated ALT (Table 3). There was no statistically significant difference between the groups in regard to the proportion of patients having a second biopsy, with 10/28 (36%), 14/59 (24%) and 148/628 (24%) having had a repeat biopsy within 5 years of their initial biopsy and prior to the onset of therapy. The rate of fibrosis progression was 0.33 ± 0.94 Ishak fibrosis points/ year for PNALT ≤ 30 patients, 0.35 ± 0.82 for PNALT ≤ 40 patients and 0.19 ±

0.48 for those with an elevated ALT. There was no statistically significant difference in either the interval between biopsies or the rate of fibrosis progression between the PNALT patients and those with an elevated ALT. The majority of patients with paired biopsies had an Ishak fibrosis score of 0, 1 or 2 on the initial biopsy. Restricting the analysis of fibrosis progression to only these patients did not alter the result. In all, fibrosis progressed in 5/11 (45%) patients with PNALT ≤ 30, 8/15 (53%) with PNALT ≤ 40 and 59/164 (36%) patients with an elevated ALT. 1/11 (9.1%), 2/15 (13.3%) and 22/164 (13.4%) patients respectively had an increase in fibrosis score of ≥ 2 points between biopsies.

When the PNALT patients with paired biopsies (n=15) were compared to the PNALT group as a whole (n=87), no statistically significant difference in terms of age, sex, ethnicity, main risk factor, or history of heavy alcohol use was identified. There was also no difference in the Ishak necroinflammatory or fibrosis score on the first biopsy. On the other hand, those patients with an elevated ALT and paired biopsies (n=162) had a significantly lower Ishak necroinflammatory (2.8 ± 1.8 vs 3.3 ± 2.2 P=0.002) and fibrosis score (0.6 ± 1.0 vs 1.8 ± 2.1 P<0.001) on the first biopsy than the elevated ALT group as a whole (n=1140). There was also no difference in the Ishak necroinflammatory (2.76 ± 1.90 vs 2.75 ± 1.81 P=0.971) and fibrosis score (0.78 ± 1.27 vs 0.54 ± 0.85 P=0.160) on the first biopsy between patients who had fibrosis progression and those who did not. This was true for both the elevated ALT and PNALT groups. Those patients with an elevated ALT and paired biopsies were also more likely to be female (P=0.005).

Figure 2.2a: Ishak Necroinflammatory score on first biopsy (when performed within one year of diagnosis with HCV)

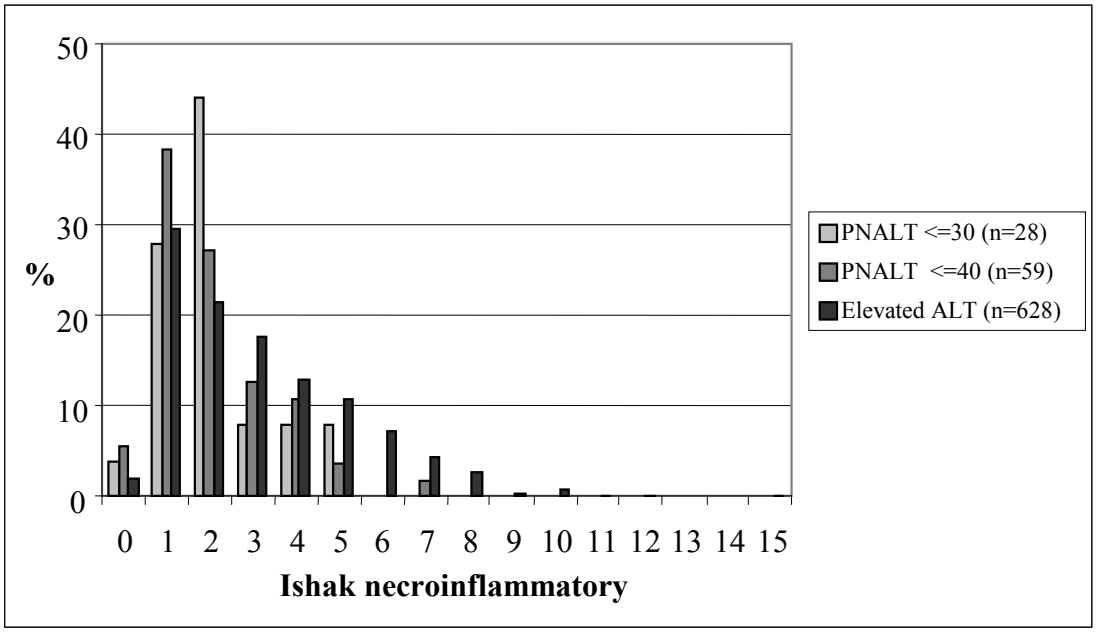
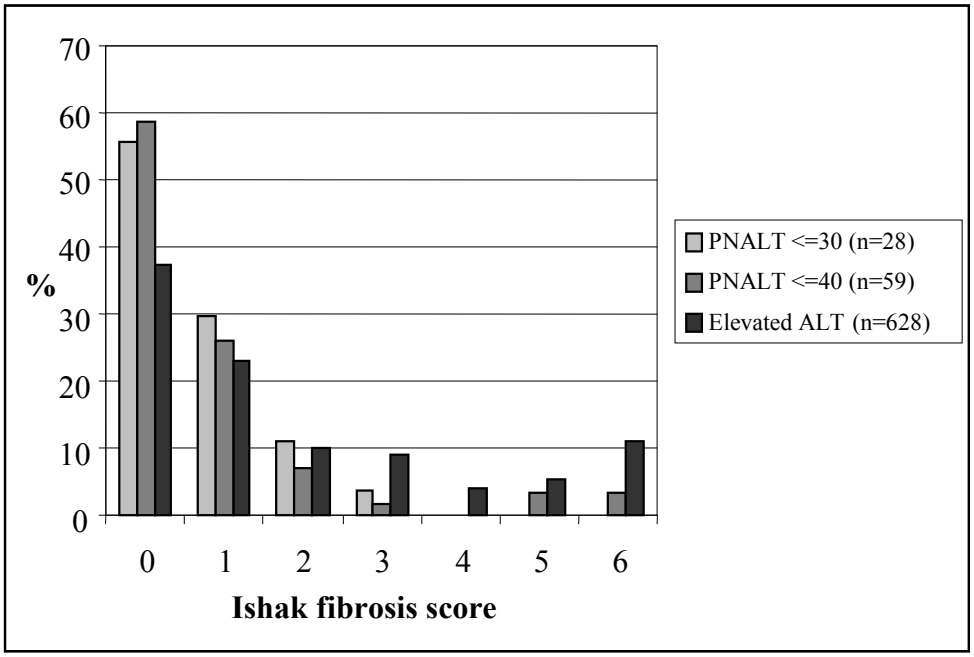


Figure 2.2b: Ishak Fibrosis score (stage) on first biopsy (when performed within one year of diagnosis with HCV)



Both Figure 2.2a and 2.2b illustrate that on an initial liver biopsy the majority of patients with either a PNALT or elevated ALT have mild Ishak necroinflammatory and fibrosis scores, but that there are proportionately more patients with higher scores amongst patients with an elevated ALT. Statistically patients with PNALT has significantly lower scores for both necroinflammation and fibrosis.

Figure 2.3a: Change in Ishak Necroinflammatory score in those with paired biopsies

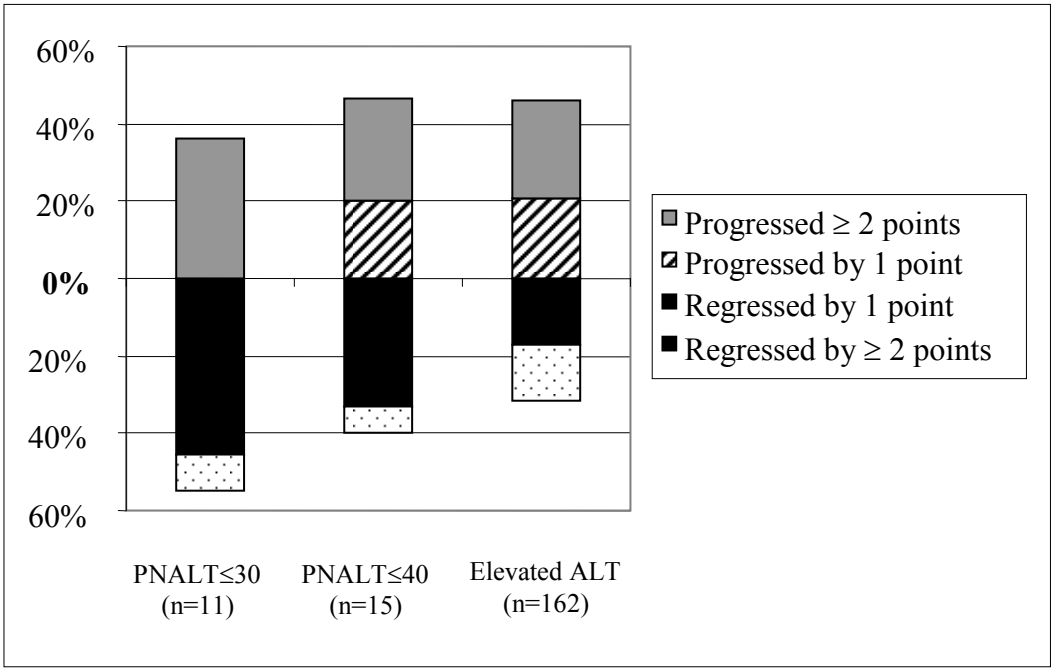
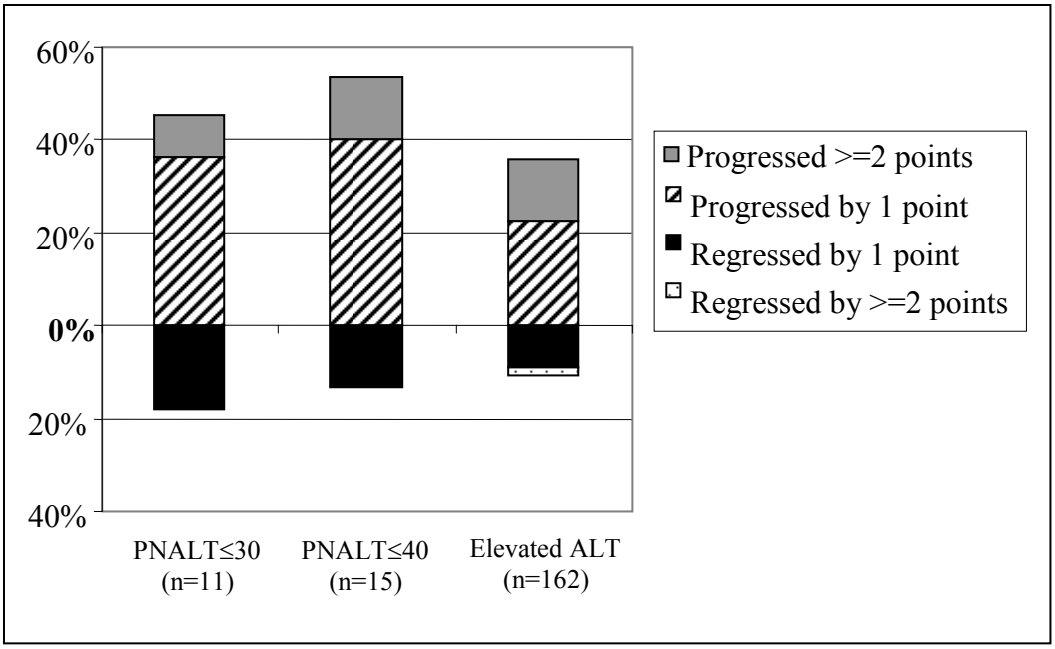


Figure 2.3b: Change in Ishak Fibrosis score in those with paired biopsies



Figures 2.3a and 2.3b illustrate that there was both progression and regression of inflammation between paired liver biopsies in those with PNALT and those with an elevated ALT. There was no statistical difference in the rates of progression when the PNALT and elevated ALT patients were compared

Table 2.3: Histological findings in those patients with paired biopsies

	PNALT ≤ 30 (n=11)	PNALT ≤ 40 (n=15)	Elevated ALT (n=162)
Age (years)	33.8 ± 9.2 34 (23-56)	34.9 ± 9.3 35 (23-56)	38.1 ± 10.4 35 (23-56)
Sex - Male:Female (% Male)	3:8 (27%)	5:10 (33%)	97:65 (60%)
Estimated duration of infection (years)	11.8 ± 5.2 10 (5-19)	13.1 ± 5.9 12 (5-22)	15.2 ± 8.7 16 (1-36)
Ever Heavy alcohol drinker (>50g/d for at least 6 months)	4 (44%)	5 (39%)	55 (43%)
BMI (kg/m^2)	22.1 (18-30) [a]	21.9 (18-26) [b]	25.5 (17-37) [a, b]
First biopsy			
Ishak necroinflammatory score	2.5 ± 1.4 2 (0-5)	2.2 ± 1.4 2 (0-5)	2.8 ± 1.9 2 (0-12)
Ishak stage	0.8 ± 1.0 1 (0-3)	0.6 ± 0.9 0 (0-3)	0.6 ± 1.0 0 (0-5)
Second biopsy			
Ishak necroinflammatory score	2.9 ± 1.8 3 (1-6)	2.7 ± 1.7 2 (1-6)	3.3 ± 2.3 3 (0-14)
Ishak stage	1.3 ± 1.6 1 (0 -4)	1.2 ± 1.4 1 (0-4)	1.1 ± 1.6 1 (0-6)
Interim between biopsies (months)	37.3 ± 19.2 31 (12-85)	35.6 ± 16.6 31 (12-85)	34.4 ± 15.4 28 (12-111)
Fibrosis progression (points/ yr)	0.33 ± 0.94 0 (-0.5-3)	0.35 ± 0.82 0.29 (-0.5-3.0)	0.19 ± 0.48 0 (-0.9-2.6)

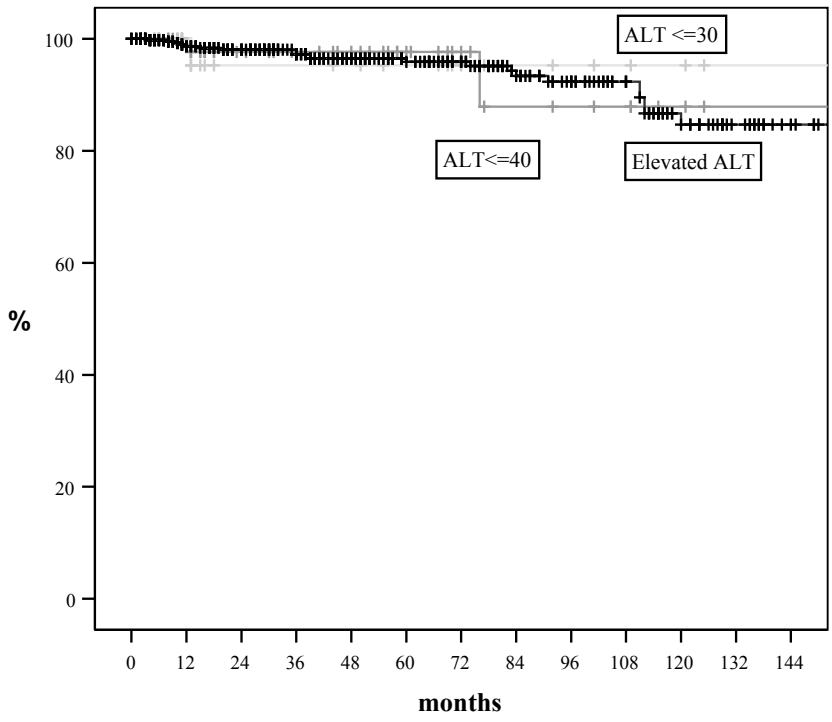
Results are Mean ± Standard deviation and Median (Range)
[a,b] Significant difference between groups with P = 0.002 (Mann Whitney test)

2.4.4 Morbidity and Mortality

No cases of Hepatocellular carcinoma (HCC) were recorded in the group of patients with a PNALT. 4/437 (0.9%) patients with an elevated ALT were diagnosed with HCC after a median of 46.5 months. Analysis of mortality of patients was restricted to patients with an Ishak fibrosis score of 0, 1 or 2 on the initial biopsy.

Figure 2.4 illustrates survival from the date of diagnosis for the three groups of patients. There was no difference in the survival probability at 5 years, with 95.2%, 97.6% and 95.9% of patients with a PNALT ≤ 30, PNALT ≤ 40 and an elevated ALT respectively being alive.

Figure 2.4: Survival from date of discovery in patients with IS stage 0,1,2



2.5 Discussion

It has been proposed that those HCV infected patients with a PNALT represent a subgroup with mild, slowly progressive disease [403-404, 406]. This study looked at whether a PNALT, defined as a normal ALT on 2 or more occasions (at least one month apart) in the 6 months following diagnosis, predicts that the ALT will subsequently remain normal. It also examined the risk of disease progression as compared to patients with an elevated ALT. In accordance with recent evidence suggesting that the standard reference range for ALT fails to identify a significant proportion of patients with liver disease [407], it also explores the natural history of patients with a PNALT using a standard threshold of 40 U/L for ALT and a lower level of 30 U/L. This evidence is supported by a study in which a fall in ALT was noted in patients with a "normal" ALT level after achieving a SVR with treatment [408]. The PNALT ≤ 30 U/L group included 25 women, 58% of the total. It was decided not to adopt the Prati et al [407] definition of a normal ALT for a woman of ≤ 19 U/L and for a man of ≤ 30 U/L, as only five of the twenty five women in this group would have been included.

Patients with PNALT were more likely to be female and to have a lower Body Mass Index (BMI) than those with an elevated ALT. The predominance of female patients amongst those with a PNALT has been reported previously[403, 405-406, 409] and is consistent with evidence from non HCV infected patients, that points to the fact that ALT levels are on average 30% lower in women [407]. The same study and others have also shown a strong correlation between ALT and BMI, even after adjustment for other potential

confounders, such as age and alcohol [407, 410-411]. An increased frequency of genotype 2 among patients with PNALT has been previously reported [409, 412]. In line with several other groups [403-404, 406] this study found no difference in the seroprevalence of genotype 2, though the number of patients with this genotype was small.

Using a standard definition of PNALT [296], 87 patients with an ALT persistently ≤ 40 U/L were identified, included in which were 43 patients in whom it was persistently ≤ 30 U/L. In contrast there were 1140 patients with at least one ALT > 40 U/L in the six months following diagnosis. This study was not designed to assess the prevalence of PNALT in HCV infected patients, but 87/ 1227 (7%) is significantly lower than the 20-50% reported by some groups [162, 398-399, 413]. Patients in this study represent those referred to secondary care from within a geographically determined area of the UK. It is to be expected that many of these patients were diagnosed with HCV following the discovery of an elevated ALT. It is, therefore, likely that our population underestimates the true prevalence of a PNALT in HCV infected patients.

This study has also shown that the majority of patients with a PNALT will ultimately have an elevated ALT. Within five years of the initial definition period for PNALT over 60% of patients with PNALT ≤ 30 U/L and PNALT ≤ 40 U/L group had a recorded ALT of greater than 30 U/L and 40 U/L respectively. This is in concordance with other studies that suggest 20-50% of patients with PNALT develop an abnormal ALT with extended follow-up [414-415]. Some groups suggest that a more stringent definition of PNALT is

needed in order to identify patients where there is a genuine PNALT. The Italian Association for the Study of the Liver recommend that the ALT be normal on tests conducted every 2-3 months for a minimum of 18 months before advocating a diagnosis of PNALT [416]. The data presented here shows that the attrition rate in both PNALT groups continued beyond 18 months and did not plateau even after five years, suggesting that very few patients maintain a PNALT throughout the course of their disease. This is consistent with evidence suggesting that the ALT fluctuates during the course of HCV infection [417]. A significant proportion (8.5%) of the patients who initially had an elevated ALT subsequently had a period where the ALT remained persistently normal for at least 6 months. Had HCV been diagnosed at that time then these patients would have been labelled as having a PNALT. The natural history of HCV would seem, therefore, to be one of fluctuating ALT levels, with many patients having periods where the ALT remains within normal limits.

In line with previous studies this study demonstrates that patients with PNALT have lower Ishak fibrosis and necroinflammatory scores on their first liver biopsy than patients with an elevated ALT [401, 413, 415]. This is despite patients with PNALT having a similar estimated duration of infection and frequency of liver biopsy as those with an elevated ALT. As has already been mentioned, patients with PNALT are more likely to be female and to have a lower BMI. The demographics of patients with PNALT are, therefore, in keeping with previous descriptions of HCV infected patients with a low risk of disease progression [111, 131].

Finally, this study has examined whether there was any biopsy-assessed difference in fibrosis progression between the PNALT groups and patients with an elevated ALT. The PNALT and elevated ALT patients with paired liver biopsies had similar necroinflammatory and fibrosis scores on their first biopsy. This reflects the fact that the repeat biopsy patients with an elevated ALT are a selected sub-group of the total cohort of patients with a raised ALT where a decision not to treat was taken after the first biopsy. We found that fibrosis progressed at a similar rate between paired biopsies in those with PNALT as compared to patients with an elevated ALT. Of the previous longitudinal studies that have specifically looked at HCV patients with PNALT, some estimate fibrosis progression on the basis of a single biopsy [404], or have not included a comparison group of patients with an elevated ALT [405]. Hui et al [406] compared fibrosis progression between liver biopsies and suggested that fibrosis progressed at a slower rate in the patients with a PNALT. The definition of PNALT used, however, incorporated an upper limit of normal for ALT of 59 IU/ml, which makes direct comparison difficult. In the present study no difference between the groups in terms of the number of patients in whom the Ishak fibrosis score increased by ≥ 2 points was observed. Such an increase is considered to be indicative of fibrosis progression rather than to any potential sampling error [418]. Patients with a PNALT would seem, therefore, to be as much at risk of disease progression as those patients with an elevated ALT who start with similar necroinflammatory and fibrosis levels. It might be argued that a median interval between biopsies of 31 months in the PNALT group and 28 months in the group with an elevated

ALT, may be too short to demonstrate significant fibrosis progression. Given that fibrosis progression in Ishak points per year was higher in the PNALT groups than in those with an elevated ALT, it seems unlikely that a longer median interval between biopsies would result in an increased rate of progression in the elevated ALT group.

Those patients in whom the risk of death or HCC was assessed, were by design those with initially mild disease (Ishak fibrosis score 0, 1 or 2 on the initial biopsy). The frequency of these events was, therefore, low and no discernable difference between the patients with a PNALT and those with an elevated ALT was demonstrated. There is evidence that HCV with a normal ALT can still be associated with liver related morbidity. In a study of 519 patients in whom the average ALT value was < 40IU/L over 10 years, the 5 and 10 year cumulative incidence of HCC was 2.0% and 11.2% respectively [419].

This study has examined the natural history of patients with a PNALT using a large geographically determined population of patients with HCV infection. The results suggest that the majority of patients with a PNALT will have had an abnormal ALT within 3 years of follow-up. These patients also demonstrate similar rates of fibrosis progression as a sub-group of HCV infected patients with an elevated ALT who are re-biopsied prior to any institution of therapy. They, therefore, warrant the same consideration with regard to treatment and the need for liver biopsy as patients with an elevated ALT. Earlier studies of standard interferon monotherapy in patients with a normal ALT were associated with a flare in serum ALT in nearly 60% of patients, leading to the

recommendation against treatment in these patients [420]. More recent studies show the efficacy of peginterferon and ribavirin in patients with a normal ALT is similar to that of patients where the ALT is elevated [421-422] and that treatment associated flares in ALT are not a significant feature [423].

Non-invasive methods of assessing liver fibrosis, including transient elastography and Doppler indices have been studied in a small number of patients with PNALT [424-426]. Liu et al [425] suggested that the splenic arterial pulsatility index was the most discrimatory of the non-invasive methods in predicting significant fibrosis (>F2). On the other hand Sebastiani et al [426] found that the performance of biochemical markers of liver fibrosis was poorer in patients with a normal ALT as compared to those with an elevated ALT. With ALT seemingly an unreliable indicator of disease progression, however, these modalities and other non-invasive investigations for fibrosis warrant further study to assess whether they are able to provide patients with information on disease severity and progression in order to allow an informed decision with regard to antiviral therapy.

Chapter 3

The natural history of Hepatitis C with severe hepatic fibrosis

3.1 Background

The seroprevalence of HCV infection is believed to have peaked in first decade of the 21st century [27]. Disease progression over many decades, however, means that the proportion of patients with cirrhosis will not peak until 2020 [27, 170], and the complications of HCV will continue to increase for many years thereafter. A better understanding of the clinical course and prognosis for patients with advanced liver disease secondary to HCV will, therefore, become increasingly important. Epidemiological studies of disease progression have shown that the patient population selected has an important bearing on the results [427]. The majority of studies examining the natural history of cirrhosis secondary to HCV have come from tertiary referral centres, have included predominantly those infected following blood transfusion and are largely from an era before the advent of effective therapy [210-211, 428-429]. With the elimination of blood as a route of transmission in the majority of western countries, patients who acquired hepatitis C from injecting drug use are now the main source of new referrals.

3.2 Aims

The aims of this study were to describe the natural history of HCV with severe fibrosis within a broad patient population that typifies those patients currently attending hepatitis clinics.

3.3 Patients and methods

3.3.1 Study population

At the time of this study (2005) there were 2003 HCV RNA positive patients enrolled into the Trent HCV study, of which 1003 had at least one liver biopsy with an Ishak disease severity score. For this study all chronically HCV-infected patients with a liver biopsy (the index biopsy) prior to January 1st 2002 demonstrating severe fibrosis (defined as Ishak stage ≥ 4) were included (n=155). Patients were then excluded if they had 1) evidence of hepatocellular carcinoma (HCC) at or within 6 months of entry into the study (n=1); or 2) co-existing liver disease that could potentially have caused severe fibrosis (n=4: 3 with active HBV infection, 1 haemachromatosis). The final cohort (n=150) contained 3 patients with a positive HBV surface antigen, but an undetectable HBV DNA. Nineteen patients had evidence of decompensated liver disease (defined below) prior to the index biopsy.

3.3.2 Follow-Up

Entry into the study was defined as the date of the index biopsy. Follow-up was until the date of death or liver transplantation, or the last recorded clinic visit prior to data collection. Data on patients were gathered from the Trent HCV study database and from review of hospital records, including laboratory databases. Baseline laboratory values were accepted if within 6 months of the index biopsy and were used in conjunction with the clinical information to calculate a retrospective Child-Pugh grade. The primary endpoint was death or liver transplantation, with death judged as being related to HCV infection if it

was associated with a progressive impairment of liver function, acute variceal haemorrhage, or the result of HCC. Secondary endpoints were HCC (diagnosed by imaging modalities) and decompensation, defined as the first appearance of jaundice (bilirubin > 50 μmol/l with no other identifiable cause), ascites (proven by paracentesis or imaging), endoscopically proven variceal haemorrhage, or hepatic encephalopathy.

109/150 patients had received at least one course of antiviral therapy, with 92 (84%) starting treatment after the index biopsy. A sustained virological response (SVR) was defined as a lack of detectable HCV RNA 6 months after completing therapy.

3.3.3 Statistical analysis

Survival analyses were performed using Life Tables to estimate 1, 3, and 5 year survival. Kaplan-Meier plots were used for the survival curves and Cox proportional hazards for predictors of death. In the Cox models survival analyses were calculated using death or liver transplant as the outcome. All variables were included in at least one model. Tests for proportionality were performed.

3.4 RESULTS

3.4.1 Patient details

Tables 3.1 shows the baseline characteristics of the 150 patients, split into those with no prior history of decompensation (n=131, Group A), and those with clinical evidence of decompensation prior to the index biopsy (n=19, Group B). Overall, the median length of follow-up was 51 months (range 1-185).

Group A

Within Group A, 69% were male. Median age at the time of index biopsy was 48 years (range 30-76), with a median estimated duration of infection of 22.2 years. Alcohol intake at biopsy varied considerably between individuals, with 10 (8%) patients consuming in excess of 80g/day. The majority of patients reduced their alcohol intake following the index biopsy, with the mean consumption falling from 22.7 to 7.8g/day.

Treatment data are shown in Table 3.2. An SVR was achieved in 32/101 (32%) patients (23% genotype 1 patients compared with 37% of genotype 2 or 3), with 28%, 33%, and 33% of patients with Ishak stage 4, 5, and 6 respectively having an SVR.

Table 3.1: Baseline characteristics of the study population at the time of the index* biopsy in all patients and those with and without evidence of prior decompensation.

	All patients (n = 150)	Group A patients with no prior history of decompensation (n = 131)	Group B patients with previous evidence of decompensation (n = 19)
Age at index biopsy (y)			
Median (range)	48 (30–79)	48 (30–76)	47 (35–79)
Sex			
Males	104 (69%)	90 (69%)	14 (74%)
Females	46 (31%)	41 (31%)	5 (26%)
Ethnicity (Grp A n = 129, Grp B n = 17)			
White	121 (83%)	106 (82%)	15 (88%)
Indian subcontinent	17 (12%)	15 (12%)	2 (12%)
Black	6 (4%)	6 (6%)	0 (0%)
Other	2 (1%)	2 (2%)	0 (0%)
Source of infection			
Ivda	66 (44%)	61 (47%)	5 (26%)
Blood transfusion	25 (17%)	22 (17%)	3 (16%)
Other	21 (14%)	19 (15%)	2 (11%)
No known risk factor	22 (15%)	18 (14%)	4 (21%)
Unknown	16 (11%)	11 (8%)	5 (26%)
Estimated duration of infection (y) (Grp A n = 82, Grp B n = 9)			
Median (range)	22.3 (3.8–51.2)	22.2 (5.1–51.2)	26.3 (3.8–33.4)
Alcohol consumption at biopsy (Grp A n = 125, Grp B n = 18)			
Nil	62 (43%)	54 (44%)	8 (42%)
Mild (< moderate levels)	43 (30%)	40 (32%)	3 (16%)
Moderate (>32 g/day for men or >24 g/day for women)	21 (15%)	20 (16%)	1 (5%)
Heavy (>80 g/day)	17 (12%)	10 (8%)	7 (37%)
Past or present history of alcohol consumption >80 g/day	54 (37%)	39 (31%)	15 (79%)
Hepatitis B status (Grp A n = 129, Grp B n = 18)			
HBsAg positive	3 (2%)	3 (2%)	0 (0%)
HBcAb positive/HBsAg negative	51 (35%)	46 (36%)	5 (28%)
HCV genotype (Grp A n = 109, Grp B n = 16)			
1	42 (34%)	38 (35%)	4 (25%)
3	65 (52%)	56 (51%)	9 (56%)
Ishak stage			
4	25 (17%)	23 (18%)	1 (5%)
5	45 (30%)	42 (32%)	2 (11%)
6	80 (53%)	65 (50%)	16 (84%)
Necroinflammatory score (Grp A n = 115, Grp B n = 19)			
Median (range)	5 (1–17)	5 (1–15)	3 (1–17)
Child–Pugh grade (Grp A n = 112, Grp B n = 17)[b]			
A	114 (89%)	104 (94%)	10 (59%)
B	11 (9%)	7 (6%)	4 (24%)
C	3 (2%)	0 (0%)	3 (18%)
Bilirubin (μg/L) (Grp A n = 129, Grp B n = 19)[b]			
Median (range)	12 (4–118)	11 (4–63)	22 (6–118)
ALT (iu/L) (Grp A n = 130, Grp B n = 19)[b]			
Median (range)	107 (19–342)	111 (19–342)	73 (30–164)
Platelets ($\times 10^9$/L) (Grp A n = 117, Grp B n = 18)[b]			
Median (range)	136 (34–589)	137 (34–589)	130 (43–211)

[a] The index biopsy refers to the biopsy at entry to the study with an Ishak stage of 4, 5, or 6.
[b] Laboratory values refer to the result nearest to the index biopsy (providing within 6 months of biopsy). The Child–Pugh grade was calculated retrospectively based on these results where available.

Group B

The main differences between Groups A and B were in alcohol consumption and in the severity of the underlying liver disease (Table 3.1). At the time of the index biopsy, mean daily alcohol consumption was more than twice that of Group A patients. 16/19 (84%) Group B versus 65/131 (50%) Group A patients were Ishak stage 6 and more were Child-Pugh grade B (24% vs 6%) and C (18% vs 0%).

Table 3.2: History of antiviral therapy for all patients and those with (Group B) and without (Group A) evidence of prior decompensation

	All patients ($n = 150$)	Group A patients with no prior history of decompensation ($n = 131$)	Group B patients with previous evidence of decompensation ($n = 19$)
Received antiviral therapy (%)	109 (73%)	102 (78%)	7 (37%)
Timing of antiviral therapy			
Pre index biopsy	5 (5%)	4 (4%)	1 (14%)
Post index biopsy	92 (84%)	88 (86%)	4 (57%)
Pre + post index biopsy (separate courses)	12 (11%)	10 (10%)	2 (29%)
Number of separate treatments			
1	74 (68%)	69 (68%)	5 (71%)
2	31 (28%)	29 (28%)	2 (29%)
⩾3	4 (4%)	4 (4%)	0 (0%)
Most recent therapy			
Standard IFN monotherapy	25 (23%)	23 (23%)	2 (29%)
Standard IFN + ribavirin	31 (28%)	28 (27%)	3 (43%)
PEG-IFN monotherapy	3 (3%)	3 (3%)	0 (0%)
PEG-IFN + ribavirin	50 (46%)	48 (47%)	2 (29%)
Treatment response ($n = 107$[a])			
Withdrawn	25 (23%)	23 (23%)	2 (29%)
Non-responder	27 (25%)	25 (25%)	2 (29%)
Relapser	22 (21%)	20 (20%)	2 (29%)
Sustained viral response	33 (31%)	32 (32%)	1 (14%)

[a] One patient remained on treatment at the end of the study and another died while on treatment.

3.4.2 Mortality/ Liver transplantation

During follow-up 50 (33%) patients reached the defined end-points of death (n=40) or liver transplantation (n=10), 33 (25%) from Group A and 17 (89%) from Group B.

Group A

Within Group A there were 25 (19%) deaths, 19 (76%) of which were directly attributable to HCV infection (n=16), or had HCV infection as a contributing factor (n=3). The probability of survival without the need for transplantation was 97%, 88%, and 78% at 1, 3, and 5 years (Table 3.3). The median interval from biopsy to either death or transplantation was 42 months (range 1-109).

Group B

The outcome was worse for the 19 Group B patients. 15 died, whilst 2 underwent liver transplantation, after a median interval of 22 months (range 7-158).

Table 3.3 Survival rates and cumulative probability of liver transplantation, HCC and decompensation for patients in Group A (no prior history decompensation)

	1 year	3 years	5 years
Survival	0.97 (0.02)	0.88 (0.03)	0.80 (0.035)
Survival with native liver	0.97 (0.02)	0.88 (0.03)	0.78 (0.04)
Cumulative probability of Liver transplantation	0/124 (0%)	3/109 (2.8%)	7/63 (11.1%)
Cumulative probability of HCC and/ or decompensation	8/124 (6.5%)*	15/115 (13.0%)	25/76 (32.9%)

Note: Values in parentheses represent standard errors unless shown as a percentage

* patients were excluded from the cohort if they developed HCC within the first 6 months of biopsy

3.4.3 Morbidity

Group A

Hepatocellular carcinoma (HCC) and/or decompensation was diagnosed in 33/131 (25%) Group A patients, after a median of 41 months (range 1 – 106). The cumulative probability of HCC and/or decompensation was 6.5%, 13%, and 32.9% at 1, 3, and 5 years respectively (Table 3.3). Of the 13 patients with HCC, 5 underwent liver transplantation, with the diagnosis having been made before surgery in all cases. At the end of the study 9/13 HCC patients had died, including 4 of the 5 transplanted patients. The median interval from diagnosis of HCC to death was 7 months (range 23 days to 48 months).

Decompensation occurred in 26 (20%) Group A patients (6 of whom also developed HCC), with jaundice (n=6), ascites (n=9), encephalopathy (n=3), variceal haemorrhage (n=5), or more than one of the above (n=3). The median interval between biopsy and the first episode of decompensation was 29 months (range 1-92). Subsequent to this, 15 patients died and 3 underwent liver transplantation. Following the first episode of decompensation, the cumulative probability of survival without liver transplantation in Group A patients was 55%, 26%, and 19% at 1, 3, and 5 years respectively. The median interval between the first episode of decompensation and death/transplantation was 10.2 months (range 0-91).

Group B

Two patients developed HCC. All patients in Group B had by definition suffered a decompensation event prior to the index biopsy and further analysis on subsequent events was not performed.

3.4.4 Factors correlating with survival

Analyses of factors correlating with death or liver transplantation were performed for Group A patients. In univariate analysis, age, Child-Pugh grade, treatment with either standard or pegylated interferon combined with ribavirin, and a response to antiviral treatment (SVR or relapser) were all significantly associated with survival. Figure 3.1a shows the effect of antiviral treatment on the probability of survival without liver transplantation. Laboratory measurements associated with an increased probability of death or transplantation were an elevated serum Bilirubin or Immunoglobulin M or A, and a low Albumin (Table 3.4). The platelet concentration at the time of the index biopsy was significantly lower in those Group A patients with Ishak stage 6 as compared to Ishak stage 4 (129.2 vs 186.5; P = 0.002). Despite this the Ishak stage (4, 5 or 6) did not influence the probability of survival (figure 3.1b). In multivariate analysis, elevated IgA or IgM levels were independent factors predictive of death or liver transplantation, whilst combination antiviral therapy was associated with survival (Table 3.5).

Figure 3.1 (a) The probability of survival without liver transplantation depending on whether patients received antiviral treatment and the response to therapy in those with no previous evidence of decompensation (Group A)

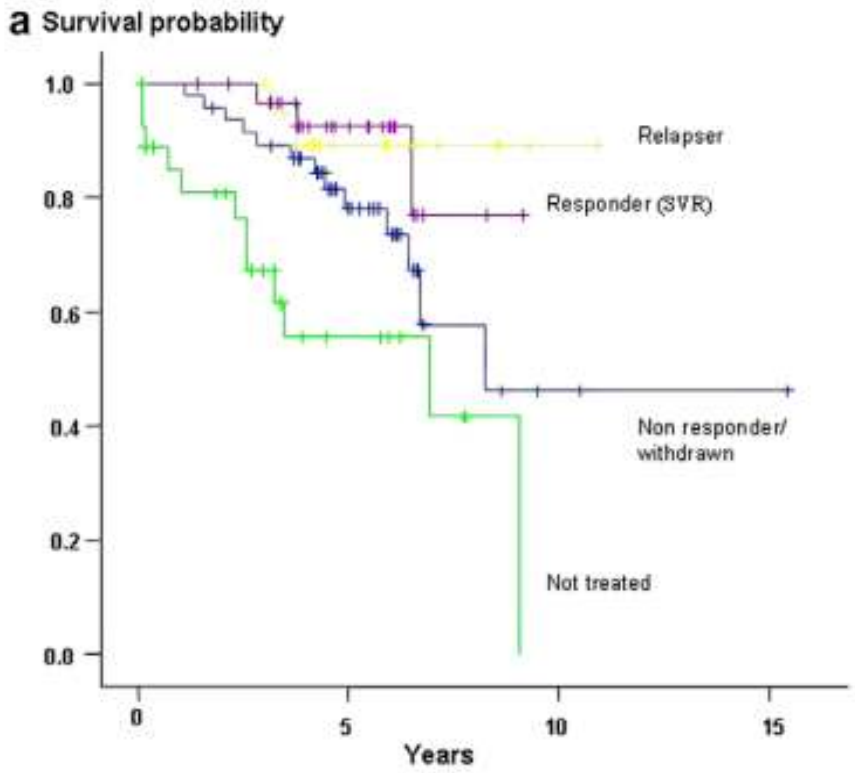


Figure 3.1 (a) demonstrates that patients who achieved a sustained virological response (SVR) or were responder-relapsers following therapy had an improved survival when compared to patients who did not receive antiviral therapy.

Figure 3.1 (b) The probability of survival without liver transplantation depending on Ishak stage at the time of the index biopsy in those with no previous evidence of decompensation (Group A)

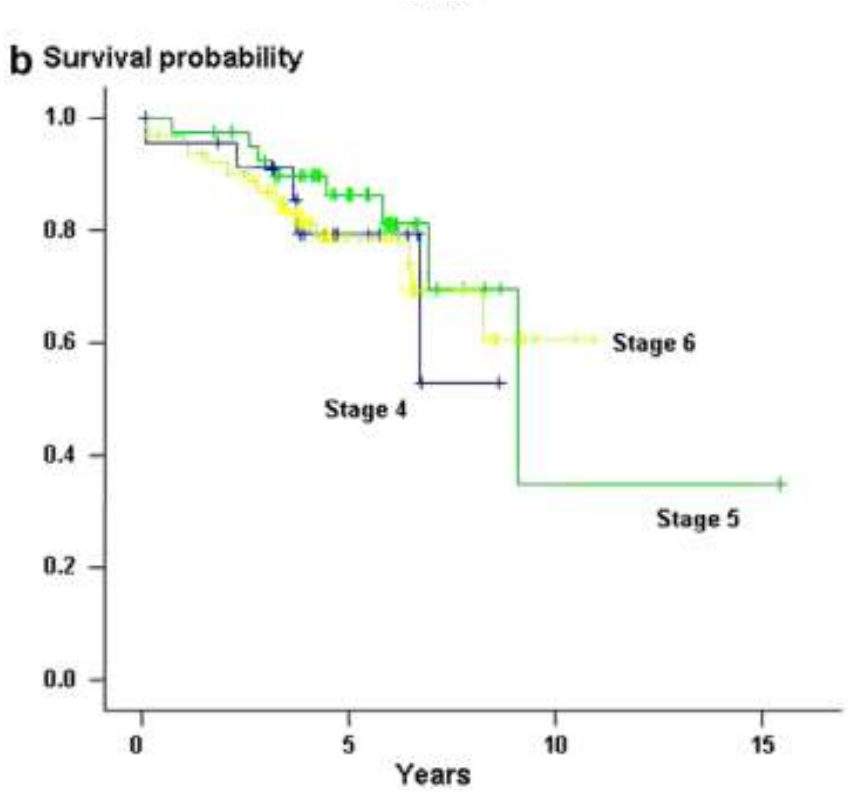


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Figure 3.1 (b) demonstrates that all patients with significant liver fibrosis on biopsy have a similar survival probability, regardless of whether their Ishak stage on liver biopsy was 4, 5 or 6.

Table 3.4 Univariate analysis of factors correlating with death or liver transplantation for patients in group A (no previous decompensation).

Variable			No. of patients	OR (95% CI)	P value
Age (per year)			131	1.04 (1.01-1.08)	0.013
Sex	Female		41	reference	
	Male		90	0.83 (0.40 –1.73)	ns
Ethnic group:	White		106	reference	
	Indian subcontinent		15	1.17 (0.40 - 3.38)	ns
	Black		6	1.48 (0.20 -11.06)	ns
	Other		2	1.56 (0.21 - 11.69)	ns
Route of infection:	No known risk factor		18	reference	
	Intravenous drug use		61	0.73 (0.26-2.06)	ns
	Blood product transfusion		22	1.36 (0.45- 4.09)	ns
Past or present history of alcohol consumption > 80g/d		No	87	reference	
		Yes	39	1.20 (0.55 – 2.60)	ns
Genotype:	1		38	reference	
	3		56	0.80 (0.32 - 1.97)	ns
Ishak stage:	4		23	reference	
	5		42	0.68 (0.23 – 2.07)	ns
	6		65	0.96 (0.35 – 2.65)	ns
Ishak necroinflammatory score (per point)			114	1.06 (0.95-1.19)	ns
Hepatitis B status:	HBsAg and HBcAb neg		80	reference	ns
	HBsAg neg HBcAb pos		46	1.01 (0.45 – 2.29)	ns
	HBsAg pos		3	2.62 (0.33 – 20.61)	ns
Immunoglobulins (per unit increase)*		IgG	111	1.05 (0.98 - 1.13)	ns
		IgM	110	1.33 (1.17 - 1.52)	<0.001
		IgA	111	1.59 (1.25-2.02)	<0.001
Antiviral therapy:	No therapy		29	reference	
	IFN monotherapy (includes PEG =3)		26	0.53 (0.23-1.23)	ns
	Standard IFN + Ribavirin		28	0.31 (0.11 – 0.91)	0.03
	PEG-IFN + Ribavirin		48	0.10 (0.03 – 0.39)	0.001
Response to antiviral therapy:	No treatment		30	reference	
	Non responder/ withdrawn/ died		49	0.46 (0.20 - 1.05)	ns
	Relapser		20	0.16 (0.03 – 0.73)	0.02
	Sustained viral response		32	0.18 (0.05 – 0.65)	0.009
Bilirubin (μg/L)* per μg			129	1.04 (1.01 – 1.07)	0.018
ALT (iu/L)* per iu			130	1.00 (0.10-1.01)	ns
Albumin (g/ L)* per g			127	0.90 (0.85 – 0.96)	0.002
Platelets (x 10^9/L)* per unit			117	1.00 (0.99-1.00)	ns
Child pugh grade	A		104	reference	
	B		7	3.07 (1.03 – 9.19)	0.04

* Laboratory values refer to the result nearest to the index biopsy (providing within 6 months of biopsy). The Child-Pugh grade was calculated retrospectively based on these results where available.

Table 3.5: Multivariate analysis of factors correlating with death or liver transplantation for patients in group A (no previous decompensation).

Variable	Number of Patients	OR (95% CI)	P value	Standardised Relative risk (standard deviation)
Age (per year)	131	1.02 (0.98 – 1.07)	ns	
Sex – (Male)	131 (90)	1.12 (0.40 – 3.11)	ns	
Immunoglobulin A	111	1.36 (1.05 – 1.77)	0.02	1.59 (1.51)
Immunoglobulin M	110	1.42 (1.16 – 1.73)	0.001	1.74 (1.6)
Antiviral therapy:				
No therapy	29	reference	ns	
IFN monotherapy (includes PEG =3)	26	0.43 (0.13 – 1.38)	ns	
Standard IFN + Ribavirin	28	0.26 (0.07 – 1.03)	0.05	
PEG-IFN + Ribavirin	48	0.05 (0.01 – 0.32)	0.001	

3.5 Discussion

This prospective study provides a clearer picture of the prognosis for individuals with chronic HCV infection complicated by severe hepatic fibrosis. By investigating a geographically determined population this study avoids much of the selection bias associated with this type of study. The aim was to examine the natural history in a group of patients that typify those attending hepatitis clinics and patients have, therefore, been included irrespective of their alcohol consumption.

The principal findings concern survival to death or transplantation and the cumulative probability of both HCC and decompensation. Those patients with prior evidence of decompensation were evaluated separately leaving 131 patients in the main analysis; these were similar in age and sex to other studies [210-211, 428-430]. The median estimated duration of HCV infection (22.2 years) is also consistent with the literature [111, 113]. In other respects, however, the patient population differed markedly from those of previous studies. The patients in each of these previous studies were predominantly blood transfusion recipients [210-211, 428-430], whereas almost half of the patients in this study acquired HCV through injecting drug use. This group has been under-represented in some previous studies, with the Eurohep series [211] including just 2% of such patients. Those studies that have focused on patients infected with HCV through injecting drug use have mainly included patients with mild disease [101].

The most important difference, however, is in the inclusion of patients who consumed excess alcohol. At entry to the study 10 (8%) Group A and 7 (37%) group B patients were consuming in excess of 80g/day of alcohol. Heavy alcohol use (> 50g/day) is associated with increased fibrosis[120], however, neither alcohol consumption at the time of biopsy or past heavy alcohol use were prognostic factors in this study. Mean alcohol consumption fell by approximately two thirds following the index biopsy, with only one patient in Group A continuing to drink in excess of 80g/day and only 6 at moderate levels (>32g/d for men or > 24g/d for women). These findings suggest that if patients diagnosed with severe fibrosis reduce their alcohol consumption, subsequent prognosis is not influenced by their prior alcohol intake. This underlines the importance of encouraging HCV infected patients to reduce their alcohol consumption, particularly those with advanced disease.

At the end of the study a quarter of those patients in Group A had died or undergone liver transplantation, with the majority of deaths judged to be related to HCV. Following a diagnosis of severe hepatic fibrosis (Ishak stage $\geq$4), the likelihood of the patient being alive at 5 years was 80%; lower than in some series [211], but consistent with the findings of a French study, which included a similar number of patients with an alcohol consumption of > 80g/day [210]. Following the first appearance of decompensation, the cumulative probability of survival fell to 19% at 5 years. This is lower than the approximately 50% 5 year survival previously reported [211, 428, 431], but two of these studies only reported on patients who remained HCC free [211, 428], while the third included only those patients where the diagnosis of HCC

was subsequent to the decompensation. Our study examined survival following decompensation irrespective of whether the patient also had HCC and the order in which they were diagnosed.

Hepatitis C is suggested to be an important cause for the increase in incidence of HCC in developed countries [197, 199, 432]. The cumulative risk of HCC in group A patients was 15.4% at 5 years, comparable with the 7-13.5% previously reported [210-211, 428-430]. The median estimated duration of infection prior to the diagnosis of HCC was 30 years. Prior HBV infection is suggested as a risk factor for the development of HCC in HCV infection [433], however, a similar proportion of patients who did not develop HCC had a positive HBcAb in this study.

Baseline patient and laboratory characteristics were assessed for their ability to define prognosis using Cox's model. Univariate analysis showed that age, together with indicators of poor liver function (Bilirubin, Albumin and the Child-Pugh grade) were prognostic factors for survival. An elevated serum immunoglobulin M or A was also associated with a worse outcome in both univariate and multivariate analyses. Hypergammaglobulinaemia is a feature of cirrhosis, with reduced antigen clearance by Kupffer cells thought to be the stimulus [434-436], suggesting it may result from rather than contribute to cirrhosis. *In vitro* and animal studies, however, demonstrate that immunoglobulins stimulate the proliferative activity of hepatic stellate cells leading to increased fibrosis [437-438]. Elevated levels of immunoglobulins have been reported in patients with HCV [439], with immunoglobulin A, G and

total immunoglobulin levels also shown to be independent predictors of hepatic fibrosis [440]. Previous natural history studies have not featured elevated immunoglobulins as potential prognostic factors, but neither do they appear to have included measurement of immunoglobulins in their design [111-113, 210-211, 428-429]. This study can not discern whether the observation that raised Immunoglobulin levels are associated with a worse prognosis results from immunoglobulin induced stimulation of hepatic stellate cells or that raised levels are simply a surrogate marker for declining liver function.

The proportion of patients who received antiviral therapy was higher (78% in Group A) than in previous studies. Advanced fibrosis is independently associated with a lower rate of SVR in response to combination antiviral therapy [285-286]. Treatment naïve patients in this study who received Pegylated Interferon and Ribavirin had an SVR of 39%, only marginally lower than for advanced fibrosis patients in the published trials [285-286]. Treatment was associated with survival in both univariate and multivariate analyses. An undetectable HCV RNA at the end of treatment was also associated with survival in the univariate analysis. A potential bias is that the untreated group may include some patients judged too unwell for treatment. Untreated patients were more likely to be Child-Pugh grade B at biopsy (13% vs 5%), genotype 1 (44% vs 33%), and to consume > 80g/day of alcohol (17% vs 6%), suggesting that the treating physician may have been more inclined to recommend treatment if the course of treatment was shorter and the likelihood of success greater. It is noteworthy, however, that treated patients with a viral response, whether sustained or not, faired better than non-responders. This suggests that

interruption of viral replication, even if only temporary, is associated with an improved prognosis, possibly through a reduction in liver fibrosis and inflammation [441-442], which in turn may reduce portal hypertension[443].

Unlike previous studies that looked only at cirrhotic patients, this study included patients with a spectrum of severe liver disease, from portal fibrosis with marked bridging (Ishak stage 4) to probable (Ishak stage 5) or definite cirrhosis (Ishak stage 6). The Ishak stage did not, however, significantly impact on the probability of survival, suggesting progression to severe fibrosis is universally associated with a poor prognosis. Sampling error is an inherent problem to staging HCV on liver biopsy [418, 444]. In 4 of the 19 patients with prior decompensation, the subsequent index biopsy did not demonstrate definite cirrhosis. This could be interpreted as evidence of sampling error, though an alternative explanation is that decompensation was secondary to alcoholic hepatitis, given that all 4 patients previously consumed alcohol in excess of 80g/ day. The significant difference in the mean platelet concentration between Group A patients with Ishak stages 4 and 6, at the time of the index biopsy, suggests that these are distinct groups of patients and that mis-scoring through sampling error was not a significant problem. Physicians need, therefore, to be aware that those patients with bridging fibrosis but not yet definite cirrhosis, are also at significant risk of morbidity and mortality from liver disease in the short as well as long term. Extending the study to include patients with Ishak stage 3, would have incorporated a further 73 patients. During an average of more than six years follow-up, six of these patients died, but none through HCV-related causes. No patients were

diagnosed with HCC or underwent transplantation. This suggests that Ishak stage 4 is the threshold at which significant morbidity and mortality from liver disease occurs.

The whole cohort also included a number of patients with evidence of decompensation prior to the index biopsy (Group B). Patients with advanced liver disease will often present with complications before a defining biopsy has been performed and this is, therefore, an important group to consider. Not surprisingly, these patients had a worse prognosis, with 89% of patients compared with 25% in Group A having died or undergone liver transplantation by the end of the study.

In conclusion, this prospective study examined the natural history of severe hepatic fibrosis secondary to HCV in a geographically determined population. It demonstrates a worse prognosis than has been reported from earlier studies using more selected patient populations. It also confirms that once decompensation develops, HCV infection is associated with a high mortality rate. Indicators of poor synthetic liver function and hypergammaglobulinaemia were found to be important prognostic factors for mortality, while combination antiviral therapy was associated with an improved survival. The results from this study add to our understanding of the natural history of HCV infected patients with severe hepatic fibrosis. It also demonstrates the importance of using data collected from a broad patient population when planning the allocation of resources to the management of hepatitis C.

Chapter 4

The natural history of hepatocellular carcinoma in patients with hepatitis C and the opportunity for early diagnosis with surveillance

4.1 Background

HCC is recognised as an increasing cause of morbidity and mortality in HCV infected patients [199-200]. HCV related HCC almost exclusively occurs in those patients with cirrhosis [106, 201], where approximately 3.7% of patients will develop HCC each year [445]. Those patients who present with symptomatic disease will usually have an advanced tumour that is not amenable to curative therapy. Such patients have a median survival with supportive care alone of just 5.4 months [446]. Tumours identified at an earlier stage may be amenable to curative treatment through resection, transplantation or local ablative therapies. Routine periodic screening of patients with HCV related cirrhosis is, therefore, recommended in UK [447], European [448] and US [449] guidelines in order to diagnose more patients at an earlier stage of disease. This recommendation is based not on randomised controlled trials of surveillance versus no surveillance, as these are lacking, but on observational studies that suggest that HCC detected during formal surveillance are smaller, more often uninodular and more likely to be amenable to curative treatment as compared to those where the diagnosis is as a result of symptoms or incidental [450]. Surveillance programmes vary, but generally incorporate one or both of α-fetoprotein (AFP) and ultrasound at an interval of between 3 and 12 months. The recommended interval is based on evidence showing that the period between undetectable HCC and a 2 cm lesion is 4-12 months [451].

4.2 Aims

The aims of this study were to:

- Examine the opportunity for and effectiveness of surveillance in the early diagnosis of HCC in HCV associated cirrhosis

- Explore factors associated with an increased risk of developing HCC within a population with cirrhosis secondary to HCV.

4.3 Patients and methods

4.3.1 Study population

At the time of this study (2007) the Trent HCV study cohort included 2184 HCV RNA positive patients, from which 46 patients with an additional diagnosis of HCC were identified. Information on HCC diagnosis and treatment and in particular whether the diagnosis was made as a result of surveillance was ascertained from review of the patient's hospital records. These were unavailable in 6 cases. Additional clinical and laboratory data were extracted from the study database.

Patients were judged to have had appropriate surveillance for HCC if they had both an AFP and ultrasound scan at intervals of not longer than 6 months, as recommended in guidance from the British Society of Gastroenterology [447]. HCC were felt to have been diagnosed at an early stage if their dimensions fell within the Milan criteria [452] (a single solitary lesion not greater than 5cm in

diameter or up to 3 nodules with none more than 3cm in diameter, together with no evidence of vascular invasion).

A further 129 patients with biopsy proven cirrhosis (Ishak stage 6) but no evidence of HCC were identified. Data at the point of diagnosis with cirrhosis was taken from the study database and compared to similar data for those patients who subsequently developed HCC.

4.3.2 Statistical analysis

Continuous variables were compared using the Mann-Whitney U-test. Categorical variables were compared using the chi-squared test or Fisher's exact test. A two-tailed p value of < 0.05 was considered significant. Survival analyses from the point of diagnosis with HCC were performed using Life Tables to estimate 1, 3, and 5 year survival. Kaplan-Meier plots were used for the survival curves.

Multiple logistic regression analyses included all HCV infected patients with cirrhosis and were performed with diagnosis of HCC as the dependent variable and included age at diagnosis, sex and those variables from the univariate analysis with a p value < 0.1.

4.4 Results

4.4.1 Patient details

The majority of the 46 patients with HCC were male (78%), with a median age at diagnosis of 60.5 years (range: 42- 83 years). The median estimated duration of HCV infection at the time of diagnosis with HCC was 24.5 years. Forty five percent of patients were current smokers and a further 20% were ex-smokers. An existing diagnosis of diabetes was present in 20% of patients at the time of diagnosis with HCC.

4.4.2 Opportunity for and effectiveness of surveillance

The opportunity for and effectiveness of surveillance was assessed in the 40 patients for whom the hospital notes were available for review. Seventy five percent of patients (30/40) had been diagnosed with cirrhosis at least 6 months prior to the diagnosis of HCC and as such there was an opportunity for surveillance. Of these a third (10/30) had a minimum of AFP and ultrasound every 6 months (appropriate surveillance), while a further 14 patients (47%) had inconsistent surveillance. In total, therefore, 80% of patients had evidence of some form of surveillance.

HCC was diagnosed as a result of surveillance in 14 patients (35%), 58% of those who were having some form of surveillance and 70% of patients who had appropriate surveillance. Thirty (75%) patients diagnosed with HCC had an ultrasound within 6 months of diagnosis, on which HCC was evident in 26/30 (87%) of cases. The median AFP at diagnosis was 54 ng/ml (range 4 -

400,000), with 21% of patients having a normal AFP and 18% a level ≥ 400 ng/ml.

For 6 patients it was not possible from either the hospital records or radiology reports to accurately stage the tumour and, therefore, to ascertain whether it fell within the Milan criteria. Forty four percent of the remaining patients had HCC that fell within the Milan criteria. Patients who were receiving some form of surveillance (55%) or appropriate surveillance (63%) were more likely than those receiving no surveillance (20%) to have earlier stage tumours. When HCC was diagnosed as a result of surveillance 85% (11/13) were within the Milan criteria, compared to 17% (3/18) when the diagnosis was incidental or the result of symptoms (P<0.001). All patients with an AFP ≥ 400 ng/ml (n=5) had tumours whose dimensions fell outside of the Milan criteria compared to 11/26 (42%) where the AFP was < 400 ng/ml.

4.4.3 Mortality

Survival from the time of diagnosis with HCC was 55%, 31% and 19% at 1, 3 and 5 years. Survival was not significantly different in those who received surveillance, but having a potentially curative treatment [liver transplantation (n=9), resection (n=2), radiofrequency ablation (n=6) or alcohol injection (n=3)] was associated with a 84%, 62% and 33% survival at 1, 3 and 5 years compared to a 1 year survival with palliative treatments (n=20) of 37% and no survivors beyond 34 months (P<0.001) (Figure 4.1).

Figure 4.1 (a) Probability of survival depending on whether or not the patient received any form of surveillance.

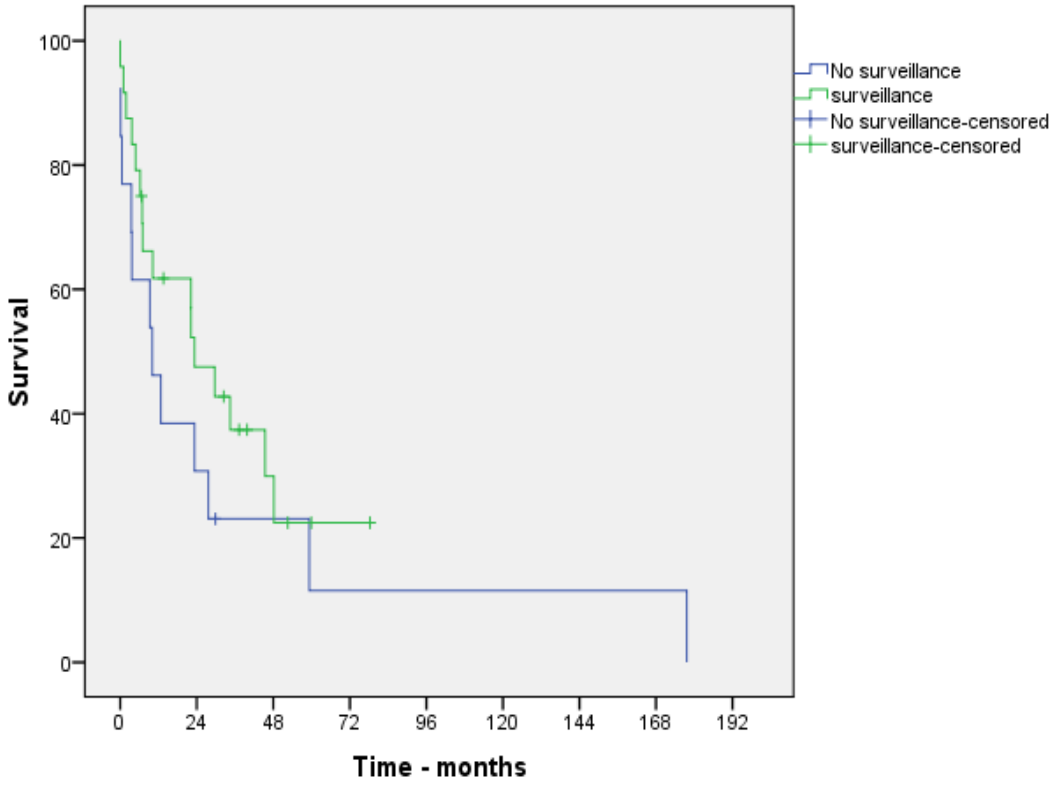


Figure 4.1 (a) illustrates that patients receiving HCC surveillance had a similar survival probability to those who were not receiving any form of surveillance.

Figure 4.1 (b) Probability of survival depending on whether the patient received a potentially curative treatment or palliative treatment

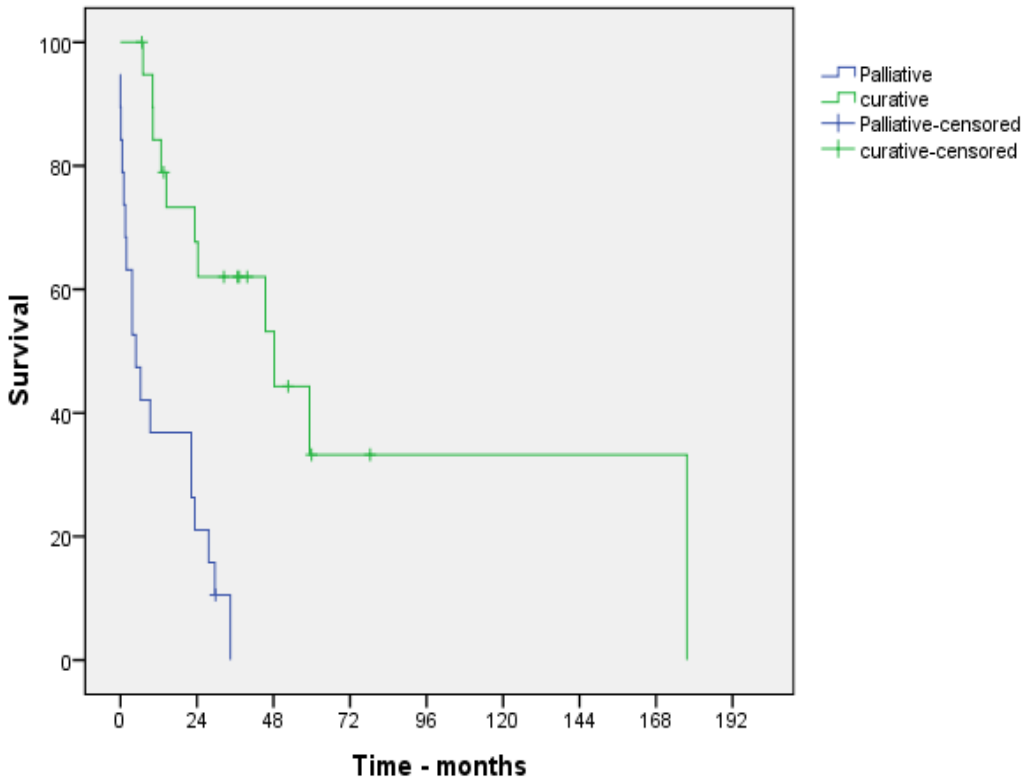


Figure 4.1 (b) demonstrates that patients who were suitable for some form of potentially curative therapy (liver transplantation, surgical resection, radiofrequency ablation or alcohol injection) had an improved survival probability as compared to those patients for whom only palliative therapies were possible.

4.4.4 Factors associated with an increased risk of HCC

Table 4.1 compares the 46 patients with HCC to 129 patients with cirrhosis, but who had not developed HCC at the time of the study. The data presented is taken from the point at which the patient was diagnosed with cirrhosis. Smoking history and information on the presence or not of diabetes which was described earlier for the patients with HCC is not recorded on the study database and was, therefore, not available for the 129 patients with cirrhosis, but no HCC. Patients with HCC were older at the time of diagnosis with cirrhosis (57 vs 46 years, P<0.001), had a higher serum IgG level (20.6 vs 17.0, P=0.007) and a lower albumin (32.4 vs 36.5, P=0.001).

Factors associated with the development of HCC in the univariate analysis included advancing age (OR 1.07 [95% CI; 1.04-1.10, P<0.001]), the estimated duration of infection (OR 1.06 [95% CI; 1.01-1.11, P =0.03]) and higher levels of IgG (OR 1.09 [95% CI; 1.02-1.17, P=0.03]). Treatment with pegylated interferon and ribavirin (OR 0.35 [95% CI; 0.15-0.81, P=0.01]) together with an SVR (OR 0.24 [95% CI; 0.07-0.88, P=0.03) were shown to be protective, as was Sheffield steatosis score 1 vs 0 (OR 0.18 [95% CI; 0.05-0.61, P =0.006]) (Figure 4.2). Those variables that were statistically significant in the univariate analysis were further considered in multivariate analysis, but none were statistical significant in the adjusted model.

Table 4.1 Baseline characteristics of the study population at the time of diagnosis with cirrhosis in those who did or did not subsequently develop HCC.

	HCC (n=46)	Cirrhotics (n=129)	P value
Age at diagnosis with cirrhosis	57 (38-78) (n=42)	46 (15-79)	P<0.001
Male	36 (78%)	92 (71%)	ns
Ethnic group		(n=128)	
White	36 (78%)	102 (80%)	ns
Indian subcont	3 (7%)	18 (14%)	
Black	5 (11%)	4 (3%)	
Other	2 (4%)	4 (3%)	
Main risk factor		(n=126)	
IVDU	14 (34%)	54 (43%)	ns
Blood Tx	13 (32%)	21 (17%)	
Other	7 (17%)	29 (23%)	
No known risk factor	7 (17%)	22 (18%)	
Ever heavy drinker	25 (61%) (n=41)	60 (57%) (n=105)	ns
Genotype	(n=24)	(n=113)	ns
1	7 (29%)	36 (32%)	
2	3 (13%)	8 (7%)	
3	12 (50%)	64 (57%)	
4 or 5	2 (8%)	5 (5%)	
Ishak necroinflammatory score	5.7 ± 5.0 (n=22)	4.8 ± 2.5 (n=79)	ns
	(n=21)	(n=77)	
Sheffield steatosis score 0	14 (67%)	24 (31%)	ns
1	4 (19%)	38 (49%)	
2	1 (5%)	9 (12%)	
3	2 (10%)	6 (8%)	
Hep B status HBsAg	0 (n=45)	6 (6%) (n=109)	ns
HBcAb	15 (47%) (n=32)	31 (43%) (n=73)	
BMI	25.9 ± 3.5 (n=11)	27.4 ± 5.8(n=79)	ns
Estimated duration of infection	24.7 ± 8.9 (n=23)	21.2 ± 10.3 (n=81)	ns
Immunoglobulins IgG	20.6 ± 7.6 (n=35)	17.0 ± 5.9 (n=72)	P=0.007
IgA	4.2 ± 2.4 (n=35)	3.9 ± 3.2 (n=72)	ns
IgM	2.1 ± 2.0 (n=35)	2.1 ± 1.3 (n=72)	ns
Antiviral therapy: No therapy	22 (48%)	45 (35%)	treatment
Standard IFN monotherapy	11 (24%)	11 (9%)	vs no
Standard IFN + Ribavirin	3 (7%)	14 (11%)	treatment
PEG-IFN + Ribavirin	10 (22%)	59 (46%)	ns
Response to antiviral therapy:			SVR vs
Non responder/ withdrawn/ died	(n=22)	(n=80)	no SVR
Relapser	16 (73%)	33 (42%)	
Sustained viral response	3 (14%)	20 (25%)	ns
	3 (14%)	26 (33%)	
Bilirubin	37.6 ± 99.3 (n=33)	19.1 ± 14.1 (n=98)	ns
ALT	116 ± 63 (n=33)	118.7 ± 71.8 (n=101)	ns
GGT	249.1 ± 318.3 (n=30)	151.1 ± 158.4 (n=79)	ns
Albumin	32.4 ± 6.0 (n=30)	36.5 ± 5.5 (n=94)	P=0.001

Table 4.2 Univariate analysis of factors correlating with development of Hepatocellular cancer in patients with cirrhosis

Variable			OR (95% CI)	P value
Age (per year)			1.07 (1.04-1.10)	< 0.001
Sex	Female		reference	
	Male		1.45 (0.65 –3.22)	ns
Ethnic group:	White		reference	
	Indian subcontinent		0.47 (0.13 - 1.70)	ns
	Black		3.54 (0.90 -13.92)	ns
	Other		1.42 (0.25 – 8.07)	ns
Route of infection:	No known risk factor		reference	
	Intravenous drug use		0.82 (0.29-2.29)	ns
	Blood product transfusion		1.95 (0.65- 5.82)	ns
Ever heavy drinker (> 80g/d)		No	reference	
		Yes	1.17 (0.56 – 2.45)	ns
Genotype:	1		reference	
	3		0.96 (0.35 - 2.67)	ns
Ishak necroinflammatory score (per point)			1.16 (0.96-1.39)	ns
Sheffield steatosis score	0		reference	
	1		0.18 (0.05-0.61)	0.006
	2		0.19 (0.02-1.67)	ns
	3		0.57 (0.10-3.23)	ns
Hepatitis B status:	HBsAg and HBcAb neg		reference	ns
	HBsAg neg HBcAb pos		1.21 (0.52 – 2.79)	ns
	HBsAg pos		0 (0– ∞)	ns
BMI			0.95 (0.83-1.08)	ns
Estimated duration of infection			1.06 (1.01-1.11)	0.03
Immunoglobulins (per unit increase)		IgG	1.09 (1.02 - 1.17)	0.03
		IgM	1.03 (0.80 - 1.32)	ns
		IgA	1.04 (0.91-1.18)	ns
Antiviral therapy:	No therapy		reference	
	Standard IFN monotherapy		2.05 (0.77-5.45)	ns
	Standard IFN + Ribavirin		0.44(0.11 – 1.69)	ns
	PEG-IFN + Ribavirin		0.35 (0.15 – 0.81)	0.01
Response to antiviral therapy:	No therapy		reference	
	Non responder/ withdrawn/ died		1.01 (0.46 - 2.22)	ns
	Relapser		0.16 (0.08 – 1.17)	ns
	Sustained viral response		0.24 (0.07 – 0.88)	0.03
Bilirubin			1.00 (0.98 – 1.02)	ns
ALT			1.00 (0.99-1.00)	ns
GGT			1.00 (1.00-1.00)	ns
Albumin			0.96 (0.91 – 1.02)	ns

4.5 Discussion

This study describes 46 HCV infected patients with hepatocellular carcinoma identified from the Trent HCV study. The proportion of male patients (78%) and the median age at diagnosis (60.5 years) are similar to other case series of patients with HCC [453]. An estimated duration of HCV infection of 24.5 years prior to the diagnosis of HCC is also in keeping with our understanding of the natural history of HCV [106, 201]. Sixty five percent of patients were current (45%) or ex-smokers (20%) at the time of diagnosis with HCC. Studies that have explored the association between smoking and HCC suggest it is not independently associated with an increased risk of HCC, but further increases the risk of HCC in both HBV and HCV infected patients. A meta-analysis of 6 studies demonstrated a multiplicative interaction between HCV and smoking [454]. In a Japanese series there was a significantly increased risk of HCC for both current (OR 9.6 [95% CI; 1.50 – 61.36]) and ex- smokers (OR 7.8[95% CI; 1.09-56.15]) infected with HCV [455]. Diabetes has also been shown to increase the risk of HCC, both independently and in synergy with HCV. Twenty percent of patients with HCC in the current series also had a diagnosis of diabetes. In a large cohort of US patients from veteran's association (VA) medical centres the incident rate for HCC was 2.39 vs 0.87 per 10,000 person years ($P<0.0001$) in diabetic and non-diabetic subjects respectively. Diabetes was associated with a hazard ratio for HCC of 2.16 (95% CI; 1.86-2.52, $P<0.001$) [456]. Another large study included 2061 patients with HCC and 6183 non-cancer controls identified through the SEER-Medicare database. Forty three percent of the patients with HCC had diabetes compared to 19% of the non-cancer patients. This represented a 2-3 fold increased risk of HCC in

diabetics, independently of other risk factors. The odds ratio for HCC in HCV infected patients without diabetes was OR 24.4 compared to OR 36.9 in HCV infected patients with diabetes [457]. The synergistic relationship is supported by the study of Chen et al which demonstrated a 3.5 fold increased risk for HCV infected patients with diabetes compared to those without diabetes [458].

Regular screening for HCC in patients with cirrhosis secondary to HCC is recommended in British [447], European [448] and US [449] guidelines. In a 2002 survey of gastroenterologists working in the UK, three-quarters undertook formal surveillance for HCC [459]. The findings were similar in an analogous US study [460]. Three quarters of the patients with HCC in the current study were known to have cirrhosis at least 6 months prior to the diagnosis of cancer and as such had the opportunity for surveillance. Some form of surveillance took place in 80% of patients, though only a third had appropriate surveillance, as recommended in guidance produced by the British Society of Gastroenterology. In a study of 13002 patients diagnosed with HCV related cirrhosis in VA medical centres in the US, 42% received at least one surveillance test within the first year, but the number having surveillance then declined over the subsequent 2-4 years of the study. Of those patients with at least 2 years follow-up, only 12% were still receiving routine surveillance, with a further 58.5% having tests on an inconsistent basis [461]. A retrospective study of 1480 HCV infected patients (US – VA medical centres) who developed HCC between 1998 and 2007 showed that annual surveillance with both AFP and ultrasound was observed in only 2% of patients[462]. An issue

with surveillance programmes is patient uptake and 3-18% will fail to comply with the programme [463].

Surveillance programmes generally utilise one or both of AFP measurement and ultrasound. AFP is produced by immature hepatocytes and can, therefore, be detected in the blood at times of liver regeneration or in the presence of HCC which are made up of transformed, often immature hepatocytes. Unfortunately the sensitivity of AFP as a diagnostic tool is rather restricted by the fact that non-AFP secreting tumours constitute approximately 20% of HCC [447] and a raised AFP can be seen during a flare in viral hepatitis [448]. A cut off for AFP of 20 ng/ml has a sensitivity of 41-65% and a specificity of 80-94% [464]. In the current study 21% of HCC had a normal AFP at diagnosis. Ultrasound as a diagnostic tool performs somewhat better with a sensitivity of 35-84% and a specificity of 92-98% [450]. The performance of ultrasound is dependent on the size of the lesion with a sensitivity of 75% for lesions greater than 5cm and less than 20% for lesions below 2cm in diameter [465]. In the present study ultrasound identified the lesion in 87% of patients who had an ultrasound at the time of or in the 6 months preceding the diagnosis of HCC.

Those that dispute the effectiveness of HCC surveillance argue that there is a lack of high quality randomised controlled trials (RCT) demonstrating that it improves patient outcomes [466-467]. A single RCT of surveillance using 6 monthly ultrasound and AFP in exclusively HBV infected Chinese patients has shown an improvement in mortality at 5 years in those receiving surveillance with a mortality ratio of 0.63 (95% CI 0.41-0.98). The mortality benefit was

attributed mainly to the detection of tumours at an earlier stage [468]. Diagnosing tumours at an earlier stage may make them more amenable to treatment, but it also introduces the potential for lead-time bias in any observed survival benefit. Observational studies have also demonstrated that tumours detected by surveillance are more likely to be solitary, at an early stage and be amenable to potentially curative treatment. Adjusted analysis to take into account lead time bias in these studies still shows that surveillance is associated with improved survival [469-471]. A study of 240 HCV infected patients with cirrhosis demonstrated that the proportion of patients with lesions that were solitary, early stage and amenable to curative therapy was 66%, 83% and 80% respectively in those diagnosed as a result of surveillance and 24%, 24% and 27% in those detected outside of a surveillance programme or scheduled doctor visits [472]. Those HCC detected as a result of surveillance tests in the present study were more likely to fall within the Milan criteria than those diagnosed outside of regular surveillance (85% vs 17%) and receiving a potentially curative treatment was associated with a better survival. Thirty percent of patients who were having appropriate surveillance with AFP testing and ultrasound every 6 months did not, however, have their tumour diagnosed as a result of a surveillance test and simply receiving surveillance was not associated with an improvement in mortality in this study. In the study of 1480 infected patients with HCC described earlier, the presence of either AFP or ultrasound surveillance during the 2 years preceding HCC diagnosis was associated with a lower mortality risk [462]. In a systematic review and economic analysis of HCC surveillance the most effective form of surveillance was AFP testing and ultrasound at 6 month intervals. Assuming a maximum

willingness to pay for a quality adjusted life year (QALY) of £30,000 the optimum surveillance strategy was dependent on the aetiology of cirrhosis. For patients with HCV cirrhosis the most cost effective strategy was triage with AFP testing every 6 months [450].

US data taken from the SEER database between 1992 and 1996 show that regardless of treatment the median survival following the diagnosis of HCC was 0.64 years with a 1 and 5 year survival of 23 and 6% respectively [473]. Trent HCV study patients faired slightly better with a 1, 3 and 5 year survival of 55, 31 and 19% following the diagnosis of HCC, though these figures are significantly less than the 86%, 49% and 24% observed for HCV infected patients in the study of Trevisani et al [474]. The latter study also demonstrated that prognosis from HCC was independent of the aetiology of cirrhosis [474].

This study also examined the characteristics of patients diagnosed with HCC as compared to HCV infected patients with cirrhosis who had not developed HCC. A multivariate model did not identify any factors that were independently associated with developing HCC. In the univariate analysis an increase in the patient's age and the duration of HCV infection were associated with an increased risk of HCC. Patients over the age of 55 years have previously been recognised to have a 2-4 fold increased risk of HCC [210-211]. The risk also increases with the length of infection with the mean estimated duration of infection in this study of 24.7 years typical of the time from acquisition of infection to cancer observed in previous studies [106, 201]. Increases in IgG levels were also associated with an increased risk of HCC. As

outlined in an earlier chapter raised immunoglobulins may be a surrogate marker for a more advanced stage of disease and also possibly stimulate the proliferative activity of hepatic stellate cells leading to increased fibrosis [437-438]. Hepatic steatosis may also have been expected to be associated with an increased risk of HCC as has been shown elsewhere [217], and given the increased risk that has been described with diabetes [456-458]. Chen et al have also shown that HCV infected patients with a BMI ≥ 30 have a 4-fold increased risk of HCC [458]. In the current study BMI did not confer an increased risk of HCC and patients with mild steatosis (stage 1) were at less risk of HCC than patients with no steatosis. The latter result is hard to explain except that 28.6% of patients with steatosis stage 1 had an SVR following antiviral therapy compared to 18.4% of those with no steatosis. This may be because of the higher frequency of genotype 3 infection (58% vs 48%), though neither association was statistically significant.

Both combination therapy with pegylated interferon and ribavirin, and a SVR were associated with a lower risk of HCC. Several studies have shown that interferon based therapy decreases the risk of progression to HCC [360-361, 475-477], particularly in those who achieve an SVR [478-479]. Recent evidence suggests this benefit is even seen in those patients who already have cirrhosis at the point of treatment [365, 453].

A major limitation of this study is the small number of patients with HCC found within the Trent HCV study cohort. This in particular effects the conclusions that can be drawn from the comparison with cirrhotic patients

without evidence of HCC. This study may have missed factors associated with a small increased risk of HCC given that wide confidence intervals are a characteristic of small studies. There was also a lack of data on smoking history and diabetes in the cirrhotic patients without HCC. This data is not recorded in the Trent study database and searching the hospital records of all the cirrhotic patients was not felt to be productive, given the small number of HCC patients and reservations regarding the size of the study. This study also only addressed the performance of a single AFP level at the time of HCC diagnosis. It would be interesting to look at trends in AFP levels to see whether an increasing trend rather than an absolute cut-off value best predicts the presence of HCC.

In conclusion this study could not demonstrate any factors in cirrhotic patients that were independently associated with an increased risk of HCC in a multivariate model. Advancing age and duration of disease were associated with an increased likelihood of HCC and combination therapy with pegylated interferon and ribavirin, together with an SVR were protective in the univariate analysis. It has also shown that the majority of patients with HCC have previously been recognised as cirrhotic and there is, therefore, an opportunity for surveillance. Guidance on HCC surveillance was, however, only variably applied in the Trent region and there was no survival benefit to having surveillance. The majority of HCC were diagnosed at an advanced stage, but were more likely to be diagnosed at an earlier stage if detected as a result of surveillance. Survival was significantly improved where patients had a potentially curative treatment. While lead time bias may contribute to the

improvement in survival this results suggests that we need to develop strategies that more reliably diagnose HCC at an earlier stage than current practices allow.

Chapter 5

A comparison of the natural history and outcome of treatment for Asian and Non-Asian hepatitis C infected patients

5.1 Background

Host factors such as age, sex, alcohol consumption and immunodeficiency have been shown to influence disease progression and/ or the outcome of treatment in Hepatitis C (HCV) infection [111, 113, 125]. Ethnicity is an important host variable, known to influence disease manifestations and progression in many diseases [480-482]. Its importance compared to other host factors in HCV infection is less clear.

African Americans appear to have lower response rates with interferon based therapies as compared to white patients [325-330]. On the other hand they are reported to have slower progression of fibrosis as compared to Non-African Americans [140, 483]. The picture for Asian patients with HCV is less clear with Asian Americans whose origins are mainly in South East Asia seeming to have a similar [329] or more favourable response to treatment [484], while a United Kingdom Asian population, the majority of whom originated from the Indian sub-continent, had a poor response to treatment as compared to white patients in one study [485].

5.2 Aims

The aims of this study were to investigate the natural history of Hepatitis C infection and the outcome of therapy in an Asian population originating from the Indian subcontinent and to compare disease progression and sustained virological response rates following antiviral therapy with a group of white patients.

5.3 Methods

5.3.1 Study Population

At the time of this analysis (2009), the Trent HCV study cohort included 2451 HCV RNA positive patients of which 2364 have a known ethnic group. Of these, 2123 were white and 120 Asian (Pakistani 83, Indian 36 and Bangladeshi 1). Disease progression was calculated on the basis of fibrosis stage/ duration of infection, in those patients who had both a liver biopsy prior to therapy and an estimated duration of infection.

491 White and 55 Asian patients had completed a course of treatment with Pegylated Interferon and Ribavirin and had a known treatment response. 51/55 Asian patients had a recorded genotype of which 46 (90.2%) were genotype 3. A comparison of treatment outcome was, therefore, restricted to the 224 White and 46 Asian genotype 3 infected patients who had received Pegylated Interferon and Ribavirin.

5.3.2 Statistical Methods

SPSS v16 was used for all statistical analyses. Continuous variables were compared using the Mann-Whitney U-test. Categorical variables were compared using the chi-squared test or Fisher's exact test. A two-tailed P value of < 0.05 was considered significant. Multiple logistic regression analyses (forwards, likelihood ratio, inclusion at $p < 0.1$) were performed with a sustained virological response (SVR) as the dependent variable.

5.4 Results

5.4.1 Patient details

Table 5.1 shows the baseline characteristics of the 2123 White and 120 Asian patients in the study cohort. There was a greater proportion of female patients (43.3% vs 30.5%, P=0.003) and Genotype 3 infection (79.2% vs 42.8%, P < 0.001) within the Asian group. There was also a significant difference in alcohol consumption with only 15.5% of Asian patients reporting they drank alcohol as compared to 56.6% of whites (P< 0.001). A past or present history of excess alcohol (> 50g/d for at least 6 months) was noted for 51.4% of white and 6.5% of Asian patients (P<0.001). Elicit use of intravenous drugs (77%) was the main risk factor for infection in whites, while place of birth (i.e. being born abroad, 67%) was the most common in the Asian patients.

5.4.2 Liver Histology/ Disease progression

1173 (55.3%) White and 76 (63.3%) Asian patients had at least 1 liver biopsy prior to receiving antiviral therapy. There was no significant difference in the proportion of liver biopsy (P=0.089). Of those biopsied, 1052 White and 69 Asian patients had at least an Ishak fibrosis stage recorded on the study database (Table 5.2).

Asian patients undergoing liver biopsy were older (43.9 ± 13.6 vs 37.9 ± 10.6 years, P < 0.001) and more likely to be female (46.4 vs 29.1%, P=0.004) than their White counterparts. The Ishak necroinflammatory (4.3 ± 2. 5 vs 3.4 ± 2.1, P<0.001), Ishak fibrosis score (3.0 ± 2.3 vs 1.8 ± 2.0, P = 0.001) and Sheffield steatosis scores (0.9 ± 0.9 vs 0.6 ± 0.8, P=0.016) were all significantly higher

in Asian patients. When analysis of steatosis score was restricted to genotype 3 patients there was no significant difference (1.0 ± 0.9 vs 0.9 ± 0.9, P = 0.369). The duration of infection at the time of liver biopsy was significantly longer in White patients (9.9 ± 8.1 vs 16.7 ± 9.6 years, P=0.002), with the major caveat that duration of infection could only be estimated in a minority of Asian patients (n = 18) due to their only risk factor being their country of birth. Only 72 White and 2 Asian patients had more than one biopsy prior to therapy and, therefore, fibrosis progression was calculated on the basis of fibrosis stage on first biopsy/ duration of infection (points/ year). The rate of fibrosis progression (0.25 ± 0.31 vs 0.16 ± 0.54 points/year, P=0.068) was similar for both groups of patients (Table 5.2).

5.4.3 Outcome of antiviral therapy

224 White and 46 Asian genotype 3 patients had completed a course of Pegylated Interferon and Ribavirin therapy. Patients within the Asian group were again more likely to be female (50% vs 32%, P=0.027), but were of similar age at the time of treatment (43.3 ± 11.7 vs 42.5 ± 9.3 years, P=0.878). Table 5.3 shows the outcome of therapy for both Asian and White patients. There was no significant difference in the number of patients achieving an SVR (78.3 vs 67.9%, P=0.162), though a greater proportion of white patients were responder relapsers (4.3 vs 18.8%, P=0.016).

Table 5.1. Characteristics of White and Asian patients within the study cohort

	White (n=2123)	Asian (n=120)	P value*
Sex - Male:Female (% male)	1476:647 (69.5%)	68:52 (56.7%)	P=0.003
Age (years) at date of discovery	36.0 ± 11.5 35 (1-82)	38.8 ± 13.1 38.5 (10-73)	P=0.024 (mw)
Main risk factor	n=2038	n=101	
IVDU	1571 (77%)	10 (10%)	IVDU vs Not
Blood Transfusion	214 (11%)	17 (17%)	IVDU
Other	141 (7%)	72 (71%)	P<0.001
- Born abroad	- 24 (1%)	- 68 (67%)	
Not IVDU, Blood, tattoo or sex	114 (6%)	4 (4%)	
Genotype	n=1557	n=96	
1	730 (46.9%)	15 (15.6%)	G1 vs not G1
2	133 (8.5%)	3 (3.1%)	P<0.001
3	666 (42.8%)	76 (79.2%)	
4	19 (1.2%)	1 (1%)	
5	8 (0.5%)	1 (1%)	
6	1 (0.1%)	0	
Hepatitis B status			
HBsAg positive	43 (2.7%) (n=1559)	4 (4.5%) (n=89)	P=0.308
HBcAb positive	407 (32.0%) (n=1273)	19 (33.3%) (n=57)	P=0.829
Drinks alcohol	n=1862	n=97	
Yes	1053 (56.6%)	15 (15.5%)	P<0.001
No	809 (43.4%)	82 (84.5%)	
Past or present history of excess alcohol (>50g/d for at least 6 months)	n=1763 907 (51.4%)	n=92 6 (6.5%)	P<0.001
BMI (kg/m^2)	n=1247 25.4 ± 4.9 24.6 (14.5-61.7)	n=76 26.2 ± 4.3 25.8 (19.7-35.5)	P=0.041 (mw)

*Chi squared unless stated. mw = Mann Whitney
** For each variable, the top line indicates mean +/- S.D., and the lower line median (range)

Table 5.2: Histological findings and rate of fibrosis progression in treatment naïve patients who underwent liver biopsy

	White (n=1052)	Asian (n=69)	P value*
Sex - Male:Female (% male)	746:306 (70.9%)	37:32 (53.6%)	P=0.004 (Chi square)
Age (years)	37.9 ± 10.6** 37 (16-85)	43.9 ± 13.6 45 (20-79)	P<0.001
Ishak score	N=991 5.1 ± 3.6 4 (0-21)	N=64 7.3 ± 4.2 4 (1-15)	P<0.001
Ishak necroinflammatory score	N=991 3.4 ± 2.1 3 (0-17)	N=64 4.3 ± 2.5 4 (1-15)	P=0.001
Ishak stage	N=1052 1.8 ± 2.0 1 (0-6)	N=69 3.0 ± 2.3 3 (0-6)	P<0.001
Sheffield steatosis score	N=872 0.6 ± 0.8 0 (0-3)	N=571 0.9 ± 0.9 1 (0-3)	P=0.016
Duration of infection (years)	N=901 16.7 ± 9.6 15.9 (0.5-52.8)	N=18 9.9 ± 8.1 8.7 (0.9-31.5)	P=0.002
Fibrosis progression (stages/yr) (Stage/duration of infection)	N=901 0.16 ± 0.54 0.07 (0-11.1)	N=18 0.25 ± 0.31 0.14 (0-1.1)	P=0.068

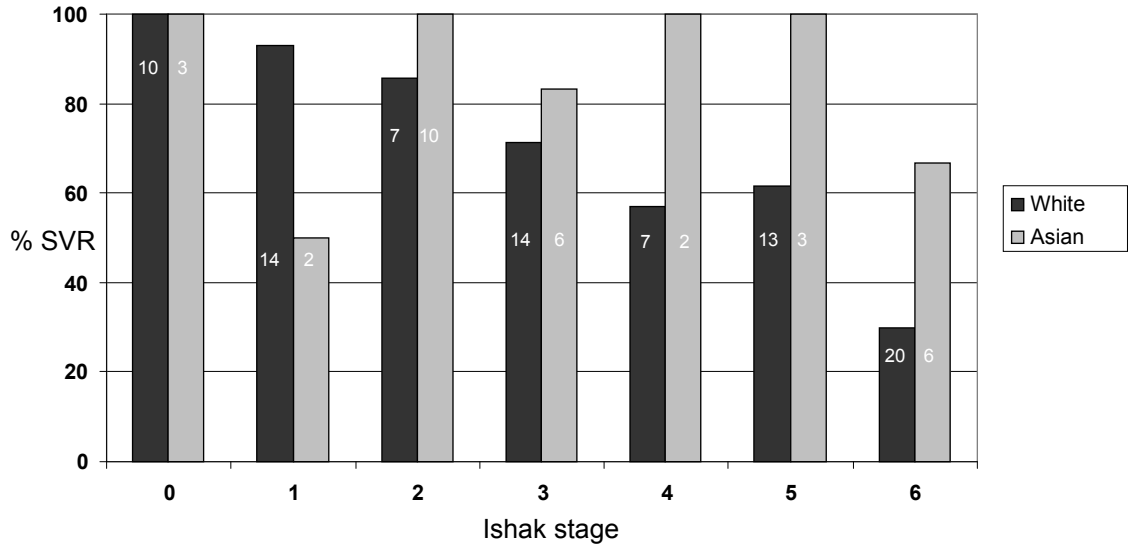
* P value calculated using Mann-Whitney test unless stated
** For each variable, the top line indicates mean +/- S.D., and the lower line median (range)

Table 5.3. Outcome of therapy for both White and Asian genotype 3 patients

	White	Asian	P value
N	224	46	
SVR	152 (67.9)	36 (78.3)	0.162
NR	6 (2.7)	3 (6.5)	0.186
RR	42 (18.8)	2 (4.3)	0.016
Treatment withdrawn	24 (10.7)	5 (10.9)	0.975

Percentages in parentheses
SVR (Sustained virological response), NR (Non-Responder), RR (Responder-Relapser)

Figure 5.1. Sustained virological response (SVR) rates in genotype 3 patients depending on Ishak stage and ethnicity



Number on bar represents number of patients

Figure 5.1 demonstrates that for White patients where the numbers were greater there was a progressive decline in the percentage of patients achieving a sustained virological response (SVR) as the Ishak fibrosis stage increased. This correlation was not observed in the Asian group, but the number of patients was smaller. When patients were grouped according to the Ishak fibrosis stage, the SVR rate amongst Asian patients was observed to be at least that of White patients for each group except Ishak stage 1 (SVR rate for Asian patients was 50% for 2 patients, i.e 1 did not achieve an SVR).

Of those completing therapy, 85 white and 23 Asian subjects had a liver biopsy in the 12 months prior to therapy. Unlike the cohort as a whole, Ishak necroinflammatory (4.8 ± 3.0 vs 4.0 ± 2.0, P =0.343) and fibrosis scores (3.5 ± 2.1 vs 3.3 ± 2.1, P =0.706) were similar for both White and Asian patients who subsequently had therapy. Figure 5.1 displays the sustained virological response depending on Ishak stage and ethnic group.

On univariate analysis (Table 5.4) increasing age and duration of infection, together with levels of fibrosis on biopsy equal and above to Ishak stage 4 were associated with a reduction in the likelihood of a sustained virological response. Higher levels of bilirubin, ALT and GGT together with lower levels of platelets were also associated with a lack of treatment success.

In the multivariate model (Table 5.5), cirrhosis (Ishak stage 6) was associated with a reduction in the likelihood of a sustained virological response as were increased levels of GGT.

Table 5.4: Univariate analysis of factors associated with an SVR

Variable		No. of patients	OR (95% CI)	P value
Age (per year)		270	0.958 (0.932-0.986)	0.003
Sex Female		95	reference	
Male		175	0.682 (0.390-1.193)	0.180
Duration of infection		207	0.958 (0.930-0.986)	0.004
Ethnic group:	White	224	reference	
	Asian	46	1.705 (0.802 –3.627)	0.166
Route of infection:	No known risk factor	15	reference	
	Intravenous drug use	159	0.345 (0.075-1.589)	0.172
	Blood product transfusion	34	0.220 (0.043-1.131)	0.070
	Other	55	0.375 (0.076-1.854)	0.229
Drinks Alcohol				
	No	133	Reference	
	Yes	123	0.960 (0.566-1.629)	0.879
Past or present history of alcohol consumption > 50g/d				
No		147	reference	
Yes		102	0.583 (0.339-1.003)	0.051
BMI (kg/m^2)		245	0.977 (0.917-1.041)	0.475
Ishak stage:	0,1	29	Reference	
	2,3	28	0.272 (0.05-1.481)	0.132
	4,5	25	0.157 (0.03-0.831)	0.029
	6	26	0.046 (0.009-0.238)	<0.001
	No biopsy taken	155	0.170 (0.039-0.745)	0.019
Ishak necroinflammatory score:	0,1,2	22	reference	
	3,4	33	2.122 (0.630-7.079)	0.221
	5,6	29	1.086 (0.341-3.456)	0.885
	7,8	10	2.286 (0.387-13.505)	0.362
	≥ 9	1		
	No biopsy taken	155	1.313 (0.516-3.340)	0.568
Bilirubin		255	0.948 (0.912-0.984)	0.005
ALT		255	0.997 (0.994-1.000)	0.07
Albumin		255	1.093 (1.029-1.160)	0.004
GGT		189	0.996 (0.993-0.999)	0.011
Platelets		170	1.006 (1.002-1.011)	0.006

Table 5.5: Multivariate analysis of factors associated with an SVR

Variable	OR (95% CI)	P value
GGT	0.991 (0.984 – 0.999)	0.036
Ishak fibrosis score 0,1	Reference	
Ishak fibrosis score 2,3	0.121 (0.005-3.218)	0.207
Ishak fibrosis score 4,5	0.056 (0.002-1.279)	0.071
Ishak fibrosis score 6	0.015 (0.001-0.381)	0.011

5.5 Discussion

Ethnicity impacts on many aspects of HCV infection; from awareness of the disease in the non-infected [486] to perception and knowledge of risk factors in those infected [484]; from rates of seroprevalence [481] to rates of testing and referral for treatment [275, 487]. Perhaps most importantly patient ethnicity has been shown to be an important predictor of disease progression and response to treatment in some populations. A number of studies have shown that African Americans have an inferior response to treatment as compared to other ethnic groups [325-330] and yet may also have a slower rate of fibrosis progression than Non-African American patients [140, 483]. The picture for Asian populations is less clear with a distinction previously being drawn by some groups between patients of South-East Asian origin who may have a more favourable response to treatment as compared to White patients [484] and patients from the Indian subcontinent where the SVR has been previously reported to be inferior [485].

The largest ethnic minority group in the United Kingdom are Asians whose origins lie in the Indian subcontinent (India, Pakistan and Bangladesh). This study aimed to compare disease progression and treatment outcome in Asian and White patients chronically infected with HCV within the Trent HCV cohort. It demonstrates significant differences in the epidemiology and presentation of HCV infection between these two ethnic groups. Asian patients are more likely to be female, older at diagnosis, to drink less alcohol and to have genotype 3 infection. Unlike White patients where the predominant risk

factor for infection is a history of injecting drug use, the majority of Asian patients lack identifiable risk factors, other than their country of birth.

There was a similar frequency of liver biopsy amongst Asian and White patients prior to therapy. There were, however, significant differences in the severity of the histological manifestations of HCV infection demonstrated. Asian patients had significantly higher necroinflammatory, fibrosis and steatosis scores as compared to Whites. Asian patients were older at presentation as compared to White patients. If we assume that for those patients where the only identifiable risk factor was having being born in a country with a high seroprevalence of HCV infection, infection was acquired at a young age, then Asian patients will also have a longer duration of infection. A longer duration of infection and the older age at presentation may explain the higher fibrosis scores on biopsy seen in Asian patients, a hypothesis indirectly supported by the observation that for the small minority (18/69) of biopsied Asian patients with dateable risk factors, the rate of fibrosis progression estimated on the basis of the initial biopsy and the estimated duration of infection, was not significantly different to that of White patients. The higher steatosis score is likely to relate to the predominance of genotype 3 infection in Asian patients, particularly given that both Asian and White patients had a similar BMI and that there was no significant difference in steatosis score when the analysis was restricted to genotype 3 patients. There is a large body of evidence linking genotype 3 infection with increased hepatic steatosis [133, 136].

The analysis of response rates to PEG-Interferon and Ribavirin combination therapy in previously treatment naïve patients was restricted to patients with genotype 3 infection, due to the low number of infections with other genotypes in Asian patients receiving treatment. There was no significant difference in SVR rates between White and Asian patients and ethnic group was not a predictor of treatment response in either the univariate or multivariate analysis. Unlike the entire cohort, White and Asian patients receiving combination treatment had similar necroinflammatory and fibrosis scores to one another. Predictors of treatment response in the univariate analysis were age, duration of infection, an Ishak fibrosis score ≥ 4, bilrubin, albumin, platelet count and GGT. In the multivariate model only cirrhosis (Ishak fibrosis score 6) and an increasing GGT were associated with a reduction in SVR rates. A low GGT has previously been shown to be independently associated with improved SVR rates [311, 347, 488]. GGT has been shown to correlate with insulin resistance and with levels of hepatic fibrosis and steatosis, all of which have been demonstrated to impact on SVR [348-349].

There is a single previous published study comparing treatment response in HCV genotype 3 infected Asian (Indian subcontinent) and non-Asian patients [485]. Patients were treated at the Queen Elizabeth Hospital, Birmingham, UK and the Asian population in their catchment area is likely to be similar to our own. Overall SVR rates for both Asian (42.1%) and non-Asian (62.1%) patients were lower in that study than we have observed. Asian ethnicity was a significant determinant of inferior SVR compared with non-Asian in the univariate analysis, but no factors achieved statistical significance in their

multivariate analysis. There was, however, a significant difference in the severity of liver fibrosis between Asian and non-Asian patients in that study with 54% of Asian patients having cirrhosis compared to 18% of non-Asian patients. In our study there were a similar proportion of cirrhotic patients within both the Asian (26%) and non-Asian (23%) group. Given that the current study demonstrates that fibrosis stage and not ethnicity is an independent predictor of an SVR, it is likely that the previous suggestion that Asian patients have an inferior response to treatment reflects the higher proportion of Asian patients with cirrhosis in that population, rather than a direct effect of ethnicity on treatment response. In a post-hoc analysis of data from an open-label parallel study of 1.5 or 1.0 μg/ kg/ wk PEG-IFN alfa-2b plus Ribavirin for 16-24 weeks published in abstract form, White and Asian (both Indian and South East Asian) genotype 2 and 3 patients also had similar SVR rates [489].

Recent data have shown that a single nucleotide polymorphism near the IL28B gene, encoding Interferon λ-3 is associated with response to treatment with Pegylated Interferon and Ribavirin [97, 334-336]. Patients with the C/C genotype are three times more likely to clear HCV relative to C/T and T/T genotypes combined. This response is independent of viral genotype [490]. IL28B genetic variation is associated with initial virological response and C/C patients have a significantly greater decline in HCV RNA from day 0-28. When stratified for race, IL28B is associated with a higher rapid virological response (RVR) in both African Americans and White patients [491]. Geographical variation in the IL28B gene supports the notion that Asian

patients should have at least as good response rates as their White European counterparts, given that both populations, unlike African Americans have a high frequency of the C/C genotype [97]. Patients with the C/C genotype have also been shown to have a lower GGT [490]. As described earlier a lower GGT was an independent predictor of SVR in the present cohort.

There was in fact a non-statistically significant trend towards a superior treatment response in Asian patients in the present study as compared to White patients. Subsequent ad-hoc analysis of 661 patients (496 White) from two multicentre trials of combination therapy (standard interferon and Ribavirin) for HCV performed in the USA showed that treatment response was highest among Asians (61%), followed by Whites (39%), Hispanics (23%) and African Americans (14%) [325]. In a logistic regression model that adjusted for factors including genotype, Asian patients continued to be more likely to respond than Whites while Hispanics and African Americans were less likely. The study was not able to provide an explanation for the observations. The Asian population was also very different to our own with their origins being in South-East Asia rather than the Indian subcontinent and a predominance of genotype 1 infection.

The results of this study refutes the idea that Asian patients with Indian subcontinent origins have a poorer response to treatment, but confirms that there are significant differences in demographics of Asian and White HCV infected patients. A limitation of the study is that reliable data was not available on compliance with treatment, need for dose reduction and viral load

at each stage of treatment, because this was poorly recorded on the database. Data was available on end of treatment response (EOTR) and unlike the study from Birmingham, UK we have not observed a large proportion of Asian patients who relapsed after an initial EOTR.

This study demonstrates that Asian patients infected with Hepatitis C have at least as good a response to treatment with Pegylated Interferon and Ribavirin as White patients. Asian patients do, however, present to medical services later in life, having been infected for longer and with a more severe histological injury than White patients. Resources should be directed towards increasing awareness of HCV amongst the Asian population, as well as educating primary care physicians in recognising that having been born in the Indian subcontinent is a risk factor for HCV infection. This should hopefully enable earlier identification of infection through opportunistic testing.

Chapter 6

Does the platelet count influence treatment success in patients with Hepatitis C?

6.1 Background

The platelet count is integrally associated with liver disease. Thrombocytopenia occurs in 64-76% of patients with liver cirrhosis and 6% of non-cirrhotic patients with chronic liver disease [492]. Both hospital [493-495] and community [496-497] based studies illustrate a positive association between anti-HCV positivity and thrombocytopenia. There is also a significant inverse correlation between the platelet count and the severity of liver disease in untreated patients with chronic HCV [498].

Several mechanisms contribute to thrombocytopenia in HCV related liver disease. Hypersplenism leads to sequestration and destruction of platelets in the spleen[499], but hypersplenism is not a consistent finding in HCV infected patients with reduced platelet counts [500-501]. Immune mediated destruction of platelets is increased in the context of HCV infection. Elevated titres of platelet associated immunoglobulin are detected in as many as 88% of patients with chronic HCV and there is an inverse correlation between platelet count and platelet survival time [494]. It is suggested that binding of HCV to platelets may induce the development of neoantigens on the platelet surface or alter the conformation of the platelet membrane promoting autoantibody formation[492]. Decreased platelet production also results from direct virus induced bone marrow suppression[502] and reduced hepatic production of thrombopoietin [503-504]. Thrombopoietin is the primary cytokine governing megakaryocyte maturation and platelet production [505]. It binds to receptors on haematopoetic stem cells and megakaryocytes promoting all stages of platelet production. It also binds to platelets, enhancing activation and function

[506]. Thrombopoietin is produced in hepatocytes and there is an inverse correlation between liver fibrosis and thrombopoietin levels [498].

Interferon induced bone marrow suppression leads to an approximately 33% reduction in platelet counts in patients treated with pegylated interferon, with levels reaching a plateau by the 12^{th} week of treatment [507]. Thrombocytopenia is most pronounced in pegylated interferon monotherapy and it is suggested that ribavirin induced anaemia may contribute to a reactive thrombocytosis, balancing out to a degree interferon induced myelosuppression [286]. Successful treatment on the other hand leads to an improvement in platelet levels [501, 508]. Product labels for pegylated interferon recommend dose reductions for patients with platelet counts between 50 and 100 x 10^9/L and suspension of treatment if levels fall below 20-25 x 10^9/L [509]. In the registration trials of pegylated interferon approximately 4% of patients required a dose modification, but less than 1% stopped treatment because of thrombocytopenia [285-287, 316]. Fried et al observed severe bleeding in association with thrombocytopenia in 1 of 453 patients treated [286]. In a study of the efficacy of pegylated interferon and ribavirin in patients with cirrhosis and a baseline platelet count of > 75 x 10^9/L, dose modifications due to thrombocytopenia were required in 18-19% (permanent in 8-13%) of patients and treatment was discontinued in 2-4% [510].

Thrombocytopenia and concern regarding the risk of bleeding complications may cause the postponement of necessary procedures and therapy, or alternatively may result in suboptimal doses of antiviral therapy. Not

surprisingly, therefore, there is considerable interest in the pharmacological treatment of thrombocytopenia. Eltrombopag is an orally bioavailable low molecular weight non-peptide growth factor, that is a selective agonist for the thrombopoietin receptor (Mpl). It has been the subject of a phase II study examining its efficacy in treating thrombocytopenia in patients with HCV related cirrhosis receiving treatment with pegylated interferon and ribavirin. 12 weeks of treatment was completed by 36-65% of patients treated with eltrombopag, compared to just 6% of controls [511]. No study has yet answered the question, however, of whether addressing thrombocytopenia with pharmacological agents increases the number of patients achieving an SVR.

6.2 Aims

This aim of this study was to determine if a reduced platelet count adversely affects the likelihood of an SVR using data from the Trent HCV study.

6.3 Methods

6.3.1 Study Population

At the time of this analysis (2008), the Trent HCV study cohort included 2184 HCV RNA positive patients. An initial 650 patients were identified from the study database as having a recorded response to treatment with pegylated interferon and ribavirin. The final study population incorporated 357 patients with at least one recorded platelet count (3505 individual platelet counts, range 1-35) taken during treatment. The majority of patients (87%) had a pre-treatment biopsy, though 27 patients had incomplete data on fibrosis stage.

6.3.2 Statistical Methods

SPSS v14 was used for all statistical analyses. Variables including the initial and minimum platelet count were assessed for their association with both SVR and completion of therapy (without dose reduction) using logistic regression. The results were expressed as the odds ratio (OR) and their 95% confidence interval (CI). For the multiple logistic regression analysis patients were stratified according to genotype. A two-tailed P value of < 0.05 was considered significant. The Pearson correlation coefficient was used to measure the relationship between the Ishak fibrosis stage and both the initial and minimum platelet count.

6.4 Results

6.4.1 Patient details

Table 6.1 shows the baseline characteristics of the study population at the start of treatment. The majority of the 357 patients were male (68%), white (87%) and acquired HCV through the use of intravenous drugs (60%). Forty one percent of patients had a past or present history of consuming in excess of 50g of alcohol/ day. Serology indicated prior Hepatitis B infection in 63 patients (31% of those with serology), while four patients (2%) had ongoing co-infection with HBV as evidenced by a positive hepatitis B surface antigen. Patients with genotype 3 (52%) outnumbered those with other genotypes (G1 35%, G2 10% and G4 3%). The majority of patients who had a pre-treatment liver biopsy had mild disease (66% stage ≤ 3), while cirrhosis was evident in 45 (16%) cases.

6.4.2 Platelet levels and SVR

The mean platelet count at the start of treatment was 176 x 10^9/L. One hundred and twenty seven (38%) patients had a platelet count below 100 x 10^9/L at the start of therapy. Overall 78% of patients completed therapy (73% without a dose reduction), while 33% of genotype 1 and 67% of genotype 2/3 patients achieved an SVR. Table 6.2a shows the number of patients with an SVR according to Ishak stage and initial platelet count. Table 6.2b and 6.2c separate patients according to genotype. A patient with genotype 3 infection, fibrosis stage 0 or 1 and platelet > 150 x 10^9/L had an SVR rate of 89%, whereas no patients with genotype 1 infection, cirrhosis and a platelet count < 50 x 10^9/L achieved an SVR. Both the initial ($r = -0.43$, $P<0.001$) and minimum ($r= -0.46$,

Table 6.1: Baseline characteristics of the study population (n=357)

Variable	
Age	43.3 ± 10.7
Male	242 (68%)
Estimated duration of infection (years) n=282	20.6 ± 10.7
Ethnic group	
White	310 (87%)
Indian subcontinent	30 (8%)
Black	7 (2%)
Other	8 (2%)
Main risk factor (n=345)	
IVDU	206 (60%)
Blood Tx	56 (16%)
Other	49 (14%)
No known risk factor	34 (10%)
Ever heavy drinker (past or present history of alcohol consumption > 50g/day) (n=336)	135 (41%)
Hep B status (n=205)	
HBsAg positive	4 (2%)
HBcAb positive (HBsAg negative)	63 (31%)
BMI (n=305)	25.6 ± 4.4
Genotype (n=352)	
1	123 (35%)
2	35 (10%)
3	183 (52%)
4	11 (3%)
Ishak necroinflammatory score (mean) (n=276)	3.9 ± 2.1
Ishak stage (n=282)	
0,1	108 (38%)
2,3	82 (28%)
4,5	47 (17%)
6	45 (16%)
Platelet count at start of therapy (x 10^9/ L)	176.3 ± 72.9

Table 6.2a: SVR according to Ishak fibrosis stage and platelet count

Ishak Stage	Platelet count (x 10^9/L)				
	All (n=330)	<50 (n=37)	50-100 (n=90)	100-150 (n=107)	> 150 (n=96)
0,1	73/108 (68)	2/3 (67)	15/19 (79)	22/42 (52)	34/44 (77)
2,3	48/82 (59)	2/6 (33)	12/23 (52)	19/27 (70)	15/26 (58)
4,5	20/47 (43)	3/8 (38)	9/20 (45)	6/15 (40)	2/4 (50)
6	15/45 (33)	4/17 (24)	8/20 (40)	2/6 (33)	1/2 (50)
No biopsy	31/48 (65)	1/3 (33)	7/8 (88)	11/17 (65)	12/20 (60)
Total	187/330 (57)	12/37 (32)	51/90 (57)	60/107 (56)	64/96 (67)

Figures in parentheses are percentage The 27 patients who had a biopsy, but on whom there was incomplete data on fibrosis stage are not included

Table 6.2b: SVR in genotype 1 patients according to Ishak fibrosis stage and platelet count

Ishak stage	Platelets (x 10^9/L)				
	All (n=112)	<50 (n=13)	50-100 (n=35)	100-150 (n=35)	> 150 (n=29)
0,1	12/34 (35)	0/1 (0)	3/6 (50)	3/14 (21)	6/13 (46)
2,3	11/31 (36)	1/3 (33)	1/10 (10)	5/9 (56)	4/9 (44)
4,5	2/16 (13)	0/3 (0)	2/9 (22)	0/4 (0)	0/0
6	2/11 (18)	0/4 (0)	2/6 (33)	0/1 (0)	0/0
No biopsy	11/20 (55)	1/2 (50)	3/4 (75)	4/7 (57)	3/7 (43)
Total	38/112 (34)	2/13 (15)	13/35 (37)	12/35 (34)	13/29 (45)

Figures in parentheses are percentage The 27 patients who had a biopsy, but on whom there was incomplete data on fibrosis stage are not included

Table 6.2c: SVR in genotype 2/3 patients according to Ishak fibrosis stage and platelet count

Ishak stage	Platelets (x 10^9/L)				
	All (n=203)	<50 (n=24)	50-100 (n=51)	100-150 (n=67)	> 150 (n=61)
0,1	56/87 (84)	2/2 (100)	11/12 (92)	18/25 (72)	25/28 (89)
2,3	35/48 (73)	1/3 (33)	10/11 (91)	14/18 (78)	10/16 (63)
4,5	18/30 (60)	3/5 (60)	7/10 (70)	6/11 (55)	2/4 (50)
6	13/33 (39)	4/13 (31)	6/14 (43)	2/4 (50)	½ (50)
No biopsy	18/25 (72)	0/1 (0)	4/4 (100)	6/9 (67)	8/11 (73)
Total	140/203 (69)	10/24 (42)	38/51 (74)	46/67 (69)	46/61 (75)

Figures in parentheses are percentage. The 27 patients who had a biopsy, but on whom there was incomplete data on fibrosis stage are not included

P<0.001) platelet count on treatment showed a negative correlation with Ishak stage.

6.3.3 Factors associated with an SVR and completion of therapy

Table 6.3 shows the results of the univariate analysis examining factors associated with an SVR and completion of therapy (without dose reduction). Increasing age and estimated duration of infection were associated with a failure to achieve SVR as was Ishak stage ≥ 4 on biopsy. Patients with cirrhosis were less likely to complete therapy than patients with lower stages of fibrosis. A higher initial platelet count was associated with both an SVR and completion of therapy, while a platelet count of < 50 x 10^9/L during treatment was associated with a failure to complete therapy or achieve an SVR.

In the multivariate model (Tables 6.4a-d) patients were stratified according to genotype. sex, age, estimated duration of infection and Ishak stage were included in the model along with initial and minimum platelet count. Age at the start of treatment (OR 0.95 [95% CI 0.903-0.999, P=0.046]) was the only independent predictor of SVR in genotype 1 patients, while the estimated duration of infection (OR 0.996 [95% CI 0.994-0.999, p=0.016]) and Ishak fibrosis stage (6 vs 0 or 1; OR 0.072 [95% CI 0.021-0.244, P <0.001]) were independent predictors of SVR in genotype 2/3 patients. No factors were independently associated with completion of therapy in genotype 1 infected patients, but Ishak fibrosis stage was associated with completion of therapy in genotype 2/3 patients (6 vs 0 or 1; OR 0.208 [95% CI 0.052-0.838, P <0.027]).

Table 6.3: Univariate analysis of factors associated with SVR and completion of therapy in 357 patients treated with pegylated interferon and ribavirin

Variable	No. of patients (%)	SVR		Completion of therapy	
		OR (95% CI)	P value	OR (95% CI)	P value
Age (per year)	357	0.97 (0.95-0.99)	0.002	1.00 (0.98-1.02)	0.762
Sex Female Male	115 242 (68)	reference 0.74 (0.47-1.16)	0.183	reference 0.97 (0.59-1.61)	0.912
Duration of infection (per month)	282	0.997 (0.995-0.999)	0.002	1.00 (1.00-1.00)	0.709
Past or present history of alcohol > 50g/d No Yes	201 (59) 135 (41)	reference 0.69 (0.45 – 1.08)	0.102	reference 0.69 (0.42-1.13)	0.140
Genotype: 1 2,3	123 (36) 218(64)	reference 4.30 (2.68-6.89)	<0.001	Reference 3.12 (1.90-5.14)	<0.001
Ishak stage: 0,1 2,3 4,5 6 No biopsy taken	108 82 47 45 48	reference 0.68 (0.37-1.3) 0.36 (0.18-0.72) 0.24 (0.11-0.50) 0.87 (0.43-1.79)	 0.199 0.004 <0.001 0.713	reference 0.85 (0.42-1.71) 0.63 (0.29-1.37) 0.50 (0.23-1.08) 0.65 (0.29-1.45)	 0.650 0.239 0.078 0.294
Initial platelets	357	1.005 (1.002-1.008)	0.002	1.003 (1.000-1.007)	0.049
Minimum platelets	357	1.006 (1.002 - 1.010)	0.004	1.001 (0.997-1.005)	0.564
Normal Platelets < 50	105 39	reference 0.25 (0.12-0.55)	0.001	reference 0.40 (0.18-0.86)	0.018
Normal Platelets <100	105 138	reference 0.54 (0.32-0.90)	0.018	reference 1.09 (0.62-1.94)	0.763
Normal Platelets < 150	105 252	reference 0.60 (0.37-0.95)	0.03	reference 1.09 (0.65-1.83)	0.733

Table 6.4a: Multivariate analysis of factors associated with a sustained virological response in Genotype 1 patients

Variable	Number of Patients	OR (95% CI)	P value
Age at start of treatment	123	0.95 (0.903 – 0.999)	0.046
Initial platelet count	123	1.006 (0.999-1.014)	0.081

Variable(s) entered on step 1: sex, initial platelet count, minimum platelet count, age at start of treatment, estimated duration of infection, Ishak Stage.

Table 6.4b: Multivariate analysis of factors associated with a sustained virological response in Genotype 2 or 3 patients

Variable		Number of Patients	OR (95% CI)	P value
Estimated duration of infection (per month)		167	0.996 (0.903 – 0.999)	0.016
Ishak stage	0,1	67	Constant	
	2,3	48	0.426 (0.252-1.191)	0.104
	4,5	30	0.359 (0.113-1.143)	0.083
	6	33	0.072 (0.021-0.244)	<0.001
	No biopsy	25	0.260 (0.073 – 0.930)	0.038

Variable(s) entered on step 1: sex, initial platelet count, minimum platelet count, age at start of treatment, estimated duration of infection, Ishak Stage.

Table 6.4c: Multivariate analysis of factors associated with a completion of therapy in Genotype 1 patients

Variable	Number of Patients	OR (95% CI)	P value
Initial platelet count	123	1.006 (0.999-1.012)	0.090

Variable(s) entered on step 1: sex, initial platelet count, minimum platelet count, age at start of treatment, estimated duration of infection, Ishak Stage.

Table 6.4d: Multivariate analysis of factors associated with completion of therapy in Genotype 2 or 3 patients

Variable		Number of Patients	OR (95% CI)	P value
Ishak stage	0,1	67	Constant	
	2,3	48	0.323 (0.090-1.164)	0.084
	4,5	30	0.396 (0.090-1.746)	0.221
	6	33	0.208 (0.052-0.838)	0.027
	No biopsy	25	0.153 (0.037 – 0.635)	0.010

Variable(s) entered on step 1: sex, initial platelet count, minimum platelet count, age at start of treatment, estimated duration of infection, Ishak Stage

6.4 Discussion

The platelet count is a recognised surrogate marker of the severity of liver disease and the results of this study confirm that platelet levels show a negative correlation with the Ishak fibrosis stage. In HCV infected patients on treatment, thrombocytopenia can also result from interferon induced bone marrow suppression. Thrombocytopenia may, therefore, prevent some patients commencing therapy or force therapy to be curtailed or continued only after a reduction in the dose of interferon. Shortening of the duration of therapy or reducing the dose of interferon will have a negative impact on the likelihood of achieving an SVR. Modifications in drug dosage or temporary cessation of therapy have the most impact when made in the first 12 weeks of therapy. Individuals who are able to maintain at least 80% of both the pegylated interferon and ribavirin dose have an early virological response (EVR) rate of 80%, compared to 30% when less than 80% of both drugs are taken. Less of one drug has a more modest impact with patients taking less than 80% of their pegylated interferon but more than 80% of the ribavirin dose having an EVR of 70% [310]. Seventy eight percent of the 357 patients treated with pegylated interferon and ribavirin in the present study completed therapy without the need for a reduction in drug dosage. A higher initial platelet count was associated with completion of therapy in the univariate analysis. The only factor shown to be independently associated with completion of therapy in the multivariate analysis was cirrhosis on liver biopsy, which predicts a failure to complete therapy in genotype 2/3 infected patients. Neither the initial platelet count nor the minimum platelet count during treatment would appear, therefore, to be independent predictors of completion of therapy.

Thirty three percent of genotype 1 and 67% of genotype 2/3 infected patients achieved an SVR. In those patients with minimal fibrosis on liver biopsy (Ishak stage 0 or 1) and a platelet count > 150 x 10^9/L the SVR rate was 77% compared to 24% in cirrhotic patients with a platelet count < 50 x 10^9/L. Both the initial platelet count and minimum count on treatment were associated with an SVR on univariate analysis. Neither, however, was independently associated with obtaining an SVR, instead the patient's age in genotype 1 and estimated duration of infection and cirrhosis on biopsy in genotype 2/3 patients were the factors associated with treatment success in the multivariate analysis.

The severity of fibrosis on liver biopsy has, as described in chapter 1, repeatedly been shown to predict the likelihood of a patient achieving an SVR. Both the initial and minimum platelet count in this study showed a negative correlation with liver fibrosis and, therefore, any association with SVR in the univariate analysis may simply reflect worsening levels of liver fibrosis in patients with lower platelet levels. An alternative reasoning is that though thrombocytopenia correlates with fibrosis stage, thrombocytopenia is as the univariate analysis suggests a cause of failure to complete therapy or achieve an SVR in patients with cirrhosis. Against this is that less than 1% of patients discontinued therapy because of thrombocytopenia in the registration trials of pegylated interferon and ribavirin. The investigators in these trials would also have been restricted by the product labels which stipulate dose reductions for patients with platelet counts between 50 and 100 x 10^9/L and suspension of treatment if levels fall below 20-25 x 10^9/L. Outside of the confines of a

clinical trial many clinicians may be happy to continue with the pegylated interferon dose unchanged in patients with lower platelet levels than the product label stipulates for dose reduction. If true then this would further reduce the impact of thrombocytopenia on SVR rates.

The strength of this study is the large amount of available data on platelet counts taken during antiviral treatment. The fact that the majority of patients had a pre-treatment biopsy also allows some distinction to be drawn between the direct impact of thrombocytopenia on SVR rates and what is simply a surrogate for the effect of severe hepatic fibrosis. The mandatory data fields recorded in the Trent study database do not, however, include a reason for a reduction in drug dose or cessation of therapy. This is a significant limitation for the present study, as the data does not tell us with certainty which patients reduced or stopped treatment as a result of thrombocytopenia. Instead we are left to draw conclusions from associations between platelet levels and SVR rates. Information on any complications resulting from thrombocytopenia is also not available.

In conclusion this study did not find the initial or minimum platelet count during antiviral therapy to be independent predictors of SVR or completion of therapy. Focusing attention on drug therapies designed to increase the platelet count during treatment may not be a valuable or cost-effective approach to improving SVR rates.

Chapter 7. Discussion

The introduction to this thesis has summarised our current understanding of the epidemiology and natural history of HCV infection. In particular it outlines the patient, environmental and viral factors that influence spontaneous viral clearance, disease progression and the success of therapy.

It has been proposed that those HCV infected patients with a persistently normal alanine aminotransferase (PNALT) represent a subgroup with mild, slowly progressive disease [403-404, 406]. Chapter 2 describes a study examining whether a PNALT predicts that the ALT will subsequently remain normal. It also explores the risk of disease progression as compared to patients with an elevated ALT. It illustrates that patients with PNALT are more likely to be female and to have a lower Body Mass Index (BMI) than those with an elevated ALT. Using a standard definition of PNALT[296], over 60% of patients subsequently had an elevated ALT within five years. The attrition rate in both PNALT groups continued beyond even more stringent definitions of PNALT [416], suggesting that very few patients maintain a PNALT throughout the course of their disease. This is consistent with evidence suggesting that the ALT fluctuates during the course of HCV infection[417].

In line with previous studies, patients with PNALT were found to have lower Ishak fibrosis and necroinflammatory scores on their first liver biopsy than patients with an elevated ALT [401, 413]. Despite the lower Ishak score on the first biopsy and a similar estimated duration of infection, fibrosis progressed at

a similar rate between paired biopsies in those with PNALT as compared to patients with an elevated ALT. There was also no difference in the number of patients in whom the Ishak fibrosis score increased by ≥ 2 points. This is considered to be indicative of fibrosis progression rather than to any potential sampling error. Patients with a PNALT would appear, therefore, to have a similar risk of disease progression as those with an elevated ALT. In order to have been included in the analysis of fibrosis progression, however, patients needed to have remained treatment-naïve. Patients with an elevated ALT who remained treatment-naïve and underwent a repeat biopsy proved on further scrutiny to be a group with milder disease as compared to the whole elevated ALT cohort.

The study of HCV infected patients with severe fibrosis on liver biopsy is described in chapter 3. It seeks to examine the natural history in a group of patients that typify those attending hepatitis clinics and avoids much of the selection bias associated with some natural history studies, by using a geographically determined population. The study's findings concern survival to death or transplantation and the cumulative probability of both HCC and decompensation. The patient population differs from those of previous studies in that almost half of patients acquired HCV through injecting drug use (previous studies were predominantly blood transfusion recipients [210-211, 428-430]) and by the inclusion of patients who consumed excess alcohol. Neither alcohol consumption at the time of biopsy or past heavy alcohol intake were found to be prognostic factors, but mean alcohol consumption fell by approximately two thirds following the index biopsy, suggesting that if patients

diagnosed with severe fibrosis reduce their alcohol consumption, subsequent prognosis is not influenced by their prior alcohol intake. This underlines the importance of encouraging HCV infected patients to reduce alcohol consumption, particularly those with advanced disease.

At the end of the study a quarter of patients had died or undergone liver transplantation, with the majority of deaths judged to be related to HCV. Following a diagnosis of severe hepatic fibrosis (Ishak stage $\geq$4), the probability of survival at 5 years was 80%, though this was only 19% if there was existing or prior evidence of hepatic decompensation. The cumulative risk of HCC was 15.4% at 5 years with a median estimated duration of infection prior to the diagnosis of HCC of 30 years. The study also differs from previous studies in that a high proportion of patients received antiviral therapy. Treatment with either standard or pegylated Interferon in combination with Ribavirin was associated with survival in both univariate and multivariate analyses. Univariate analysis using Cox's model also showed that age, together with indicators of poor liver function (Bilirubin, Albumin and the Child-Pugh grade) were prognostic factors for survival. An elevated serum IgM or IgA was also associated with a worse outcome in both univariate and multivariate analyses. This observation may arise from Ig-induced stimulation of hepatic stellate cells [438, 512] or raised Ig levels may simply be a surrogate marker for declining liver function (reduced antigen clearance by Kupffer cells [434-436]).

The study of 46 HCV infected patients with Hepatocellular carcinoma (HCC) is described in chapter 4. Patients diagnosed with HCC had an estimated duration of infection of 24.5 years. Diabetes was present in 20%, while 65% were current or ex-smokers. Both are recognised risk factors for HCC. Seventy five percent of patients had been diagnosed with cirrhosis at least 6 months prior to the diagnosis of HCC and as such there was an opportunity for surveillance. Surveillance was variably applied with 80% of patients having some form of surveillance performed, but only a third had AFP testing and an ultrasound at 6 monthly intervals, as recommended in guidance produced by the British Society of Gastroenterology [447]. Twenty one percent of patients with HCC had a normal AFP at diagnosis. Tumours were more likely to fall within the Milan criteria (85% vs 17%) and patients more likely to receive a potentially curative therapy if HCC were detected as a result of surveillance. Nevertheless 30% of patients having appropriate surveillance did not have their tumours diagnosed as a result of surveillance practices. The 1, 3 and 5 year survival was 55, 31 and 19% and having had surveillance was not associated with a significant survival benefit. This suggests that new strategies to diagnose HCC at an earlier stage are needed in HCV infected patients.

In comparison with a cohort of cirrhotic patients without HCC, increasing age and duration of infection, together with an elevated IgG were associated with an increased risk of HCC. Combination therapy with pegylated interferon and ribavirin, and separately an SVR were associated with a reduced risk. A multivariate model, however, did not identify any factors independently associated with development of HCC.

Several studies have shown that African Americans have an inferior treatment response as compared to other ethnic groups [325-330]. The picture for patients from the Indian subcontinent, which constitutes the main ethnic group in the UK, is less clear. The study described in chapter 5 examines the natural history and outcome of therapy for 2123 white and 120 Asian patients with HCV. The Asian patients were more likely to be female, to be older at time of diagnosis, to drink less alcohol and to be infected with genotype 3 than their white counterparts. Asian patients also had more severe disease on initial liver biopsy, but were older and are likely to have been infected with HCV for longer. An analysis of response to treatment was restricted to those patients with genotype 3 infection. Ethnicity was not associated with the outcome of treatment and in the multivariate analysis only cirrhosis on biopsy and an increasing GGT predicted treatment failure. Contrary to a previous report [485], patients from the Indian subcontinent do not have an inferior response to treatment. They are, however, diagnosed with HCV later in life and attention should focus on increasing awareness of HCV in the Asian population.

Study 6 looked at factors associated with a sustained virological response in 357 patients treated with pegylated interferon and ribavirin. In particular it focused on whether thrombocytopenia at beginning of or during treatment independently influenced the likelihood of success. Seventy eight percent of patients completed therapy without a reduction in drug dosage. The only factor independently associated with completion of therapy was cirrhosis on biopsy in genotype 2/3 infected patients. Thirty three percent of genotype 1 and 67% of

genotype 2/3 infected patients achieved an SVR. Increasing patient age in genotype 1 infection and increasing duration of infection and cirrhosis in genotype 2/3 were independently associated with treatment failure. The initial or minimum platelet count did not independently predict either completion of therapy or SVR. Focusing attention on increasing the platelet count during treatment may, therefore, not improve SVR rates.

As a collection these studies add to our understanding of the epidemiology and natural history of HCV infection. They utilise the Trent HCV study database which thanks to the efforts of the enthused individuals who form the study's steering committee and the hard work of its data entry clerks, is well maintained with low levels of missing data. That said any epidemiological study is only as good as the quality of the data collected. Data fields that at the study's outset may not have seemed important to include are later seen to be relevant, as our knowledge of HCV infection increases. An example would be our awareness now of the increased risk of HCC seen in HCV infected patients with diabetes, but information on diabetic status was not available for the study in chapter 4. Patients are enrolled with informed consent and some patient populations such as those with undiagnosed infection who present with HCC may be less likely to enter the study. Histological data obtained from liver biopsy features heavily in most of the data chapters comprising this thesis. A valid criticism of the statistical analysis of histological data is that the Ishak scores have in some instances been treated as a linear scale rather than distinct categories in order to generate annual fibrosis progression rates for comparison between patient groups. Though such analysis features prominently in several

peer reviewed published studies of the natural history of HCV [111-113, 116], the methodology can be justifiably criticised given that neither fibrosis progression in HCV nor progression through the Ishak stages is linear.

There is still a great deal to understand about why there exists such variability in disease progression in HCV infection and why some patients clear the virus with treatment and others do not. We have had a glimpse of the future of natural history studies of HCV infection in the work described in chapter 1 examining the role of genetic polymorphism at IL28 in determining the likelihood of spontaneous clearance or treatment response. Future research must link epidemiological data to a biobank of patient blood samples in order to further probe the role of both host and viral genetics on the outcome of HCV infection.

8. References

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Appendix 1: Trent HCV Study group

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Professor WL Irving, Professor of Virology (Chair)
Prof KR Neal, Professor of Public Health Medicine & Epidemiology
Dr SD Ryder, Consultant Hepatologist
Dr BJ Thomson, Senior Lecturer in Infectious Diseases
Dr A Zaitoun, Consultant Histopathologist

Sheffield Teaching Hospitals NHS Trust

Dr A Dube, Consultant Histopathologist
Dr D Gleeson, Consultant Gastroenterologist
Dr M Karajeh, Consultant Gastroenterologist
Prof S Green, Consultant in Infectious Diseases
Prof G Kudesia, Consultant Virologist, Sheffield Virology Consortium
Prof MW McKendrick, Consultant in Infectious Diseases
Mr R Poll, Consultant Nurse for Viral Hepatitis
Dr A Vedio, Staff Grade, Infectious Diseases

University Hospitals of Leicester NHS Trust

Dr T Delahooke, Consultant Hepatologist
Dr A Grant, Consultant Hepatologist
Dr A McGregor, Consultant Histopathologist
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Appendix II:

Previous publication of work presented in this thesis

Abstracts

Lawson A, Taguri N, Rye K**,** Hagan S, Zaitoun A, Irving WL, Neal KR, Ryder SD.
The Natural history of Hepatitis C with severe hepatic fibrosis.
Hepatology. 2004; 40, (4), suppl 1 A685

Lawson A, Ryder SD, Neal KR, Irving WL
Experience of Hepatocellular carcinoma in a large cohort of HCV infected patients: Can we predict it, can we find it?
Presented at the British Association for the Study of the Liver (BASL) annual meeting, London 2007.

Lawson A, Ryder SD, Neal KR, Irving WL.
Alanine Aminotransferase (ALT) as a marker of severity in Hepatitis C: Are patients with a persistently normal ALT really a group with mild disease.
Journal of Hepatology. 2008; 48, suppl 2 S277

Lawson A, Austin AS, Harrison G, Neal KR, Ryder SD, Irving WL
Does the platelet count influence treatment success in patients with hepatitis C?
Hepatology. 2008; 48 (4) Suppl 1 A883

Papers

Lawson A, Hagan S, Rye K, Taguri N, Zaitoun A, Neal KR, Ryder SD, Irving WL.
The Natural history of Hepatitis C with severe hepatic fibrosis.
Journal of Hepatology. 2007; 47:37-45

Lawson A on behalf of the Trent HCV study group
Hepatitis C infected patients with a persistently normal ALT: Do they exist and is this really a group with mild disease. Journal of Viral Hepatitis 2010; 17 (1) 51-8

Lawson A on behalf of the Trent HCV study group
A comparison of the natural history and outcome of treatment for Asian and Non-Asian hepatitis C infected patients. Journal of Viral Hepatitis 2011; 18 (7) e270-277

Review articles

Lawson A, Ryder SD
Progression of hepatic fibrosis in chronic hepatitis C and the need for treatment in mild disease. Eur J Gastroenterol Hepatol. 2006 Apr; 18 (4):343-347

Reports

Hepatitis C in England: An update 2007. London: Health protection Agency Centre for Infections. December 2007. (Contributor to report)

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