The Natural History and Treatment of Hepatitis C in the South-East of Scotland.

By

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To My Wife

Diane

Declaration

I hereby declare that this thesis is based on data collected and analysed by myself, except where acknowledged, and that the thesis is exclusively of my own composition. It has not been submitted previously for a higher degree. This work was carried out in the University of Edinburgh's Department of Medicine and subsequently the Liver unit, in the Royal Infirmary of Edinburgh, Scotland.

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Abstract

Background: Since the discovery of Hepatitis C there have been studies of the natural history of the infection. The outcome is dependent on whether the research is performed in the community or in hospital and where the patients are drawn from. Scotland is a country with a low prevalence of Hepatitis C infection but Edinburgh has a large intravenous drug using population in whom the prevalence is high. The Royal Infirmary of Edinburgh is the main tertiary centre for the assessment and treatment of these patients.

Aim: Describe the characteristics of those patients that have ongoing Hepatitis C infection that have been referred and assessed. Describe the progression of the disease to cirrhosis and its complications. Identify the independent factors that influence this progression. Describe the outcome of treatment with interferon monotherapy, combination standard interferon and ribavirin and combination pegylated interferon and ribavirin, and identify the predictors of response.

Methods: All patients that have been referred to the Royal Infirmary of Edinburgh and assessed between 1990 and 2004 with Hepatitis C infection. Retrospective analysis of the patient's case notes, laboratory, pathology and endoscopy records was performed. There was entry of data into a specially constructed Microsoft Access relational database. Kaplan-Meier analysis was used to describe progression. Cox regression analysis was used to identify independent predictors of progression. A sustained viral response was the primary end-point of the treatment studies, with binary logistical regression to identify predictors of this outcome. Documentation of adverse events for each treatment was made.

Results: Six hundred and ninety-four patients were identified that have ongoing infection. This cohort was made up of a significant proportion of middle-aged men who have acquired the infection less than 20 years ago, principally through intravenous drug use, who have a significant history of alcohol abuse. At least 22% of patients have had cirrhosis diagnosed clinically, although only about half of these have had it confirmed by biopsy or laparoscopy. In 12% of patients at least one complication of cirrhosis has been recorded. Grade 2 oesophageal varices have been found in about 7% of patients overall, but only half of these have bled. A major complication of cirrhosis has occurred in 10.5% and Hepatocellular carcinoma (HCC) in 3.3%. So far only 13 patients have been transplanted. Eleven percent of the cohort has died and in those in whom the cause is known, liver-related death is twice as common as non-liver-related death. It has not been possible to establish a median time from infection to cirrhosis or its complications but it appears to be in excess of 35 years. The age of the patient and previous alcohol intake of greater than 50 units per week for more than five years independently influences progression. A steady improvement in the efficacy of treatment with the introduction of each new treatment regime has been confirmed. In interferon naïve patients, treated with pegylated interferon and ribavirin, the sustained viral response rate was 29.0% and 59.3% for genotype 1 and genotype 2 or 3 infections respectively. Significant side effects occurred with treatment that necessitated both dose reduction and sometimes its termination. About 45% of all patients referred and assessed each year were deemed suitable for treatment and listed.

Conclusion: Chronic Hepatitis C infection is a significant health problem in Edinburgh with large numbers being referred for assessment, treatment and

management of the complications of cirrhosis. The natural history of the infection and how it is influenced by therapy is becoming clearer, in particular the influence of alcohol and the age of the patient. Treatments are effective, although do have significant side effects that affect compliance.

Aims and structure

This thesis aims to describe the characteristics and outcomes of those patients that have ongoing Hepatitis C infection that have been referred and assessed to the Royal Infirmary of Edinburgh. It aims to give insights into the natural and therapy-influenced history of similar cohorts of patients with Hepatitis C infection and to compare the results with the current medical literature in this field of study.

The first part of this thesis (**Chapter 1; Introduction**) provides a general background of the virology and molecular biology of the Hepatitis C virus. The immunological response of the host is described in detail, as well as the pathological consequences of infection. The epidemiology in the United Kingdom and worldwide is also discussed. The current literature on the natural history of the infection is analysed to highlight the factors that are thought to influence progression to cirrhosis and its complications. The final section describes treatment of Hepatitis C infection with interferon-based treatments and includes a synopsis of the major randomised controlled trials in this area.

The second part of the thesis is based on the retrospective and prospective analysis of all patients that have been referred to the Royal Infirmary of Edinburgh for assessment of their Hepatitis C infection from the Edinburgh 'EH' postcode area. There is a description of data collection on this cohort and the typical management of these patients (**Chapter 2; Methods**). There is a description of data collection on a second cohort of patients that have had combination standard interferon and ribavirin in other liver units around the United Kingdom.

The basic characteristics, demographics and outcomes of all Hepatitis C Ribonucleic acid polymerase chain reaction positive patients in the Edinburgh cohort are

documented (**Chapter 3**). Correlation between various features of the cohort, such as previous alcohol intake and gender, are explored. The factors that influence the time of presentation are also described.

The progression of infection to uncomplicated cirrhosis either clinically diagnosed or biopsy proven is described (**Chapter 4**). The factors that influence this progression are explored. There is also a discussion concerning the most appropriate method of statistical analysis and its limitations.

The progression to the complications cirrhosis from infection or from uncomplicated cirrhosis (either clinically diagnosed or biopsy proven) is described (**Chapter 5**). Identification of the independent factors that influence this progression is also attempted.

The final analytical work covers the outcome and side effects of treatment with interferon monotherapy, combination standard interferon and ribavirin and combination pegylated interferon and ribavirin (**Chapter 6**). The outcome of treatment with combination standard interferon and ribavirin in other United Kingdom liver units and is compared and contrasted with the Edinburgh cohort. Identification of the predictors of response and side effects is attempted.

A detailed critical discussion of the work is included at the end of each chapter. There is also a final overall discussion highlighting the main results and insights made by the thesis (**Chapter 7; Discussion**).

Finally a description is given of the Hepatitis C management database that was constructed to facilitate data collection for the thesis (**Appendix**). This application is a major positive outcome of the thesis, as it is currently being used as an audit tool in all the major liver units in Scotland.

Abbreviations

The abbreviations frequently used and / or novel to this thesis are listed below.

ALT	Alanine aminotransferase
CSAGS	Confidentiality and Security Advisory Group for Scotland
DNA	Deoxyriboneucleic acid
ECM	Extracellular matrix
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
HSC	Hepatic stellate cells
IDU	Intravenous drug use
IFN	Interferon
IRF	Interferon regulatory factor
ISG	Interferon stimulated gene
MHC	Major Histocompatibility Complex
NANB	Non A Non B
NFκB	Nuclear factor κB
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PCT	Porphyria Cutanea Tarda

PEG-IFN	Pegylated interferon
PNALT	Persistently Normal Alanine aminotransferase
RCT	Randomised controlled trial
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SVR	Sustained viral response
UK	United Kingdom
USS	Ultrasound scan

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Chapter 1 Introduction

1.1 Discovery of Hepatitis C virus

For many years prior to the discovery of Hepatitis C virus (HCV) its existence had been assumed. It was known that patients undergoing blood transfusion could develop a hepatitis that was negative on serological testing for Hepatitis A virus and Hepatitis B virus (HBV). This was therefore termed Non A Non B (NANB) hepatitis. In 1989 the genome of this virus was cloned and sequenced and soon after an ELISA test for its viral proteins was produced (Choo et al. 1989). The virus was then officially given the name Hepatitis C virus.

1.2 Virology of Hepatitis C Virus

1.2.1 Classification

HCV is a member of the Flaviviridae family. Other members of this family include the Yellow fever virus and Dengue virus. HCV is the only member of the Hepacivirus genus of this family. It is a small, enveloped, positive-sense, single stranded Ribonucleic Acid (RNA) virus.

1.2.2 Molecular biology of Hepatitis C virus

1.2.2.1 The genome and its products

The HCV genome consists of approximately 9600 nucleotides. It has a single open reading frame about 9000 nucleotides in length. This is flanked at each terminus by untranslated regions including control elements required for translation and replication (Kong & Sarnow 2002). Translation of this produces a polyprotein of 3010 or 3011 amino acids that is cleaved into 10 structural and non-structural proteins by a combination of host and viral proteases (Kato et al. 1990).

1.2.2.1.1 Untranslated regions

The 5' untranslated region is highly conserved (Bukh et al. 1992) and has been of great importance in the development of diagnostic assays for the detection of HCV viraemia. It contains an internal ribosome entry site that controls translation and has sequences that can directly interact with the 3' end of the viral genome or indirectly with RNA-protein as replication signals (Zhang et al. 1999).

In the 3' untranslated region, there is a non essential short variable region, a polypyrimidine stretch that must be more than 26 nucleotides long and finally a highly conserved sequence of 98 nucleotides (X domain), which constitutes the 3' terminus of the HCV genome (Okamoto et al. 1991),(Chayama et al. 1994) . The latter two regions are required for genomic replication.

1.2.2.1.2 Structural protein region

The amino-terminal one third of the polyprotein encodes the virion structural proteins.

The Core protein is highly basic and is associated with the viral genomic RNA to form the nucleocapsid (Takeuchi et al. 1990). It is a highly conserved protein, contains several B-cell epitopes and has been very important in the development of serological tests for HCV (Akatsuka et al. 1993).

A lipid-containing envelope derived from the host membrane surrounds the nucleocapsid. There are two structural N-glycosylated proteins (E1 and E2) in the envelope. They have a hydrophobic portion that anchors them to the membrane and they function as heterodimers that are stabilized by noncovalent interactions. There is a high degree of heterogeneity in the part of the genome that encodes these proteins. In particular, the N-terminus of the E2 protein, and this has been designated the

"hypervariable region 1". In genotype 1b isolates a "hypervariable region 2" has also been described downstream of the first on the E2 protein (Chayama et al. 1994).

After the structural proteins, comes the small integral membrane protein, p7, which appears to function as an ion channel (Pavlovic et al. 2003). The structural proteins mature by signal peptidase cleavages.

1.2.2.1.3 Non-structural protein region

The rest of the polyprotein is processed into six non-structural (NS) proteins that coordinate the intracellular processes of the virus life cycle.

The C-terminal two-thirds of NS2 protein contains the catalytic triad of a cysteine protease. Cleavage of the NS2/3 junction requires these residues as well as the downstream expression of the NS3 serine protease domain. Cleavage of N2/3 is partially dependent on the presence of microsomal membranes, implying a cellular cofactor is required for processing. It is enhanced by Zn^{2+}, which has a structural role in stabilising the NS3 fold (Stempniak et al. 1997).

The NS3 serine-protease activity cleaves NS3/4A, 4A/4B, 4B/5A, and 5A/5B junctions of the polyprotein (Hahm et al. 1995). It forms a stable complex with NS4A protein. The C-terminal of the NS3 protein contains an NTPase and an RNA-helicase. The latter unwinds the duplex RNA during replication; hydrolysis by the NTPase supplies the energy for this process (Zhang et al. 2005).

NS4A is a small protein that anchors NS3 to cellular membranes to allow its serine protease to function (Bartenschlager et al. 1995).

The integral membrane protein NS4B induces ultrastructural vesicular structures, termed 'membranous webs' to form within the endoplasmic reticulum and these been proposed to serve as a scaffold for replication complex assembly (Egger et al. 2002).

NS5A can be phosphorylated on multiple serine residues by cellular kinases (Reed et al. 1997), but the role of remains unknown. Hyperphosphorylation appears to reduce replication levels (Appel et al. 2005). NS5A associates with membranes through an N-terminal alpha helix. The structure suggests it may have a role in binding RNA (Tellinghuisen et al. 2005). A region on NS5A has been labelled the interferon-sensitive determining region, after some research that suggested that mutations in this region appeared to correlate with the sensitivity of HCV genotype 1b isolates to interferon (IFN) treatment (Sato & Enomoto 1996). NS5A has been found to interact with the interferon-induced cellular protein kinase PKR (Gale, Jr. et al. 1998) providing a possible mechanism for this observation. These findings however were not replicated in European (Khorsi et al. 1997) or North American (Hofgartner et al. 1997) patients.

NS5B protein is an RNA-dependent RNA polymerase (Lin et al. 1994). NS5B has a typical 'right hand' polymerase structure, with catalytic sites in the base of the palm domain, surrounded by thumb and finger domains (Penin et al. 2004). NS5B is anchored to the membrane by a C-terminal peptide region (Schmidt-Mende et al. 2001).

1.2.2.1.4 Genomic variations

HCV is characterised by extensive genetic heterogeneity. HCV isolates have been classified into 6 major genotypes and over 100 subtypes (Simmonds et al. 1994). There is up to 35% variation in the genome between the most different isolates. In an infected individual, HCV circulates as quasispecies, which is a mixture of closely related but distinct genomes, differing by up to 2% (Enomoto et al. 1994). These arise as a result of error prone replication by the viral polymerase that lacks a

proofreading function. They can accumulate over time during the infection or can occur from the onset of the infection due to simultaneous transmission of multiple viral species (Laskus et al. 2004). They can also occur early in the treatment of the infection (Abbate et al. 2004). The heterogeneity of quasispecies can be found throughout the genome but certain regions are hypervariable, such as the N-terminus of the E2 protein.

1.2.2.2 Life cycle

After entry into the bloodstream, by the methods described in the epidemiology section, the virus enters cells to begin replication of the virus. There is ongoing controversy as to which cells are susceptible to infection.

1.2.2.2.1 Cell tropism

1.2.2.2.1.1 Hepatocytes

Although the primary site of replication is definitely the liver, it has been very difficult to demonstrate the presence and the precise location of the RNA transcription and the translated viral proteins. The traditional methods of immunohistochemistry for viral proteins (Sansonno & Dammacco 1993) and in situ hybridization and digital image analysis of liver biopsies (Gosalvez et al. 1998) have produced mixed results.

1.2.2.2.1.2 Extrahepatic

Extrahepatic replication has been proposed to occur at low levels in a number of other tissues. During replication of the virus, a negative strand of RNA is produced from the positive strand. Therefore the traditional method used to investigate the sites of HCV replication has been to detect this negative strand. However some caution in

interpretation of these studies should be observed, as extensive artifactual detection of negative strand RNA, due to self-priming and mispriming events can occur. Both artifacts can be dramatically reduced (mispriming) or eliminated (self-priming) using CAP-based Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay, which uses a primer located in the nucleocapsid part of viral genome outside the highly structured 5' untranslated region (Lerat et al. 1996) or *Thermus thermophilus* (Tth)-based RT PCR (Radkowski et al. 2002).

Using the CAP-based RT-PCR assay, 8% of Peripheral Blood Mononuclear Cells (PBMC) were observed to have the negative strands while in sera and fresh bone marrow cells none were detected (Lerat et al. 1996). Occult HCV infection, characterised by the presence of HCV-RNA in the liver, in the absence of anti-HCV and serum HCV-RNA, is also documented. In these patients negative strands of HCV RNA have been found in 3% of PBMC (Castillo et al. 2005;Otsuka et al. 2005). Thus, they could be potentially infectious.

The brain is another postulated site of replication due to the observed neuropsychological changes seen patients infected with HCV (Forton et al. 2002). One study has documented the presence negative strands RNA in three out of six post mortem patients have using Tth-based RT PCR (Radkowski et al. 2002). In two of these patients, the viral genomic sequence was different from RNA isolated from the same patient's serum. In one of these patients the same sequence negative strand was also found in a lymph node. The authors suggest that HCV can replicate in the central nervous system, probably in cells of the macrophage/monocyte lineage.

Overall extrahepatic HCV replication remains controversial.

1.2.2.2.2 Cell entry

The mechanism by which HCV enters cells is not known, but it is likely that E1 and E2 are involved. The cell surface molecule CD81 has been identified as a possible binding receptor for E2 (Pileri et al. 1998). It is expressed on a variety of cell types including hepatocytes. It has also been suggested that HCV can form complexes with very low density lipoproteins or low density lipoproteins and enter via the low density lipoprotein receptor (Agnello et al. 1999). Other candidate HCV receptors have also been identified including scavenger receptor class-B type-1 (Bartosch et al. 2005), L-SIGN and DC-SIGN (Cormier et al. 2004).

The bound virus is probably then internalised and fusion of the viral and cellular membranes occurs, presumably triggered by the low pH of the endocytic compartment. This leads to the release of a single-stranded, positive–sense RNA genome into the cytoplasm.

1.2.2.2.3 RNA translation

The positive–sense RNA genome initially serves as a messenger RNA for translation of the viral polyprotein. The HCV internal ribosome site within the 5' untranslated region binds 40S ribosomal subunits directly, bypassing the requirement for pre-initiation factors. This complex recruits eukaryotic initiation factor 3 and the ternary complex of Met-tRNA-eIF2-GTP to eventually become the translationally active 80S complex (Ji et al. 2004). This produces the polyprotein that is then proteolytically cleaved to produce the 10 viral proteins described above.

1.2.2.2.4 RNA replication

As for all positive-strand RNA viruses, HCV RNA replication occurs in association with altered cytoplasmic membranes. As mentioned, NS4B can induce this membranous web formation. The lipid composition of these membranes affects the efficiency of replication suggesting membrane fluidity is important (Kapadia & Chisari 2005). Little is known about the process of RNA synthesis but if it is similar to related viruses it is likely to be semiconservative and asymmetric with the positive-strand serving as a template to make a negative-strand intermediate. The negative strand then serves as a template to produce multiple genomes. This replication probably involves *de novo* initiation by a multiprotein complex (replicase) including the NS5B coded RNA polymerase. As mentioned above both the 3' and 5' untranslated regions are involved in efficient RNA replication, as are NS3 and NS5A. Adaptive mutations in these and NS4B and NS5B have been shown to enhance replication in 'subgenomic' replicons in cell culture by up to 10,000-fold (Blight et al. 2000).

1.2.2.2.5 Virion assembly and release

The process of virion assembly and release has been hampered by the lack of a cell culture system that can mimic this process *in vitro*. Although adaptive mutations of the genome can produce replication of replicons in cell culture, they appear to prevent virion release. However recently an isolate from a patient in Japan (JFH-1) has been shown to produce infectious particles in cell culture (Wakita et al. 2005) and undoubtedly this will lead to further insights to this process. Core protein is localised to the cytoplasmic surface of the endoplasmic reticulum and lipid droplets

(Yasui et al. 1998). Its central hydrophobic domain allows membrane association and the N-terminal region interacts with the RNA genome. The envelope glycoproteins E1 and E2 require complex folding that involves a number of endoplasmic reticulum resident chaparones. Virions presumably form by budding into the endoplasmic reticulum and leave the cell through the secretory pathway. Despite the difficulty in demonstrating the presence and the location of the HCV replication, it does appear that HCV virions turn over rapidly (with a half-life of about three hours) and up to about 10^{12} viruses are produced every day in an infected person (Neumann et al. 1998).

1.3 Immunology of Hepatitis C

Following inoculation, HCV triggers both innate and adaptive immune responses. Despite this the virus is able to persist and cause chronic infection in approximately 50 to 80 percent of patients (Wiese et al. 2005), (Niederau et al. 1998). These immune responses are now starting to be characterised and the strategies by which the virus evades them, are being better understood.

1.3.1 Innate immune response

When HCV infection occurs within the liver a series of intracellular events are triggered that protect the infected cells and the surrounding tissues. This response is initiated when a pathogen-associated molecular pattern (PAMP) presented by the infecting virus (such as single strand RNA or polyuridine signatures), is recognised and engaged by specific PAMP receptor factors (Toll-like receptor 3 and Retinoic-acid-inducible gene 1) expressed in the host cell, triggering signals via a number of Interferon regulatory factors (IRF) and Nuclear factor κB (NFκB) that induce the

expression of antiviral effector genes (IFN-α / IFN-β and cytokines/chemokines respectively) (Iwasaki & Medzhitov 2004). IFN-α and IFN-β released then binds its common receptor and activates a number of Interferon stimulated genes (ISG) via the Jak-STAT pathway (Sarcar et al. 2004). These ISG products impart regulatory functions that limit HCV replication through processes that include disruption of viral RNA translation and inhibition of antigenomic strand RNA synthesis (Wang et al. 2003).

HCV proteins can interfere with these pathways. The NS3/4A protease can inhibit the IRF (Foy et al. 2005) and the NFκB (Li et al. 2005) pathways triggered by PAMP receptor activation. HCV Core protein induces Suppressor of Cytokine Signalling proteins that inhibit the Jak-STAT pathway (Bode et al. 2003). HCV protein leads to expression of high levels of protein phosphatase 2A which may also inhibit this pathway (Duong et al. 2004). NS5A and E2 proteins bind protein kinase R, and inhibit its catalytic activity, which may allow HCV to evade in part the translational-suppressive actions of IFN and protein kinase R dependent signalling processes that amplify the host response to infection (Taylor et al. 1999). NS5A can also induce Interleukin 8 expression through transactivation of the interleukin 8 promoter (Polyak et al. 2001). The process by which Interleukin 8 antagonises the action of IFN is not known. RNase L is an ISG product that should be able to cleave HCV genomic RNA at specific dinucleotide sites into non-functioning nucleolytic products. Genotype 1 RNA sequences, in general, have fewer of these cleavage sites compared with genotype 2 or 3 and may explain some of the resistance of this genotype to IFN based treatment (Han et al. 2004).

In acutely infected chimpanzees HCV triggers a strong IFN-α and IFN-β release within the liver, however the strength of this response is no different between those animals that clear the infection acutely and those that do not (Bigger et al. 2004). This might suggest either no role in determining outcome of the acute infection or the determinant having its effect downstream of the IFN receptor by the methods described above. In chronic human infection, there is evidence for a clear absence or only low level of IFN-α and IFN-β expression (Mihm et al. 2004). This suggests that at this stage in the disease, there maybe suppression of the IRF pathways by the method described above. However, despite this, some of these patients still have abundant ISG expression. It has been suggested that the explanation for this, is that cellular stress from fibrosis and / or cirrhosis maybe sufficient to stimulate ISG expression (Smith et al. 2003). Secretion of IFN-γ by hepatic effector T cells and Natural Killer cells also contribute to the level of ISG expression (Frese et al. 2002). Overtime, the host's innate immune system pressure may also drive the outgrowth or selection of quasispecies with variations in the proteins discussed above, that are better able to resist the actions of IFN and thereby maintain persistence (Farci et al. 2000).

1.3.2 Adaptive immune response

1.3.2.1 Humoral immunity

Virus–specific antibodies can be detectable 7-8 weeks after HCV infection (Pawlotsky 1999). There is evidence that antibodies can neutralise HCV infectivity *in vitro* prior to inoculation into chimpanzees (Farci et al. 1994). The outcome of acute infection in humans has been predicted by the occurrence of sequence changes in the hypervariable region 1 of glycoprotein E2, which is the major target of the

antibody response, and this occurred simultaneously with antibody seroconversion, presumably reflecting immune selective pressure (Farci et al. 2000). However in both chimpanzees and humans, clearance of acute infection can occur without antibody production (Post et al. 2004) and the presence of antibodies do not prevent reinfection (Farci et al. 1992). Therefore their role remains unclear.

Antibodies to HCV proteins wane over time in those that have cleared the infection. In the German Anti-D single source cohort, HCV antibodies were undetectable in 21% patients after 25 years of infection (Wiese et al. 2005), although T-cell memory responses remained and are therefore a better marker of previous infection (Takaki et al. 2000).

1.3.2.2 T-cell immunity

1.3.2.2.1 Acute infection

HCV RNA genomes appear in the serum within a few days of inoculation, and peak at 6 to 10 weeks irrespective of outcome (Abe et al. 1992). In successful clearance of the virus in acute infection, $CD8^+$ cytotoxic T-cell responses are vigorous and generally target multiple major histocompatibility complex (MHC) class I-restricted epitopes in structural and non-structural HCV proteins (Lechner et al. 2000). Expansion of these cells coincides with the rise in the serum transaminases suggesting the damage to the liver is immunopathological. At the same time strong, sustained $CD4^+$ T helper proliferation to multiple MHC class II-restricted epitopes on the structural and non-structural viral proteins is required for clearance (Cramp et al. 1999). Work in chimpanzees suggest a further complexity in that the response initially focuses on a limited number of dominant epitopes and then spreads to additional targets only after viraemia is mostly controlled (Shoukry et al. 2004).

With control in viraemia there is a contraction in the $CD4^+$ and $CD8^+$ T-cell responses almost certainly by programmed cell death. If no rebound viraemia occurs durable T-cell memory populations occur within the liver (Takaki et al. 2000). Although chimpanzees that have resolved acute infection are susceptible to reinfection there is a marked reduction in the duration and the peak of viraemia (Bassett et al. 2001) and this coincides with massive $CD4^+$ and $CD8^+$ T-cell recall responses (Shoukry et al. 2003). Pre-treatment with anti-$CD4^+$ (Grakoui et al. 2003) and anti-$CD8^+$ (Shoukry et al. 2003) antibodies prolonged viraemia on reinfection and led to persistence of the virus. In injecting drug users, previous clearance of the infection also provided immunity on re-exposure unless co-infected with Human Immunodeficiency Virus (HIV) (Mehta et al. 2002).

1.3.2.2.2 Chronic infection

If the immune response does not conform to the description above, there is a higher chance that the viraemia either does not fully come under control or relapses after a period of control (Abe et al. 1992). This will usually lead to viral persistence and chronic infection, where the level of the viraemia may vary from person to person (typically 10^3 to 10^7 genomes per ml of serum) but remains remarkably constant over time in an individual. It is not clear whether in chronic infection $CD8^+$ cytotoxic T-cells contribute to the control of replication, due to conflicting evidence on correlations with viral load (Freeman et al. 2003b), (Nelson et al. 1997b). The activity of $CD8^+$ cytotoxic T-cells in liver biopsies correlates with both Alanine aminotransferase (ALT) serum levels and Histologic Activity Index and tend to be found in areas of lobular and piecemeal necrosis (Leroy et al. 2003). Intrahepatic pro-inflammatory cytokine (such as IFN-γ) messenger RNA levels have been

correlated with severity of portal inflammation and liver fibrosis (Napoli et al. 1996). It is unclear if T-cells are the major source of these cytokines or whether it is from the other inflammatory cell types, such as macrophages, recruited to the liver and the degree that these cells mediate tissue injury (McGuinness et al. 2000).

1.3.2.3 Evasion of the adaptive immune response

The outcome of acute infection therefore seems to be decided in the first six months. It may be that the mechanism of evasion needs only to act for a time during this critical period to be sufficient to alter the timing or vigour of T-cell immunity and favour persistence. However there will need to be mechanisms that can explain the antigen-specific defect in adaptive immunity that lasts for decades and appears to be exclusive to HCV that occurs in chronic infection.

1.3.2.3.1 Viral determinants

Mutational escape of HCV epitopes: The virus's ability to generate genomic diversity has been one of the theories put forward to explain how the virus evades the control exerted by the adaptive immune system. $CD8^+$ T-cell mediated selection pressure against HCV has been demonstrated in the chimpanzee model (Weiner et al. 1995). In human infections from a single source there is also evidence of escape mutations in targeted MHC class I.-restricted epitopes (Tester et al. 2005). It is possible that $CD8^+$ T-cells that achieve viral clearance target a set of protective epitopes functionally constrained from mutation, although the evidence so far is against this (Lauer et al. 2002). It is unlikely that this method of evasion is the only mechanism at work, as some individuals who develop persistent infection have multi-specific $CD8^+$ cytotoxic T-cell responses and many T-cell epitopes are intact in

persistently replicating genomes and do not mutate despite and intense focal response (Urbani et al. 2005).

Deletion or anergy: Recent studies of HCV-specific $CD8^+$ T-cells have indicated that these cells may be functionally impaired, or anergic in chronic infection (Wedemeyer et al. 2002). However, $CD8^+$ T-cell function has also been shown to be impaired in patients that have cleared the infection (Gruener et al. 2001). Virus specific $CD4^+$ T-cell responses are generally weak or absent when assessed using functional methods of identification (Rosen et al. 2002). But it is unclear whether they are present but functionally impaired or have been lost from the immune response.

Regulatory T-cell populations: Certain HCV specific $CD8^+$ T-cells found in the liver in chronically infected patients produce the cytokine interleukin 10 which downregulates T-cell responses (Koziel et al. 1995). In the peripheral circulation of these individuals the proportion of $CD4^+CD25^+$ regulatory T-cells is elevated compared with recovered or uninfected individuals (Cabrera et al. 2004). These cells can suppress HCV specific $CD8^+$ T-cells activity *in vitro* (Boettler et al. 2005).

Impaired antigen presentation: Dendritic cells are the main presenters of antigen in the liver. Natural Killer cells are potent regulators of dendritic cell function, and there is evidence of inhibition of these cells by HCV E2 protein (Tseng & Klimpel 2002). It has also been suggested that viral proteins may directly inhibit dendritic cell maturation and function but these findings have been inconsistent and permanent global defects in chronic infection are not seen (Larsson et al. 2004).

Impaired T-cell recruitment: An inability of effector T-cells to move into the infected liver has been proposed with limited experimental evidence (Thimme et al. 2002).

1.3.2.3.2 Host determinants

Major Histocompatibility Complex: An individual's MHC alleles do appear to affect the likelihood of viral clearance, in particular the DRB1*01 allele in an Irish anti-D cohort (Barrett et al. 1999). There are other MHC allele associations with clearance but none are universal (Cramp et al. 1999).

Co-infection: Acute HCV infection in patients infected with HBV that are Hepatitis B surface antigen (HBsAg) positive and have active HBV replication, usually have transient HCV viraemia with poor antibody responses to HCV (Chu et al. 2002).

Immunosuppressive medication: In a renal transplant recipient, who acquired HCV post transplant and who had their immunosuppression medication stopped after failure of the graft, spontaneously cleared the infection (Somsouk et al. 2003).

1.4 Pathology of Hepatitis C

1.4.1 Histopathology of the liver in chronic infection

The classic histological presentation of HCV infection is portal lymphocyte infiltration leading to lymphoid follicles or aggregates in approximately 50% of cases (Scheuer et al. 1992). Bile duct damage is generally lymphocytic and non-destructive and is seen in 30% (Lefkowitch & Apfelbaum 1989). There also can be varying degrees of lobular lymphocyte infiltration, with both lobular and piecemeal necrosis (Dhillon & Dusheiko 1995). The later two features are the main determinants of the grading of the severity of the necroinflammatory component of the hepatitis, used in

scoring systems mentioned below. Steatosis is seen in approximately 60% of cases and is usually macrovesicular, mild and non-zonal (Hourigan et al. 1999).

The fibrosis tends to develop from the portal tracts with portal spurring, progressing to portal-portal bridging and then cirrhosis once nodular regeneration of the hepatocytes occurs (Gerber et al. 1992). This pattern of fibrosis progression is recognised in the various scoring systems that have developed to stage the disease. The three commonly used ones have been the Histological Activity Index (Knodell et al. 1981), the Ishak system (modified histological activity index) (Ishak et al. 1995) and the METAVIR system (French METAVIR Cooperative Study Group 1994).

1.4.2 Pathophysiology of liver disease during chronic infection

The mechanisms responsible for the onset and progression of hepatic lesions during chronic HCV infection are not fully understood. As previously stated HCV is not thought generally to be cytopathic, largely due to the lack of correlation of viral load to severity and prognosis of liver disease (Fanning et al. 1999). The only lesion that has been attributed to a direct pathogenic effect of the virus is steatosis in HCV genotype 3 infections (Kumar et al. 2002), whilst in the other genotypes steatosis is seen less frequently and is usually associated with the insulin resistance of the metabolic syndrome (Fartoux et al. 2005b). In transgenic mice models that either overexpress HCV Core or NS5A protein, accumulation of intracytoplasmic lipid droplets occurs (Moriya et al. 1997). The core protein has been shown to inhibit the function of microsomal triglyceride transfer protein, a major regulator of hepatic assembly of Very Low Density Lipoproteins (Perlemuter et al. 2002). HCV Core protein has also been observed to induce mitochondrial injury resulting oxidative stress which then perturbs lipid peroxidation (Okuda et al. 2002). HCV genotype 3 in

particular has also been shown to induce transcription of a number of genes involved in lipid metabolism, including Stearoyl coenzyme A desaturase 4, a rate limiting enzyme in the synthesis of monounsaturated fats (Bigger et al. 2001). The situation in the immunocomprimised patient pre- or post-transplant is poorly understood as higher levels of virus replication are seen and these are associated with severer liver lesions and a poorer outcome (Tolan et al. 2001). This has been stated as evidence of a direct cytopathic effect of the virus.

As previously stated in the immunology section, the activity of $CD8^+$ cytotoxic T-cells is important in removal of virus-infected cells but also bystander cells, and is therefore the mediator of damage in the liver in chronic infection. This is through TNF-α-, *fas*- and perforin-mediated mechanisms (Ando et al. 1997).

Fibrosis is the main complication of HCV in the liver and is a wound healing response to injury. It results from a dynamic mechanism of gene transcription and synthesis of extracellular matrix (ECM) components (collagen, laminin, fibronectin and proteoglycans) that are deposited within the liver parenchyma (Rockey 2000). Hepatic Stellate cells (HSC) are the main ECM producing cells and reside in the space of Disse. Following chronic injury, HSCs activate or transdifferentiate into myofibroblast-like cells, acquiring contractile, proinflammatory and fibrogenic properties (Marra 1999). When activated they migrate to the sites of tissue repair. Platelet Derived Growth Factor produced by Kuppfer cells is the main mitogen for activated HSCs (Lau et al. 2005). Myofibroblasts derived from small portal blood vessels also have fibrogenic potential when liver injury occurs periportally (Knittel et al. 1999).

Damaged hepatocytes and activated Kuppfer cells release reactive oxygen species and fibrogenic mediators such as Transforming Growth Factor β1 and Tumour Necrosis Factor α that activate HSCs in HCV infection (Nelson et al. 1997a). Apoptosis of damaged hepatocytes stimulates the fibrogenic actions of liver myofibroblasts (Canbay et al. 2004). There is also some very preliminary evidence of a direct effect of HCV on HSCs. When adenoviruses encoding core and nonstructural proteins (NS3-NS5) were used to express HCV proteins in HSCs, it induced proinflammatory actions, such as increased the secretion of bioactive Transforming Growth Factor β1 and the expression of procollagen α1 and intercellular cell adhesion molecule type 1 (Bataller et al. 2004).

Activated HSCs secrete inflammatory chemokines, express cell adhesion molecules, and modulate the activation of lymphocytes (Vinas et al. 2003) which amplifies and perpetuates the process. As well as the fibrogenic factors such as Transforming Growth Factor β1, vasoactive substances (angiotensin II, norepinephrine) (Bataller et al. 2003) and adipokines (leptin and adiponectin) (Ikejima et al. 2002) are required for the development of fibrosis.

Finally, removal of excess collagen can occur by various matrix metalloproteinases and is regulated by Tissue inhibitor of metalloproteinase type 1 and Transforming Growth Factor β1 (Yoshiji et al. 2002). These pathways may be involved in the reduction of fibrosis that has been observed following successful clearance of HCV post treatment (Poynard et al. 2002b).

1.4.3 Development of Hepatocellular carcinoma

Epidemiologic, clinical, and virologic data have shown a close association between chronic infection with HCV and the development of HCC (Bruix et al. 1989). In many countries of the developed world, HCV infection accounts for more than half of the cases of HCC (Hasan et al. 1990). HCC usually arises after 2-4 decades of infection, typically in the context of underlying cirrhosis (Kiyosawa et al. 1990). Viral hepatitis greatly influences the incidence of somatic and genetic events in hepatocytes, by increasing cell turnover. A direct role for viral proteins has been the suggested as the mechanism for carcinogenesis but this still remains controversial. Core protein has been shown *in vitro* to modulate the expression of the $p21^{WAF1}$ gene, which is a major target of the tumour suppressor protein p53. It causes proliferation or apoptosis of the hepatocyte depending on whether the Core protein is in the cytoplasm or the nucleus (Kwun & Jang 2003). HCC has also been shown to occur in transgenic mice expressing the Core protein or the full-length HCV polyprotein (Lerat et al. 2002). NS3 can form a complex with p53 and modulate its activity and repress $p21^{WAF1}$ gene expression (Kwun et al. 2001). NS5A appears to interact with cellular signalling apparatus and cell cycle-regulatory kinases including p53 and $p21^{WAF1}$ (Majumder et al. 2001). These block the apoptotic cellular response to persistent HCV infection suggesting a potential role in the pathogenesis of HCC. However the influence of overexpression of these proteins that occurs these models is unknown, when compared with human infection.

1.5 Epidemiology of Hepatitis C

1.5.1 Prevalence and incidence

The prevalence varies widely from country to country depending on the prevalence of the risk factors for infection.

1.5.1.1 United Kingdom

A recent study on sera from residual specimens from adult patients submitted to laboratories in England and Wales, estimated the prevalence in England and Wales to be 1.07% and not changing significantly from 1986 to 1996 (Balogun et al. 2002). The prevalence rate in health blood donors in the West Midlands ranges from 0.01 to 0.02% (Mutimer et al. 1994). In a study on 224,000 consecutive blood donors from the North-West of England, it was found to be 0.04% (McLindon et al. 1995). If an overall estimated prevalence rate of 0.5% were taken, then there would be approximately 250,000 HCV antibody positive people in England and Wales. Up to the end of 2004 only 49,527 positive blood samples had been reported, with 8,084 reported in 2004 (Health Protection Agency 2005). This figure is rising year on year. In Scotland the overall prevalence rate has been estimated to be 1% (Drug Misuse Information Strategy Team 2005), giving approximately 50,000 patients being HCV antibody positive. Up to the end of 2003, 18,109 positive blood samples had been reported, with 1,779 reported in 2003 (Scottish Centre for Infection and Environmental Health 2004). This figure is now falling year on year. In Lothian up to the end of 2003, 2,461 positive blood samples had been reported, with 103 reported in 2003 (Scottish Centre for Infection and Environmental Health 2004).

A study in the United Kingdom (UK) estimated the rate of seroconversion rate in repeat blood donors in England found it to be 0.26 per 100 000 person years (95%

confidence interval 0.15 to 0.43) (Soldan et al. 1998). There is, of course, predonation screening of blood donators to exclude people with high-risk behaviours. This will therefore lead to an underestimation of the true incidence. Despite this 5 of the 14 people who seroconverted had these high-risk behaviours.

1.5.1.2 Worldwide

Similar prevalence rates as the UK are seen in Northern Europe (Trepo & Pradat 1999). In Southern Europe it is estimated to be between 1-2.4% (Kondili et al. 2002). In Italy, the adult incidence of HCV is reported to be between 14 to 50 cases/100 000 person years (Kondili et al. 2002), (Mazzeo et al. 2003). The rate rises in Equatorial Africa to 6% with the highest recorded in adults over 50 years old in Egypt at 41% (bdel-Wahab et al. 1994).

A study in the United States obtained serum samples from 21,241 persons six years old or older who participated in the third National Health and Nutrition Examination Survey, conducted during 1988 through to 1994 (Alter et al. 1999). The overall prevalence of anti-HCV was 1.8%, corresponding to an estimated 3.9 million persons nationwide (95% CI, 3.1 million to 4.8 million) with HCV infection. Sixty-five percent of the persons with HCV infection were 30 to 49 years old. Seventy-four percent were positive for HCV RNA, indicating that an estimated 2.7 million persons in the United States (95% CI, 2.4 million to 3.0 million) were chronically infected.

1.5.2 Genotype distribution

Phylogenetic analysis of the Non-Structural-5 region of the genome allows classification of HCV into six major genotypes and a series of subtypes (Simmonds et al. 1994). Using this method, the prevalence of each of these genotypes can be determined around the world (Smith et al. 1995).

1.5.2.1 United Kingdom

In a study on 567 patients across England and Wales, the majority of HCV infections were types 1a (32%), 1b (15%), or 3a (37%). The genotype distribution in individual risk groups was similar to the overall genotype distribution except for haemophilia patients, in whom the frequencies were 1a (39%), 1b (23%), and 3a (21%) (Harris et al. 2000). In a report on 104 patients referred to a teaching hospital in the North-East of England there was a higher proportion that were Genotype 1 (69%) (Watson et al. 1996).

1.5.2.2 Worldwide

There are clear differences in the distribution of the genotypes around the world. Genotypes 1a, 1b, 2a, 2b, 2c and 3a account for about 90% of the HCV infections in North and South America, Europe, Russia, China, Japan and Australia / New Zealand (Smith et al. 1995). In the United States, HCV genotypes 1a and 1b are the predominant genotypes in patients with chronic hepatitis C and account for greater than 40% of isolates (Zein et al. 1996). Genotype 1b accounts for the majority of infections in Southern and Eastern Europe, China and Japan. Genotype 3a is common throughout America and Europe. Genotype 4a accounts for most of the infections in Egypt. The subtypes of Genotype 4 make up the majority of infections in Central Africa. About 50% of infections in South Africa are Genotype 5a. Genotype 6 isolates are found mainly in South-East Asia (Simmonds et al. 1996). The risk group and age of the patient may also determine the genotype prevalence. In particular Genotype 3a is commoner in isolates from younger people in western countries, especially amongst intravenous drugs users (Simmonds & Smith 1997).

1.5.3 Transmission

The modes of transmission reflect HCV being a blood borne virus.

1.5.3.1 Intravenous drug abuse

The high prevalence (20 to 90%) of the HCV infection in populations using intravenous drugs clearly establishes this as a route of transmission (Roy et al. 2002). In 773 drug users who had hepatitis serology performed between January 1992 and April 1996 in Liverpool, 67% were HCV antibody positive (Lamden et al. 1998). Undoubtedly there have been changes in the incidence of HCV infection over time. In the pre-HIV era (1975-1985) sharing of needles and equipment was widespread in the Intravenous drug use (IDU) populations. Acquisition of HCV infection was rapid with seroprevalence rates of 54%, 78%, 83% and 94% among users of less than a year, 1 year, 5 years and more than 10 years respectively (Thomas et al. 1995a). Between 1986 and 1995 harm reduction schemes were introduced and there has been some evidence that incidence and prevalence rates fell. In a study to ascertain the HCV antibody prevalence among injectors from Glasgow between 1990 and 1995, sera from injectors who had undergone HIV antibody testing were tested anonymously for HCV antibody (Goldberg et al. 1998). The prevalence of HCV antibody in Glasgow fell significantly among IDUs of all ages (90% to 77%), IDUs aged 15 to 19 years (92% to 29%), and IDUs aged 20 to 24 years (91% to 65%). Since 1995, there is evidence that the incidence and prevalence may have plateaued or started to increase again. The above study was repeated in Glasgow, Lothian, Tayside and Grampian, in the late 1990s. There were no significant changes in prevalence in each of the four regions among those aged less than 25 years (Glasgow 1997-00: 43% to 41%; Lothian 1997-9: 13% to 17%; Tayside 1997-9: 45% to 35%;

Grampian 1996-9: 28% to 29%). Among those aged greater than 25 years, significant decreases in prevalence were only observed in Glasgow (1997-00: 79% to 72%, p = 0.03) and Lothian (1997-9: 54% to 45%, p = 0.05) (Hutchinson et al. 2002). In San Francisco between 1997 and 1999, IDUs aged less than 30 years old had an estimated incidence rate of 25.1 per 100 person-years (95% CI, 18.7-32.9 per 100 person-years) (Hahn et al. 2002). The incidence of HCV infection among new injecting drug users in London (aged below 30 years or had been injecting for six years or fewer) has recently been estimated to be 41.8 cases per 100 person-years (Judd et al. 2005), with a baseline prevalence of 44%.

1.5.3.2 Blood products

NANB post transfusion hepatitis had been recognised for many years prior to the discovery of HCV. In the United States prior to 1985 the incidence of was 8-10 per 100 persons transfused (Alter 1994). Once the serological test for HCV became available it become clear that HCV was the main aetiological agent (Alter et al. 1989). Transmission by this route has been extremely rare since the introduction of screening of donors using the antibody test in late 1991. With the HCV antibody test there is still a potential 10-week window from infection to antibody production when infection may be missed (Soldan et al. 1998). HCV RNA testing of donations reduces this window down to 17 days (Flanagan & Snape 1998). In the UK, the frequency of HCV infectious donations was 1 in 520,000 during 1993-98 and fell to 1 in 30 million during 1999-2001 when all donations were tested for HCV RNA (Soldan et al. 2003).

Patients that have received transfusion with pooled blood products, such as Factor VIII concentrates and Anti-D prior to 1985, unfortunately have high prevalence rates

for HCV (70 to 90%) (Jacyna et al. 1990). A single infected donation could contaminate numerous recipients of the pooled product. In 1985/86 a heat inactivation step was introduced and was shown to reduce the risk of NANB hepatitis to less than 1% of transfusions (Study Group of the UK Haemophilia Centre Directors on Surveillance of Virus Transmission by Concentrates 1988). Since then donor and pool product screening for antibody and HCV RNA has eliminated these products as a source (Makris et al. 1993).

1.5.3.3 Body tattooing and piercing

There is clearly a theoretical risk of transmitting the virus using unsterilised equipment. However despite one report that claimed it explained 41% of all cases (Haley & Fischer 2001), case-control studies in the United States have found no association with tattooing, acupuncture, or ear piercing (Alter 1999).

1.5.3.4 Sexual

HCV can be transmitted sexually however the route is not efficient. In long-term studies of heterosexual couples in relationships of 15-20 years duration, the prevalence among sexual partners of HCV infected persons was 0 to 2.5% with the majority of couple not using condoms (Bresters et al. 1993), (Neumayr et al. 1999). In a survey of from genitourinary clinics in the UK, 17,586 specimens from 1995, showed an adjusted prevalence of HCV antibody in of 1.03% (95% CI: 0.89 to 1.16) overall and 0.65% (95% CI: 0.51 to 0.78) among those who did not report injecting drug use. One study has suggested that transmission from an infected male to an uninfected female partner maybe more efficient that the other way round (Thomas et al. 1995b). The prevalence of the infection in homosexual / bisexual men is usually reported as higher than the general population at 4% in the United States and Europe

(Tedder et al. 1991). In a recent study of Scottish genitourinary clinics, the non-injecting prevalence rates for homosexual / bisexual males was 0.6%, for heterosexual males 0.8%, for heterosexual females 0.3% (Goldberg et al. 2001).

1.5.3.5 Vertical

The risk of transmission from mother to child in those that are HCV RNA PCR positive is about 5% (Conte et al. 2000). The risk increases if the mother is known to have injected drugs (9%) (Resti et al. 2002) or if she is co infected with HIV (16%) (Papaevangelou et al. 1998). If there is intrapartum infantile exposure to maternal blood or a higher level of HCV viraemia, the risk is also increased (Steininger et al. 2003). A study of HCV prevalence among Scottish childbearing women found a rate of 0.29-0.40% (Hutchinson et al. 2004). Based on reported rates of mother to child transmission, 8-11 paediatric infections are expected per annum. Only 24% and 46% of mothers were estimated to have been diagnosed prior to pregnancy and birth, respectively (Hutchinson et al. 2004). Breast-feeding is not thought to confer a risk of transmission (Spencer et al. 1997).

1.5.3.6 Household contacts

Anecdotal reports of transmission by exposure of broken skin or mucous membranes to someone else's blood such by sharing shaving equipment have been made but are very rare (Tumminelli et al. 1995). Being a sibling or a non-sexual household contact does increase a person's risk of HCV infection, but it is always difficult to be certain of absence of other shared known risk factors (Ackerman et al. 2000).

1.5.3.7 Occupational

Within a health care setting, a percutaneous injury with a sharp implement that has previously been used on a known HCV infected patient can transmit infection. The

incidence of seroconversion following these needlestick injuries is on average 1.8% (0% to 7%) (Centers for Disease Control and Prevention 1998). The prevalence of antibodies to HCV was 0.28% in healthcare workers in the West of Scotland (Thorburn et al. 2001). The rate did not vary according to the degree of patient exposure or to duration of potential exposure. In dental surgeons in the same area, prevalence of HCV antibodies was 0.1% (Roy et al. 2003). These rates are not significantly greater than the estimated prevalence of HCV infection in the local population.

1.5.3.8 Iatrogenic

In countries and previous eras, where adequate sterilisation of medical equipment that is used for invasive procedures is or was not practised, the risk of transmission is high (Frank et al. 2000). Even in countries where this is not the case occasional transition can occur due to breakdown in usual practise (Esteban et al. 1996). Dialysis units are the commonest setting for patient-to-patient nosocomial infection. A surveillance study in the United States dialysis units found a seroprevalence rate of 10%, with an annual incidence of 3% (none of these had received a transfusion or used injection drugs) (Niu et al. 1993). The mechanisms of transmission in this setting is believed to be breaches in routine dialysis unit procedures and precautions (Kellerman & Alter 1999). Other documented nosocomial routes of transmission that have been documented are endoscopes and multidose vials.

1.5.3.9 Sporadic

The proportion of patients in whom it is not possible to identify a route of transmission has fallen over the years to less than 10% of cases in the United States (Alter 1999). Most people in this category are associated with low socioeconomic

level and may report high-risk behaviour such as imprisonment, multiple sexual partners and use of non-injection illicit drugs. Therefore they may be secondary to occult or undisclosed percutaneous exposure.

1.6 Natural History of Hepatitis C

1.6.1 Methods of study

Characterising the natural history of any infection requires establishing accurately the onset of infection, identifying and evaluating the full spectrum of the acute disease, tracking the process to resolution or development of complications, evaluating outcome without treatment and comparison with a properly matched control group without the infection. Unfortunately there are a number of inherent difficulties in studying the outcome of HCV infection: the onset is not recognised in the majority, it is generally asymptomatic, the progression is slow and many patients have undergone various treatments as they became available.

1.6.2 Acute Infection

In the early years after discovery of the virus most researchers felt that following a mild acute infection in which a minority are symptomatic (Koretz et al. 1993), a majority (80 to 90%) then go on to develop chronic infection (Niederau et al. 1998). These studies were particularly susceptible to referral bias, loose definitions of acute infection and lack of a control group. Recent studies have challenged this view. In the past there have been a number of occasions when a large group of individuals have been exposed to the virus at one time. Studying such a cohort decreases the influence of referral bias, and gives much more accurate figures on those that go on

to clear the virus. One study of this type concerns mothers given contaminated anti-D injections in Ireland between 1977 and 1978 (Kenny-Walsh 1999). A screening program of the 94% of those mothers potentially involved was performed. Seven hundred and four people were found to have HCV antibodies. Of these, only 390 (55%) were HCV PCR positive. A history of icteric hepatitis was reported in 20.6% of PCR negative and 3.4% of PCR positive women after inoculation (Barrett et al. 2001).

In Germany there is a similar cohort of 2867 women exposed to HCV through contaminated anti-D injections between 1978 and 1979 (Wiese et al. 2005). Cases were initially identified using repeated Alanine transferase (ALT) screening. It has been possible to re-examine 1980 women (70% of the original cohort) 25 years after the initial exposure. An acute hepatitis was seen in 93%. Of those that had an acute hepatitis, 25% were icteric and 35% were asymptomatic. At 25 years of the 1833 people that had the hepatitis, 86% remained positive for antibody and 48% had become HCV RNA PCR negative spontaneously and 6 % with IFN treatment.

In another study 14 patients with acute hepatitis C were identified among 29 healthy volunteers participating in 2 consecutive pharmacokinetics studies (Larghi et al. 2002). At presentation, all 14 patients tested HCV PCR positive. Infection resolved spontaneously in 8 patients, HCV RNA becoming undetectable by 4 to 5 months after the presumed time of infection in 5 of them and by 8, 13, and 24 months in the remaining 3. Six patients developed chronic infection. Liver biopsies performed in 9 subjects who were HCV PCR positive 6 months after diagnosis revealed that the prevalent histological finding was lobular inflammation.

In a further study of 60 patients in hospital practise, diagnosed with acute HCV infection (on the basis of being HCV antibody negative but HCV PCR positive on a first sample and subsequently becoming HCV antibody positive), 85% were symptomatic at presentation (Gerlach et al. 2003). Spontaneous clearance was observed in 52%, usually within 12 weeks after the onset of symptoms, whereas all asymptomatic patients developed chronic hepatitis C.

1.6.3 Chronic Hepatitis C

The vast majority of cases of patients diagnosed with HCV infection have chronic infection at presentation and the acute infection has gone unrecognised.

1.6.3.1 Rate of progression to cirrhosis

One of the most important questions regarding chronic infection is whether it will eventually progress to cirrhosis in all cases.

1.6.3.1.1 Retrospective / cross-sectional studies in liver clinics

Numerous retrospective / cross-sectional studies have been performed recruiting patients with established chronic liver disease due to HCV from liver clinics in tertiary hospitals. Unfortunately few have been controlled. The first large series analysed the biopsies of 1157 French patients with chronic hepatitis C who had a probable date of infection (Poynard et al. 1997). As with most studies of this kind, the investigators took the first year of active intravenous drug use or the time of exposure of a known risk factor such as blood transfusion. The median time from infection to biopsy in the French cohort was 12.4 years. The median time to histologically confirmed cirrhosis was 30 years estimated by Kaplan-Meier analysis, with 17.9% of the cohort being cirrhotic. Without treatment 33% had a median time

to cirrhosis of less than 20 years, 31% greater than 50 years. Cox regression analysis identified the age at infection of greater than 40 years, male sex and a daily alcohol intake of greater than 50g predicted early cirrhosis development. The researchers constructed a model to predict the median time to biopsy proven cirrhosis based on the independent variables identified. This is 12 years in men infected after the age of 40 who drink greater than 50 grams of alcohol per day compared with 42 years in women infected before the age of 40 who do not drink. They were able to stage the fibrosis in all these patients using the METAVIR scoring system (0=no fibrosis to 4=cirrhosis) and then calculate a median rate of fibrosis. They validated this method by analysing a subset of patients who had repeat biopsies. The figure they produced was 0.133 METAVIR fibrosis units per year for the cohort as a whole. This cohort of patients has subsequently been expanded to include 2313 liver biopsies but drew similar conclusions (Poynard et al. 2001).

1.6.3.1.2 Prospective studies in the community

Analysis of the Irish Anti-D cohort provides a method of minimising the influence of referral bias, as the patients are recruited from the community. Therefore we know accurately who was at risk, the number infected and when they were infected. Three hundred and seventy-six of 390 HCV RNA PCR positive patients have been followed up for a median 17 years (Kenny-Walsh 1999). Currently only 2% have been found to be cirrhotic and there have been no liver related deaths or development of HCC. The explanation for this low progression rate lies in this cohort's known risk factors for progression. Only 5% of this cohort drank more than 14 units of alcohol weekly, the mean age at infection was 28 years and obviously all were female. The French study would estimate the median time to a biopsy of cirrhosis to be 42 years

(Poynard et al. 1997). Therefore at 17 years it is unlikely that a significant number would have progressed to cirrhosis. However 51% had evidence of fibrosis on their biopsies and the mean progression per year of the cohort based on the numbers given for each stage of fibrosis was 0.048 METAVIR units per year, which is approximately half that predicted by the French group. There is therefore still discrepancy between the two studies. In the German Anti-D cohort at 25 years after infection only 1.5% had developed precirrhotic stages, 0.5% had overt cirrhosis (Wiese et al. 2005).

There have been two other studies that are more representative of the typical HCV infected population in most industrialised countries (young, intravenous drug using and coexisting alcohol abuse) and have also been community based and prospective. The first, based in the United States, enrolled between 1988-1989, 1667 persons aged 17 years or older with a history of injection drug use and an HCV antibody–positive test result during follow-up (Thomas et al. 2000). Of these 722 remained persistently HCV RNA PCR positive. The mean duration of infection was 23 years and 6.5% of this group were cirrhotic at the end of follow-up. A second cohort from Australia, were drawn from patients admitted to hospital with acute viral hepatitis from 1971 through 1975. Stored sera availability from this time enabled testing to identify 238 persons who were anti-HCV-positive on admission. Systematic approaches were used to locate 95 patients in this the cohort. Of these 51 (54%) remained HCV RNA PCR positive at follow-up. At a mean duration of infection of 23 years 7.5% were cirrhotic (Rodger et al. 2000). Clearly in this study it is not possible to be sure that all these patients did have acute HCV due to the difficulty of making this diagnosis,

however this would mean the numbers progressing to cirrhosis would be an overestimate.

1.6.3.1.3 Paired biopsy studies

Another way to assess progression of fibrosis is to follow a cohort of patients after an initial biopsy and then repeat it in all patients after a specified time interval. This was performed in a cohort of 123 patients with chronic HCV that underwent 2 liver biopsies 4-212 months (mean, 44 months) apart without intervening treatment (Ghany et al. 2003). Liver histology was graded using the Ishak modified Histology Activity Index (score, 0-18) and fibrosis staged using a scoring system of 0 (no fibrosis) to 6 (cirrhosis). 48 (39%) showed progression in fibrosis scores, 46 (37%) showed no change, and 29 (24%) showed improvement. Of those with worsening fibrosis, 75% had a 1-point increase and 25% a 2-point or greater increase in scores, and 9% showed progression to cirrhosis. The overall rate of progression was 0.12 fibrosis units per year, a rate that predicts progression to cirrhosis in 50 years if progression was linear. The rate of fibrosis progression was variable, and it was higher among older patients, those with higher serum alanine and aspartate aminotransferase levels, and those with the most extensive periportal necrosis on initial liver biopsy. A similar study was performed on 180 patients with a mean time interval between biopsies of 3.67+/-2.69 years. This showed overall no significant progression (Zarski et al. 2003). The Trent HCV study group in England performed a similar paired biopsy study to look at the rate of progression of fibrosis (Ryder et al. 2004). The median time interval between biopsies was 30 months. Two hundred and fourteen patients with predominantly mild liver disease and who had not had treatment were included. There was worsening of the fibrosis in 33% by at least one

point in the Ishak score and 10.7% progressed by more than two points. The major criticisms of this type of study is whether it is possible to detect and quantify small changes in the stage of fibrosis accurately enough over this short time interval, with the discrete and bounded nature of the fibrosis score? Use of a single pathologist helps to decrease interobserver variability but there were no measures of intraobserver variability in these studies. This is important if the median change is small. Another major concern is whether selection was involved in choosing those that underwent the initial biopsy and did all those that had a first biopsy have a second or were they again selected? This could introduce significant bias. In those studies where there is a considerable variation in the time between biopsies suggests the influence of clinical decisions rather than a true prospective study.

1.6.3.2 Variations in the rate of progression over time

Does fibrosis progress in a linear, exponential or episodic manner? The French group plotted mean fibrosis score against patients grouped by duration of infection and produced a linear plot (Poynard et al. 1997). However their subsequent analysis with the larger cohort and a different statistical method, suggested four distinct phases of linear fibrosis (Poynard et al. 2001). During the first 10 years from infection there was little if any progression (except in those infected after the age of 50 years old). The second period lasted approximately 15 years with a slow regular progression of fibrosis. The third period lasted approximately 10 years, with an intermediate rate of progression. The final period was the last five years, which was associated with the most rapid progression. This finding would certainly be in keeping with an older age at infection hastening fibrosis progression. The authors themselves however do recommend caution in their analysis of the data, as those that have had the infection

for between 20 and 40 years make up a small proportion of the total cohort. Interpretation also depends on the fibrosis staging score itself being a linear scale, but as yet it is unclear how much collagen has to be laid down to progress from one stage to another. We will only get answers to this question when a technique is developed which can quantify this accurately.

1.6.3.3 Established major predictors of progression to cirrhosis

1.6.3.3.1 Alcohol

As alcohol can cause cirrhosis alone it has been important to establish if its effect in patients with HCV is purely additive or synergistic. An Italian study established the interaction between Lifetime daily alcohol intake (LDAI) and HCV showed an additive structure for LDAI below 50g/day and a multiplicative structure for consumption greater than 125g/day (Corrao & Arico 1998). A more recent study based on 800 patients that had detailed LDAI recorded and had had liver biopsies for assessment of HCV infection report conflicting results. On univariate analysis heavy alcohol intake (>50g/day) was associated with an increase in mean fibrosis but was not seen in light or moderate alcohol use (Monto et al. 2004). For each category of alcohol intake, a spectrum of fibrosis was observed, suggesting that other factors are also influencing fibrosis. In this study there was no correlation between alcohol intake and inflammation or ALT. Alcohol did not display an independent association with fibrosis in multivariate models in this study, although the statistical model chosen to perform the analysis could be criticised for being inappropriate. Another study demonstrated that both activity and fibrosis gradually increase according to the amount of alcohol ingested, and that even moderate alcohol consumption, as low as 31-50g/day in men and 21-50g/day in women, may aggravate histological lesions in

patients with chronic hepatitis C (Hezode et al. 2003). This was also seen in a paired biopsy study looking at fibrosis progression with moderate alcohol intake (less than 40g/day) (Westin et al. 2002), however it was not seen to have an effect in other similar studies (Wright et al. 2003), (Ryder et al. 2004).

1.6.3.3.2 Older age at infection

An older age at infection does appear to lead to a faster fibrosis progression (Poynard et al. 1997). In paired biopsy studies this has also been seen (Wright et al. 2003), or instead the age at first biopsy appears to have more influence (Ryder et al. 2004). This may be due to the age of the liver rather than the age of the patient, as it appears that HCV progresses faster in a transplanted liver from an older donor (Berenguer et al. 2002).

1.6.3.3.3 Co-infection with Human Immunodeficiency Virus

HIV co-infection with HCV is not unusual. HCV related cirrhosis is now becoming the major cause of mortality in HIV cohorts (Jaggy et al. 2003), (Salmon-Ceron et al. 2005). Although a study in a US urban cohort contradicts this (Sulkowski et al. 2002). In a retrospective cross-sectional study the median time from infection to cirrhosis was 6.9 years and 23.2 years, in HIV antibody positive and negative patients (Pol et al. 1998). There is a meta-analysis to quantify the effect of HIV coinfection on progressive liver disease in persons with HCV. Eight studies were identified that included outcomes of histological cirrhosis and they had a pooled relative risk of 2.07 (95% CI, 1.40-3.07) (Graham et al. 2001). The Trent group in another paired biopsy study has found that HIV infection accelerates the rate of fibrosis progression by 1.4 fold, and the development of advanced fibrosis threefold

(Mohsen et al. 2003). A low CD4 cell count was independently associated with advanced disease and correlated with higher histological index, which suggests that early antiretroviral therapy may be of benefit in slowing HCV progression in co-infected patients. In another single-centred, cross-sectional study the length of time on HAART was inversely related to the stage of fibrosis (Tural et al. 2003). Reassuringly protease inhibitor therapy does not appear to accelerate progression to HCV-related cirrhosis (Benhamou et al. 2001).

1.6.3.3.4 Hepatic steatosis

As mentioned in the pathology section steatosis of the liver is common in HCV infection. Naturally researchers have tried to discover whether its presence affects progression of the disease. Fibrosis on biopsy was found to correlate significantly with body mass index and steatosis (Hourigan et al. 1999). In a paired biopsy study, worsening of steatosis was observed in 34% of patients, stability in 50%, and improvement in 16%. Worsening of steatosis but not worsening histological activity, was significantly associated with hepatic fibrosis progression in patients with or without steatosis at diagnosis (Castera et al. 2003). In a similar study progression of fibrosis occurred in 16% of patients after a median delay of 65 months. Cumulative probabilities of the progression of fibrosis at 4 and 6 years were 5.2% and 19.8%, respectively. In multivariate analysis, steatosis was the only independent factor predictive of progression of fibrosis (Relative Risk, 4.8; CI, 1.3-18.3). Probability of progression of fibrosis was significantly related to the percentage of hepatocytes with steatosis. This observation was independent of alcohol intake, genotype and insulin resistence (Fartoux et al. 2005a). Other studies have found an association between insulin resistence and fibrosis (Hui et al. 2003b).

1.6.3.4 Postulated / minor predictors of progression to cirrhosis

1.6.3.4.1 Co-infection with Hepatitis B virus

Continuing or past HBV co-infection is common in HCV infected patients. There is an increased risk of cirrhosis in co-infected patients that are HBsAg positive and HBV Deoxyribonucleic acid (DNA) positive compared with HBsAg negative HCV patients (Zarski et al. 1998). In HBsAg positive co-infected patients those with HBV DNA detectable in the serum have higher inflammation and fibrosis scores on biopsy compared with those that do not, despite having a lower level of HCV viraemia. Another study confirmed the lower levels of both HCV RNA and the HBV DNA in co-infection compared with single infections (Jardi et al. 2001). In Hepatitis B core antibody (HBcAb) positive but HBsAg negative patients, in 46% of patients HBV DNA was present in the liver and this correlated with increased risk of cirrhosis compared with those in whom it was not detected (Cacciola et al. 1999). This occult co-infection also been observed in haemodialysis patients and the DNA found represents a YMDD mutant in 50% of cases (Besisik et al. 2003). However there appears to be no difference in progression to advanced fibrosis between Anti-HBc antibody positive and negative patients (Myers et al. 2003).

1.6.3.4.2 Sex of patient

Retrospective studies have reported that men infected with HCV are more likely to progress to advanced fibrosis than women (Poynard et al. 1997). While in the paired biopsy studies it has been demonstrated in some (Wright et al. 2003), but not others (Zarski et al. 2003), (Ryder et al. 2004).

1.6.3.4.3 Activity of hepatitis on biopsy

The large retrospective studies have not established a relationship with the necroinflammatory score (Poynard et al. 2001), although smaller ones have suggested a link (Monto et al. 2004), (Asselah et al. 2003). The paired biopsy studies are similarly divided between those that have shown a relationship (Ryder et al. 2004), and those that have not (Zarski et al. 2003).

1.6.3.4.4 Iatrogenic immunosuppression

In view of the findings in HIV coinfected patients it has been suggested that iatrogenic immunosuppression may hasten progression. However in a paired biopsy study (37 months apart) comparing renal transplant recipients and with a matched group of controls without renal disease, fibrosis progression was slower and the final stage was lower in the transplant group (Alric et al. 2002).

1.6.3.4.5 Major Histocompatibility Complex alleles

The individual's MHC alleles do affect the outcome of the infection, although as with viral clearance, the positive predictive value of the presence or absence of a particular allele in predicting progression is very low (Cramp et al. 1998), (McKiernan et al. 2000).

1.6.3.4.6 Haemochromatosis mutations

The role of coexisting Haemochromatosis (HFE) mutations remains controversial. In one study the presence of HFE mutations was independently associated with iron loading (in serum and liver) and advanced fibrosis in patients with compensated liver disease from chronic hepatitis C, especially after controlling for duration of disease (Tung et al. 2003). They suggest that HFE mutations accelerate hepatic fibrosis in

hepatitis C but may not be responsible for progression to end-stage liver disease. Another study has shown increased liver fibrosis and cirrhosis in heterozygotes for the common mutations compared with those that do not have the mutations (Erhardt et al. 2003). However a study performed in Glasgow concluded that although patients with chronic HCV infection frequently have elevated serum iron markers, elevated liver iron concentrations are uncommon in their cohort (Thorburn et al. 2002). They felt the mutations do not have a role in the accumulation of iron or the progression of liver disease in HCV infection.

1.6.3.4.7 Factor V Leiden polymorphism:

In a single study possession of the factor V Leiden polymorphism significantly increased the risk of rapid disease progression in HCV (Wright et al. 2003), but this has yet to be confirmed by other groups.

1.6.3.4.8 Smoking

Smoking has been correlated with increased histological activity but not stage of fibrosis and appears to be independent of the effect of alcohol (Hezode et al. 2003), (Pessione et al. 2001).

1.6.3.4.9 Patients with persistently normal ALTs

Some patients with HCV antibody have a persistently normal ALTs (PNALT). If followed-up, 21% of the patients had a slight increase in serum ALT levels, but histological lesions remained stable (Martinot-Peignoux et al. 2001). The presence of a PNALT however does not exclude the presence of significant liver disease (Puoti et al. 2002). In a paired biopsy study those with a PNALT with an initial fibrosis of F0 or F1 were less likely to develop progression of fibrosis than those with an elevated

ALT, although patients with PNALT may have histologically and clinically progressive disease (Hui et al. 2003a). These patients are also at risk of ALT flares, which can lead to progressive liver disease (Rumi et al. 2002). Hence, extended surveillance of HCV antibody positive patients with consistently normal or minimally elevated ALT values is warranted.

1.6.3.5 Factors that have not been shown to influence progression

In the large cross-sectional studies the genotype of the virus and the absolute level of the viraemia were not shown to be independent predictors of progression in Western populations. Only one paired biopsy study has suggested that a non-1 genotype, may hasten the rate of fibrosis (Wright et al. 2003).

1.6.4 The rate of progression to the complications of cirrhosis

The previous studies concentrate on the length of time it takes to progress to cirrhosis, however arguably the most important end-points are liver decompensation, development of HCC, liver failure and death or transplantation. There have been a number of studies describing the natural history of a large cohort of compensated cirrhotics with HCV. One followed 112 patients for a median of 4.5 years but included 49 patients who were treated with IFN. The cumulative probabilities for decompensation and development of HCC were 22.2% and 10.1% in five years, with an estimated yearly incidence of 4.4% and 2.0% respectively (Hu & Tong 1999). The cumulative survival probability was 82.8% from entry and 51.1% from decompensation in five years. Independent variables predicting decompensation were albumin level and older age at infection. As albumin is a known marker for the severity of cirrhosis, its influence may simply have been due to this (those with lower albumins had more advanced cirrhosis at recruitment for the study and required less

progression to get reach decompensation). The second study followed 103 patients for a median of 3.4 years (Serfaty et al. 1998). Fifty-nine patients were treated with IFN. The cumulative probabilities for decompensation and development of HCC were 20% and 11.5% (with an annual incidence of 3.3%) respectively at four years. Absence of treatment with IFN was the only independent variable predictive of progression to these two end-points. As there would have been selection criteria for those that received IFN, this study is deeply flawed. In another study 255 biopsy-proven cirrhotics, with no complications of cirrhosis and no other potential causes of chronic liver disease were followed for a median period of 7 years (Fattovich et al. 2001). Genotype 1b was present in 69%. Kaplan-Meier 5-year risk of HCC was 6% and 4% for patients infected by type 1b and non-1b, respectively (P=0.8); for decompensation 18% and 7% (P=0.0009) and for event-free survival 79% and 89% (P=0.09), respectively. Finally in an Italian study of 254 uncomplicated compensated cirrhotic patients (biopsy-proven and clinically determined) that were followed-up for a median of 91 months, 30.7% developed at least one complication of cirrhosis. HCC developed in 20.5% and was the commonest first complication. Ascites developed in 17.7% and 4.7% had a variceal bleed. Progression of Child's stage was seen in 18.1% and 16.3% died a liver-related death (Benvegnu et al. 2004). During follow-up 45.3% received IFN based therapy and a lower rate of complications was seen in this group (25.2% versus 35.9%).

The longest running and largest retrospective-prospective study looking at mortality has been performed by the National Heart, Lung and Blood Institute (Seeff et al. 1992). Five hundred and sixty-eight patients who received a transfusion at time of cardiac surgery and subsequently developed a post-transfusion hepatitis were

recruited between 1968 and 1980. Two hundred and eight patients had samples available that retrospectively confirmed them to be HCV antibody positive. These were compared with a control group of 379 patients who also underwent cardiac surgery and received a transfusion but did not develop hepatitis and were HCV antibody negative. At a mean of 18 years from the time of transfusion all-cause mortality in the HCV group was 49% while 54% in the matched controls. This difference was not significant. At approximately 25 years of follow-up, the All-cause mortality was 67% among the hepatitis C-related cases and 65% among the controls (P=Non-significant) (Seeff et al. 2001). Liver-related mortality was 4.1% and 1.3%, respectively (P=0.05). Follow-up of the 90 alive HCV cases revealed viraemia with chronic hepatitis in 38%, viraemia without chronic hepatitis in 39%, anti-HCV without viraemia in 17%, and no residual HCV markers in 7%. Clinically evident liver disease was observed in 86% with cirrhosis, but in only 23% with chronic hepatitis alone. A major criticism of the study is the high mortality in both groups, due the medical problems and age of the cohort, and that this may mask small differences in early mortality due to HCV. However due to the age of the cohort it would have been expected there would have been faster progression.

In the UK there is an ongoing study organized at the Public Health Laboratory Service in England looking at 924 transfusion recipients infected with the HCV traced during the HCV lookback programme (Harris et al. 2002). These have been matched to 475 transfusion recipients who tested negative for antibodies to HCV (controls). After 10 years of infection the All cause mortality was not significantly different between patients and controls. Patients were more likely to be certified with a death related to liver disease than were controls, but although the risk of death

directly from liver disease was higher in patients than controls this difference was not significant. Forty per cent of the patients who died directly from liver disease were known to have consumed excess alcohol. Clinical follow up of 826 patients showed that liver function was abnormal in 307 (37.2%), and 115 (13.9%) reported physical signs or symptoms of liver disease.

In the Irish Anti-D cohort study no patient had developed liver decompensation or HCC at 17 years (Kenny-Walsh 1999). In the German Anti-D cohort study only 0.35% had developed liver decompensation and had died of this and one HCC had been diagnosed at 25 years (Wiese et al. 2005). In the American community based study at mean duration of 23 years, 2.4% had developed a complication of cirrhosis but no HCC had been diagnosed. In a multivariate model, the risk of a complication of cirrhosis was higher for persons aged 38 years or older at enrollment (adjusted relative incidence, 3.67; 95% CI, 1.96-6.88) and those that reported ingestion of more than 260 g of alcohol per week (adjusted relative incidence, 3.60; 95% CI, 1.73-7.52) (Thomas et al. 2000). In the Australian acute hepatitis study at a mean duration of infection of 23 years, 6% had a complication of cirrhosis but again no HCC had been diagnosed (Rodger et al. 2000).

1.6.4.1 Predictors of progression to the complications of cirrhosis

As can be seen from the studies above, the variables that predict progression to complications of cirrhosis are not as clearly defined as those that predict the progression to cirrhosis. It is likely, however that there will be common factors.

In a case controlled study in the US, a synergistic rather than an additive interaction were observed between heavy alcohol consumption and chronic hepatitis virus infection and diabetes mellitus in those that developed HCC (Hassan et al. 2002).

Obesity does not appear to be an additional risk factor for HCC in HCV (Nair et al. 2002). Porphyria Cutanea Tarda (PCT) may increase the risk of a patient developing an HCC (Fracanzani et al. 2001).

1.6.4.2 Symptoms of chronic HCV infection

The symptoms related to chronic HCV infection are very non-specific. In a study of these symptoms at presentation of 1614 patients with HCV infection, fatigue was present in 53% of patients (Poynard et al. 2002a). In 17% of patients fatigue was severe and impairing activity. Five other symptoms had a prevalence above 10% including, in decreasing order: arthralgia, paresthesia, myalgia, pruritus, and sicca syndrome. The presence of fatigue, was associated with female gender, age over 50 years, cirrhosis, depression and purpura. The prevalence of fibromyalgia (as defined by the association of fatigue with arthralgia or myalgia) was 19%. There was no significant association between fatigue and the following characteristics: viral load or genotype, alcohol consumption, abnormal thyroid function, and type and level of cryoglobulinaemia. One study found that fatigue was associated with high circulating leptin levels even after correction for fat mass (Piche et al. 2002).

Patients with HCV infection have been shown to have slight but significant neurocognitive impairment even before the onset of cirrhosis (Kramer et al. 2002), (Hilsabeck et al. 2002). Using proton magnetic-resonance spectroscopy, elevations in basal ganglia and white matter choline/creatine ratios can be demonstrated in patients with histologically-mild hepatitis C, compared with healthy volunteers and patients with hepatitis B. This elevation is unrelated to hepatic encephalopathy or a history of intravenous drug abuse or by depression, or fatigue (Forton et al. 2001), (Forton et al. 2002).

It has been shown clearly that chronic HCV infection with or without cirrhosis causes a reduction in the usual measures of quality of life (Foster et al. 1998), (McHutchison et al. 2001b). This reduction can occur in the absence of major cognitive impairment (Cordoba et al. 2003).

In early cirrhosis the only symptoms experienced can be the ones mentioned above. However with the onset of portal hypertension and liver failure new symptoms can occur such as abdominal distension with ascites, peripheral oedema with low serum albumin, vomiting blood with oesophageal varices or portal hypertensive gastropathy, excessive drowsiness and day-night reversal with encephalopathy or right upper quadrant pain with HCC.

1.6.4.3 Extra hepatic manifestations

As well as the neuropsychiatric complications that may lead to the non-specific symptoms mentioned above, there are also some specific extra hepatic disease associations with chronic HCV infection. In a study of male veterans in the US, significant associations were found between HCV infection and PCT, lichen planus, vitiligo, cryoglobulinaemia, membranoproliferative glomerulonephritis, and Non-Hodgkin's Lymphoma (El Serag et al. 2002).

Approximately 40% of patients with chronic HCV infection develop detectable serum cryoglobulins or cryoprecipitates, although most do not show clinical or physical signs of syndromic cryoglobulinaemia. These are commoner in cirrhotic patients but causality is unknown (Kayali et al. 2002).

1.7 Treatment of Hepatitis C

In the last 10 years considerable progress has been made in the management of chronic HCV infection. The objectives of treatment are (a) prevention of the

occurrence of cirrhosis and its complications (b) reduce the extrahepatic manifestations (c) prevent transmission of virus to other people. The history of treatment started before discovery of the virus with the use of IFN monotherapy to treat Non-A, Non-B hepatitis (Hoofnagle et al. 1986). This was continued with the discovery of the virus but markedly improved with the introduction of ribavirin to the regime. The most recent advance is the change to Pegylated interferon (PEG-IFN) with ribavirin and this is now the established treatment of choice in the UK (Booth et al. 2001), (National Institute for Clinical Excellence 2004) and worldwide (National Institutes of Health 2002).

1.7.1 Interferon monotherapy

Interferon monotherapy was the first treatment tried and although the initial trials demonstrated promising efficacy, ultimately this was not borne out in clinical practice. Over 50 randomised controlled trials (RCTs) were performed comparing it with placebo but usually with small numbers of participants. However there are two good meta-analysis' that have been performed (Thevenot et al. 2001), (Myers et al. 2002).

1.7.1.1 Pharmacology

IFNs are naturally occurring proteins secreted by many mammalian cells (Isaacs & Lindenmann 1957). Alpha IFN, a type 1 IFN actually represents a family of more than 20 subspecies of proteins and glycoproteins. The two main forms that have been thoroughly assessed are IFN alpha-2b and IFN alpha-2a. Both are produced by recombinant DNA techniques using a strain of *Escherichia coli* bearing a genetically engineered plasmid containing an IFN alpha-2b or IFN alpha-2a gene from human leucocytes. They differ from each other by a single amino acid in the peptide

sequence (Emanuel & Pestka 1993). It is usually administered by subcutaneous injection. Maximum serum levels occur 3 to 12 hours after injection and serum levels are usually below the limit of detection by 16 hours (Wills 1990). The elimination half-life of IFN is 2 to 3 hours and clearance is largely by renal catabolism.

1.7.1.2 Mode of action

As previously described IFN alpha binds a common receptor and activates over a hundred Interferon stimulated genes (ISG) via the Jak-STAT pathway (Sarcar et al. 2004). These ISG products impart regulatory functions that limit HCV replication through processes that include disruption of viral RNA translation and inhibition of antigenomic strand RNA synthesis via 2',5'-oligoadenylate synthetase (Wang et al. 2003). Other ISGs are involved in lipid metabolism, apoptosis, protein degradation and inflammatory cell responses (DE Veer et al. 2001). Type 1 IFNs promote memory T-cell proliferation, prevent T-cell apoptosis and stimulate natural –killer cell activation and dendritic-cell maturation (Tilg 1997). IFN also upregulates the production of MHC class-I and class-II peptides and might promote a T-helper-1 over a T-helper-2 phenotype (Lechner et al. 2000). The methods by which the virus evades these actions have been previously described in the immunology section.

1.7.1.3 Efficacy

1.7.1.3.1 Duration of treatment

In the Cochrane review on IFN naïve patients, the meta-analysis revealed a significant difference in Sustained viral response (SVR) between those treated thrice weekly dosage of 3 MU for 12 or more months compared with 6 months (14% (95% CI 11 to 19%) and 7% (95% CI 5 to 11%) (Myers et al. 2002).

1.7.1.3.2 Dose

In the Cochrane review SVR for thrice weekly 6 MU versus 3 MU for at least 12 months was 43% (95% CI 32 to 55%) and 25% (95% CI 17 to 37%) respectively (Myers et al. 2002).

1.7.1.3.3 Genotype

Analysis of the IFN monotherapy trials reveals a significant difference between the SVR for genotype 1 infection (18.1%) and the non-genotype 1 infections (54.9%) (Davis & Lau 1997).

1.7.1.3.4 Cirrhotic patients

SVR were seen in 17% of treated patients with varying regimes (Myers et al. 2002).

1.7.1.3.5 HIV co-infection

There were no large RCTs in patients with co-infection with HIV, however smaller studies suggested similar efficacy as long as the CD4 count remained preserved (Mauss et al. 1998).

1.7.1.3.6 Other predictors of response

A younger age at infection and a lower viral load were also found to be predictive of a SVR (Chemello et al. 1995).

1.7.1.4 Assessment of response

Retrospective analysis of monotherapy trials suggested that non-response to IFN monotherapy could be predicted at 12 weeks of treatment by the ongoing presence on HCV RNA on PCR testing (McHutchison et al. 1998a). Treatment could be

discontinued in these patients. However the variability in the sensitivity of the PCR assay at the time of these studies made interpretation of these data difficult.

1.7.1.5 Long-term outcome

The long-term outcome of patients who are sustained responders to IFN monotherapy has been documented. The proportion that remains HCV RNA PCR negative has ranged from 96% at a mean follow-up of 4 years to 92% (Marcellin et al. 1997) at a mean of 5.4 years (Reichard et al. 1999).

There is evidence that treatment with IFN monotherapy irrespective of whether a SVR is achieved reduces the risk of decompensation and HCC. In a study of a total of 286 sustained virological responders, the rate of decompensation after five years of follow up was 1.0% (95% CI 0.0-2.3) and none developed HCC. Survival was comparable with the general population, matched for age and sex, the standard mortality ratio being 1.4 (95% CI 0.3-2.5) (Veldt et al. 2004). A meta-analysis of all the IFN monotherapy trials in cirrhotic patients suggests a slight preventative effect on HCC development but the magnitude is probably low and may be spurious due to confounding influences (Camma et al. 2001). There is also a large retrospective cohort study of 2889 patients with histological-proven chronic HCV (2430 patients received IFN therapy, and 459 patients were untreated). It demonstrated that the risk of death was reduced, compared with untreated patients, among IFN-treated patients (risk ratio for overall death: 0.37; CI: 0.24-0.60; for liver-related death: 0.28; CI: 0.16-0.49) and among sustained responders (risk ratios for overall death: 0.15; CI: 0.06-0.34 and for liver-related death 0.05; CI: 0.01-0.22). The risk of liver-unrelated deaths remained unchanged (Yoshida et al. 2002).

1.7.1.6 Adverse events

A wide array of side effects has been described with IFN treatment. The prevalences quoted are for 3 MU three times a week for six months and based on a meta-analysis of the monotherapy trials (Poynard et al. 1996). The early flu-like side effects are common (41%) and predictable. These include fever, fatigue, headache, myalgias and arthralgia. Although problematic they do not usually necessitate dose modification. Neuropsychiatric side effects such as depression (7%) and irritability can also occur. Dermatological adverse events include alopecia (16%) and worsening of psoriasis. The common laboratory abnormalities are due to the myelosuppressive actions of IFN. These include neutropenia (9%), thrombocytopenia (8%) and anaemia. They are usually mild and well tolerated but may necessitate dose reductions or discontinuation. IFN can precipitate autoimmune phenomenon such as thyroiditis (2%) leading to hypo- or hyperthyroidism. Severe and even life-threatening idiosyncratic side effects occur in 0.1% to 1% of patients; these include hepatic decompensation, retinopathy, acute renal failure, suicidal ideation and dilated cardiomyopathy and pulmonary interstitial fibrosis. These may be irreversible. Overall the rate of dose reductions is 9% and discontinuation is 4% for 3 MU three times a week. Higher doses of IFN cause higher rates of adverse events than standard doses (Poynard et al. 1996). The contraindications of therapy are based on the known side effects and are relative.

1.7.2 Combination standard interferon and ribavirin

Two large randomised controlled trials established that combination IFN and ribavirin was an effective treatment for chronic HCV infection in IFN-naive patients (McHutchison et al. 1998b), (Poynard et al. 1998). The standard regime was IFN

alpha 3MU three times a week and ribavirin in divided doses of 800 to 1,200mg/day depending on the weight of the patient.

1.7.2.1 Pharmacology of ribavirin

Ribavirin is a water-soluble nucleoside analogue of guanosine with activity against DNA and RNA viruses (Sidwell et al. 1972). It is well absorbed orally and cleared by the kidney.

1.7.2.2 Mode of action of ribavirin

Ribavirin monotherapy was associated with improvements in serum ALT but viral RNA levels long term did not change (Dusheiko et al. 1996). The addition to IFN alpha treatment however led to an improvement in the SVR rates by reducing the relapse rate at the end of treatment (McHutchison et al. 1998b). When combination is compared with IFN alpha monotherapy, there appears to be no effect on the first and second phases of viral kinetics but it does reduce the rebound in viral levels seen before the second dose of IFN and this was dependent on the concentration of ribavirin (Pawlotsky et al. 2004).

Ribavirin is phosphorylated intracellularly to form a triphosphate. If this is misincorporated by the HCV RNA polymerase, it can lead to early chain termination and inhibition of replication. However, this appears to only occur at very high concentrations (Maag et al. 2001). Ribavirin monophosphate is also a competitive inhibitor of inosine monophosphate dehydrogenase, which leads to depletion of the GTP necessary for viral RNA synthesis (Lau et al. 2002). Mutagenesis and error catastrophe has been suggested as another mechanism of action. There is some evidence of that as it increases the mutation rate and thereby decreases replicative fitness of the virus, making it more susceptible to the actions of IFN alpha (Dixit et

al. 2004). However this is disputed by some researchers (Pawlotsky et al. 2004). Several studies have suggested that ribavirin can alter the T-helper-1 / T-helper-2 balance, favouring the former and therefore increasing the chance of viral clearance (Cramp et al. 2000). The mechanism by which this occurs is not known.

1.7.2.3 Efficacy

1.7.2.3.1 Duration of treatment and genotype

Treatment of genotype 1 patients for 24 weeks and for 48 weeks produces a SVR of between 16-18% and 28-31% respectively. For genotype 2 or 3 patients it is 64-69% and 64-66% respectively (McHutchison et al. 1998b), (Poynard et al. 1998). Therefore patients infected a genotype 2 or 3 virus require only six months of treatment, while those with a genotype 1 virus that have a negative serum HCV RNA PCR test at six months do benefit from a further six months of treatment (McHutchison et al. 1998b), (Poynard et al. 1998). These SVRs are significantly better than those achieved by standard IFN monotherapy.

1.7.2.3.2 Cirrhotic patients

Due to the small number of cirrhotics in the RCTs comparing standard IFN and ribavirin with IFN monotherapy, little can be concluded except that a higher stage of fibrosis did reduce SVR and SVR was better in those patients treated with combination treatment (McHutchison et al. 1998b), (Poynard et al. 1998). In the RCTs comparing it with PEG-IFN and ribavirin, the SVR with standard IFN and ribavirin in cirrhotics was 33%.

1.7.2.3.3 HIV coinfection

The overall SVR for standard IFN alpha-2a and ribavirin for 48 weeks in co-infected patients is 8% for genotype 1 and 27% for genotype 2 or 3 (Torriani et al. 2004).

1.7.2.3.4 Other predictors of response

In both the 6 month and 12 month treatment subgroup binary logistical regression identified that genotype 3 infection, a low degree of fibrosis at baseline, female sex, baseline viral load of $2x10^6$ copies per millilitre or less as independently predictive of a SVR (McHutchison et al. 1998b), (Poynard et al. 1998). The European randomised controlled trial also identified young age as an independent predictor of response (Poynard et al. 1998).

1.7.2.4 Assessment of response

Subsequent analysis of the main RCTs has shown that a negative serum HCV PCR test at 6 months of treatment, is the earliest time response can be assessed to ensure that no eventual sustained responders are discontinued too early (McHutchison et al. 2001a).

1.7.2.5 Adverse events

Flu-like side effects predominate with fatigue occurring in 70% but are no more frequent than in IFN monotherapy. Ribavirin is known to concentrated in red blood cells, causing haemolysis and a mean drop of 3g/dl in haemoglobin in the first 4 weeks of treatment (Poynard et al. 1998). The addition of ribavirin increases the prevalence of itch and rashes compared with IFN monotherapy (20% versus 9%) (McHutchison et al. 1998b), (Poynard et al. 1998). Dose reduction occurred in 13%-17% of patients, with anaemia triggering this in 7-9% of patients. Overall

discontinuation of therapy due to a severe adverse event occurred in 8% for 24 weeks of treatment and 19-21% for 48 weeks of treatment was seen in the trials with depression being the commonest cause (McHutchison et al. 1998b), (Poynard et al. 1998).

As ribavirin is teratogenic in animal studies, pregnancy is an absolute contraindication to treatment. Due to the risk of anaemia with the treatment end-stage renal failure, severe anaemia and haemoglobinopathies should only be treated with PEG-IFN monotherapy. For the same reason a history of ischaemic heart disease and cerebrovascular disease is a relative contraindication.

1.7.2.6 Long-term outcome

It has been assumed that the persistence of the SVR following combination treatment will be equivalent to that of IFN monotherapy. The only data so far reported, followed up 25 sustained responders of which 94% remained HCV RNA PCR negative at a mean of 9.4 months (Barnes et al. 1999).

1.7.3 Pegylated Interferon monotherapy

1.7.3.1 Pharmacology

Standard IFN can be modified by the addition of a polyethylene glycol moiety. This pegylation achieves a slower rate of clearance and a longer half-life and therefore more stable levels in the blood and a weekly dosing regime. There are currently two PEG-IFNs, PEG-IFN alpha 2a (covalently bonded to a branched 40KD molecule) and PEG-IFN alpha 2b (non-convalently bonded to a straight 12KD molecule). They have distinct pharmacokinetics and pharmacodynamics (Wang et al. 2000). The PEG-IFN alpha 2a has a smaller volume of distribution and a longer half-life but is less bioavailable. For PEG-IFN alpha 2b the maximum serum levels occur 48 to 72

hours after injection. The elimination half-life of IFN is approximately 40 hours and clearance is only 30% by renal catabolism. The remaining clearance may be affected by binding to IFN receptors and non-specific hepatic metabolism (Glue et al. 2000). The recommended dosing regimes differ in that PEG-IFN alpha 2a is a fixed dose of 180μg once a week and PEG-IFN alpha 2b is 1.0 μg/kg once a week.

1.7.3.2 Efficacy

1.7.3.2.1 Duration of treatment and genotype

The main RCTs comparing PEG-IFN alpha 2a monotherapy (180μg) with standard IFN monotherapy for 48 weeks demonstrated SVRs rates of 39-44% versus 8-19% respectively (Zeuzem et al. 2000), (Heathcote et al. 2000).

In the main study that compared PEG-IFN alpha-2b (0.5, 1.0, or 1.5 μg /kg) with standard IFN alpha-2b for 48 weeks demonstrated the SVR was not dose-related above 1.0 μg /kg. For this dose, the SVR in comparison with standard IFN for those with genotype 2 or 3 and a low viral load was 62% versus 36%. For those with genotype 1 and a high viral load it was 8% versus 2% (Lindsay et al. 2001).

1.7.3.2.2 Cirrhotic patients

In patients with cirrhosis or bridging fibrosis, 180 μg of PEG-IFN alpha-2a is significantly more effective than standard IFN alpha-2a (SVR of 30% versus 8%) (Heathcote et al. 2000).

1.7.3.2.3 HIV coinfection

The overall SVR 180 μg of PEG-IFN alpha-2a for 48 weeks in co-infected patients is 31%, but 21% for genotype 1 and 57% for genotype 2 or 3 (Torriani et al. 2004).

1.7.3.2.4 Other predictors of response

Multiple logistical regression has identified that a genotype other than 1, no cirrhosis or bridging fibrosis at baseline, baseline viral load of $2x10^6$ copies per millilitre or less, body-surface area of less than $2m^2$ and pretreatment ALT quotient greater than 3 were independently predictive of a SVR (Zeuzem et al. 2000).

1.7.3.3 Assessment of response

Response can be assessed by a quantitative HCV RNA PCR at 12 weeks which either shows a two log reduction from the start of treatment or that it has become negative (Zeuzem et al. 2000). If this does not occur, treatment can be discontinued, as there is no prospect of achieving a SVR.

1.7.3.4 Adverse events

In the RCTs comparing PEG-IFN monotherapy with standard IFN monotherapy the frequency and severity of the adverse events were similar in both groups and typical of those previously reported for monotherapy. Dose modification due to neutropenia occured in 11% of patients on PEG-IFN compared with 7% on standard IFN but there were no discontinuations. Overall the discontinuation rate was 7% and dose reduction rate 19% over 48 weeks of treatment (Zeuzem et al. 2000).

1.7.3.5 Long-term outcome

There are no studies only looking at the long-term outcome with PEG-IFN monotherapy.

1.7.4 Combination pegylated Interferon and ribavirin

The introduction of PEG-IFN in combination with ribavirin is now the recommend treatment for chronic HCV infection due to improved efficacy (Manns et al. 2001) especially in the less responsive genotype 1 subgroup.

1.7.4.1 Pharmacology

Each PEG-IFN alpha molecule has been combined with ribavirin. It does not appear to affect the pharmacokinetics or pharmacodynamics of the individual molecules. The same dosing regimes used in monotherapy are used in the combination with PEG-IFN alpha 2a. While PEG-IFN alpha 2b is given at 1.5 μg/kg once a week in the combination.

1.7.4.2 Assessment of response

In genotype 1 patients response can be assessed by a quantitative HCV RNA PCR at 12 weeks which either shows a two log reduction from the start of treatment or that it has become negative (Lee et al. 2002). If this does not occur, treatment can be discontinued, as there is no prospect of achieving a SVR.

1.7.4.3 Efficacy

1.7.4.3.1 Duration of treatment and genotype

The RCTs have established that genotype 1 and 4 patients should be treated for 48 weeks and 24 weeks for the other genotypes (Manns et al. 2001), (Fried et al. 2002). More recently there has been evidence that with genotypes 2 or 3 the same SVR rates can be achieved with 12 weeks treatment compared with 24 weeks in those that have an early viral response at four weeks (Mangia et al. 2005)

The regimes described above achieve a SVR rate of 50% in the genotype 1 or 4 and 80% in genotype 2 and 3 irrespective of the type of PEG-IFN used (Manns et al. 2001), (Fried et al. 2002). There are currently no head to head RCTs comparing the efficacy.

1.7.4.3.2 Cirrhotic patients

Patients with bridging fibrosis or compensated cirrhosis do achieve lower SVR rates (50-70% for genotype 2 and 3, and 20-30% for genotype 1) (Manns et al. 2001), (Fried et al. 2002). At present there is little data on the effect of reducing the complications of cirrhosis.

1.7.4.3.3 HIV co-infection

Treatment for 48 weeks achieves a SVR of 60% in genotype 2 and 3 and 14-29% in patients with genotype 1. For patients with genotype 1 infection and low HIV viral load (less than 800,000 IU/ml) the SVRs are higher at around 60% (Chung et al. 2004), (Torriani et al. 2004).

1.7.4.3.4 Other predictors of response

A high HCV viral load, age over 40, male sex and more extensive fibrosis or cirrhosis on histology is associated with lower SVR rates (Manns et al. 2001), (Fried et al. 2002). A meta-analysis of ethnic differences showed that patients of African American or Hispanic origin had lower SVRs than Caucasians or Asian groups. Asian patients have a much better response to treatment than any other ethnic group (Hepburn et al. 2004). A weight greater than 75 kg has been shown to lower SVR in a number of studies (Shepherd et al. 2004). Patients on a stable methadone program have been shown to achieve lower but reasonable SVRs (Genotype 1 or 4; 38% versus 55%; Genotype 2 or 3: 48% versus 57%). More patients drop out in the first eight weeks compared with controls (24% versus 8%) but thereafter compliance is the same (Mauss et al. 2004). Co-infection with HBV has not been shown to affect

SVRs (Liu et al. 2003). A median daily alcohol use >30 g/day has been associated with failure to respond to treatment (Chang et al. 2005).

1.7.4.4 Effect on histology

In the patients that had pre- and post-treatment liver biopsies, 73% had an improvement in their necro-inflammatory score. In only 8% did fibrosis worsen and overall the fibrosis progression rate fell. Reversal of cirrhosis was observed in 49% of patients with baseline cirrhosis (Poynard et al. 2002b). In genotype 3 patients, SVR is associated with disappearance of steatosis on biopsy (Poynard et al. 2003).

1.7.4.5 Effect on extra hepatic manifestations of HCV infection

A study looking at all sustained viral responders following HCV treatment found it was associated with an improvement in fatigue (35% versus 22%) and cryoglobulin (33% versus 6%), but fatigue frequently persists despite a virologic response (Cacoub et al. 2002). Overall health related quality of life has been shown to improve in those with a SVR compared with those that do no achieve this (Hassanein et al. 2004).

1.7.4.6 Adverse events

The adverse event profile of PEG-IFN plus ribavirin and standard IFN plus ribavirin is similar. There are no new or unique adverse events, and there are no differences between the difference forms of PEG-IFN, with the same percentage of severe adverse events requiring discontinuation (9.7% v 9.6%), with depression being the commonest cause (Manns et al. 2001), (Fried et al. 2002). In the trials a further 12.3% withdrew for other reasons. Overall dose reduction occurred in 8.2%, with neutropenia being the commonest cause. It was also commoner with the PEG-IFN combination versus standard IFN combination (3.5% versus 1.6%). There is a

significant increase in injection-site reactions (4.9% versus 3.6%) but this does not limit therapy.

1.7.4.7 Long-term outcome

Long-term data is sparse but it appears that viral relapse is uncommon after SVR. A recent abstract presented at the annual meeting of the American Association for the Study of Liver Diseases showed that 99.2% of patients with a SVR remained HCV RNA PCR negative at five years (Swain et al. 2005).

1.7.4.8 Cost effectiveness

PEG-IFN plus weight-based dosing of ribavirin compared with no antiviral therapy, increased life expectancy by 4.7 years (Siebert et al. 2003). Compared with standard IFN and weight-based dosing of ribavirin, it increases it by 1 year with incremental cost-effectiveness ratios of 6600 euros per Quality-adjusted life year. Subgroup analysis by genotype, viral load, sex and histology demonstrated it to remain cost-effective compared with other well-accepted medical treatments.

Chapter 2 Methods

2.1 Edinburgh cohort studies

2.1.1 Patients

Patients in the Edinburgh cohort were identified by review of outpatient, inpatient, liver biopsy and endoscopy records of the Centre of Liver and Digestive Disorders in the Royal Infirmary of Edinburgh. The diagnosis of HCV was confirmed by retrospective review of the patient's hospital casenotes and hospital laboratory computer records. The specific inclusion and exclusion criteria for each study are described in the methods section of each chapter.

2.1.2 Hepatitis C management database

A Microsoft Access database application was created to enable clinicians to manage, audit practise and collect epidemiological and natural history data on a hospital cohort of patients with HCV infection.

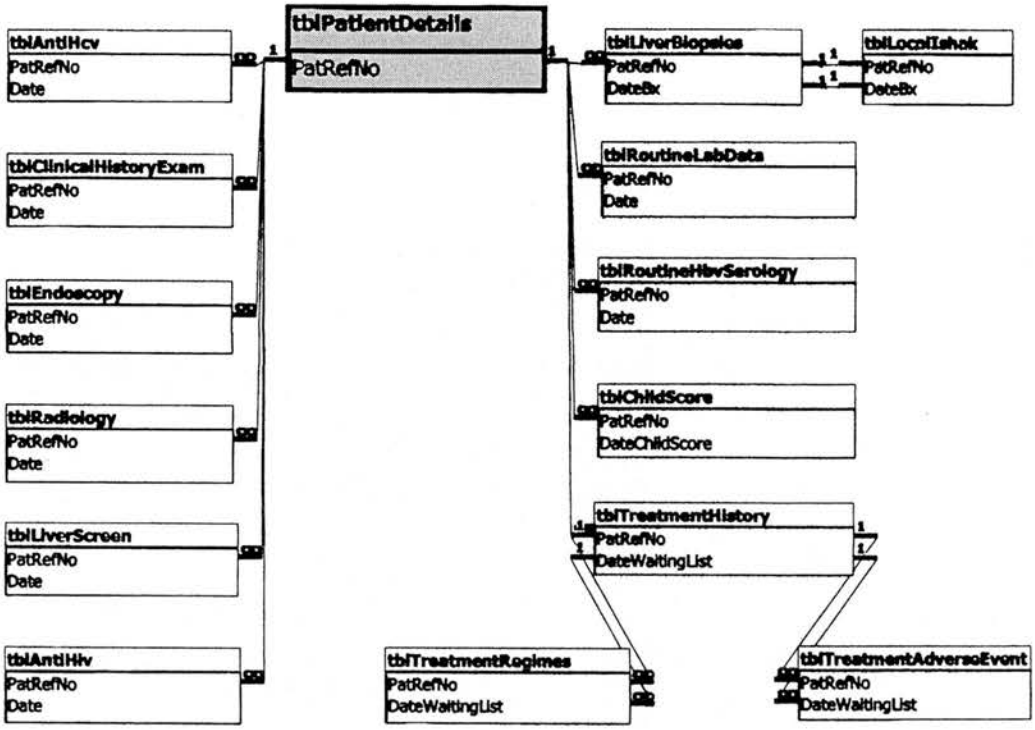
2.1.2.1 Main features

- Providing rapid patient-specific information on all patients. This includes past and present clinical status, investigations and treatments.
- Identifying, tracking, summarising auditing patients undergoing treatment.
- Surveillance management for patients at risk of HCC and varices.
- Provide descriptive statistics on the population.

2.1.2.2 Basic database design

The database application was created using Microsoft Office 2000 developer edition. It is a Microsoft Access relational database based around tables of fields that are related by a patient reference number (PatRefNo). Figure 1 shows the main tables and how they relate to each other.

Figure 1 Relationships of the database tables



'tblPatentDetails' is the key table and contains a single record for each patient. In the other tables each patient may have more than one record and therefore its uniqueness is determined by the combination of the PatRefNo and another field such as the date a test took place, if for example the table records blood results.The database user interface was created using database objects called 'Forms'. Forms can display the fields that make up the tables described above. They can be used for data entry or display of data that has already been entered. The field boxes on the forms can also be programmed, using visual basic for applications, to perform different actions depending on the data that has been entered into it. Forms can also have command buttons on them that can be programmed to perform actions, such as opening other forms or modifying data. Queries are another database object that can be used to view, change, and analyze data from one or more tables. Numerous queries have been designed and used as a source of records for the forms, charts and reports of the

database. All these features have been utilised in the database created. A full description of the database structure and features are given in appendix 1.

2.1.3 Data collection

All details on the patients were collected and entered onto the Edinburgh Hepatitis C database by retrospective review of the patient's hospital casenotes, hospital computer laboratory and pathology records and endoscopy records. Once the database was established in the outpatient clinics, where a patient's hospital record review was complete, data such as the clinical findings from the last clinic visit for were added in real time.

2.1.4 Standard patient assessment and follow up protocols

2.1.4.1 Initial assessment

History

Documentation of probable source of infection, probable date of infection (taken as the first year of active intravenous drug use or the first date of exposure to a known risk factor such as blood transfusion), the date of last exposure to a known risk factor for HCV infection, current alcohol intake, previous maximum alcohol intake sustained for greater than 5 years (one unit is equivalent to 9.6g of alcohol), onset of any symptoms suggestive of a complication of cirrhosis, sex and date of birth.

Clinical examination

Detection of the presence or absence of ascites, encephalopathy, peripheral oedema, splenomegally, hepatomegally and jaundice.

Laboratory assessment

- Liver enzymes including ALT.

- Full blood count and Prothrombin time.
- Qualitative HCV RNA reverse transcription by nested PCR using 5' noncoding region specific primers as previously described (Chan et al. 1992) was undertaken.
- Genotyping (HCV RNA reverse transcription by nested PCR using 5' noncoding region specific primers followed by restriction fragment length polymorphism analysis (Simmonds et al. 1994) was not performed on all patients due to changing policies over the period of study.
- Serological markers for HBV and HIV infection were detected with standard assays.
- Other liver diseases were excluded using standard assays.
- Serum Hyaluronic acid was assayed using the Chugai Hyaluronic acid test (Chugai Diagnostics Science CO., produced by REAADS Medical Products Inc., Westminster, Colorado, USA). A threshold of 100ng/ml was used to determine the presence of cirrhosis except when excluded by subsequent investigations. This threshold has been demonstrated to have 92% specificity and 72% sensitivity for diagnosing cirrhosis in patients with HCV infection (Plevris et al. 2000).

Other investigations

- The presence or absence of cirrhosis was determined by diagnostic laparoscopy and histology in the majority up until 2001.
- Ultrasonography of the abdomen was performed in the majority. Ultrasonic diagnosis of cirrhosis was used except when excluded by subsequent investigations.

2.1.4.2 Follow-up assessments

Patients were followed-up at regular outpatient appointments or during inpatient admissions. Documentation of further biopsies, onset of complications of cirrhosis, date of death or transplantation was made.

2.1.4.3 Surveillance

Hepatocellular carcinoma

Surveillance for HCC is carried out in cirrhotic patients with six monthly alpha-fetoproteins and ultrasound scan (USS). HCC is confirmed by Computerised Tomography scanning or laparoscopic ultrasound.

Oesophageal varices

Surveillance for oesophageal varices is carried out at diagnosis of cirrhosis and two yearly in those without varices and six monthly in those that have grade 1 varices. Grade 2 and above were treated prophylactically (Beta-blockers, banding or nitrates). These episodes were recorded on the database.

2.1.5 Treatment

2.1.5.1 Antiviral treatment

After initial assessment a decision was usually made on the suitability for treatment. As there were changes in the available treatment over the study period, the criteria on which this decision was made also varied. Different treatments also had different protocols of follow up and blood test sampling. However, usually the HCV RNA PCR was checked before treatment and at six months after the end of treatment to assess whether the patient was a sustained responder. Some patients also had a PCR checked after 12 weeks of treatment and / or at the end of treatment to assess whether they were a responder.

A full blood count and liver enzymes were checked every two weeks. Adverse events on treatment and what action and its effect were recorded. If the patient discontinued treatment, the reason for this was recorded.

If the patient was not deemed suitable for treatment or the patient refused treatment the reason for this was recorded on the database.

Categories of responder

All patients whilst on treatment were categorised with a responder status.

- **Currently on treatment** if the patient is on treatment.
- **Non-responder** is a patient who has never normalised his ALT or become PCR negative or has not had a 2 log reduction in PCR titre at 12 weeks.
- **Relapser** is either a patient who has normalised his ALT or become PCR negative but has relapsed on treatment necessitating discontinuation of treatment or who has responded up to the time of discontinuing treatment but then relapsed in the six months from discontinuation.
- **End of treatment responder but not 6/12** is a patient who responded up to the time of discontinuing treatment but has not been followed up for six months from the time of discontinuation.
- **Sustained responder** is a patient who was a responder who has remained HCV PCR negative and normal ALT beyond six months from the time of discontinuing treatment.
- **Late relapser** is a sustained responder who has subsequently relapsed.
- **Treatment incomplete** is a patient in whom treatment was discontinued prematurely and the patient has relapsed.

- **Awaiting start of treatment** for those patients currently the waiting list for treatment.

2.1.5.2 Oesophageal variceal bleeds

The practice for treating oesophageal variceal bleeds changed throughout the period of the study. It included variceal band ligation, injection sclerotherapy, use of vasoactive drugs and Transjugular Intrahepatic Porto-Systemic Shunts (TIPSS). Episodes of variceal bleeding and their treatment were recorded.

2.1.5.3 Liver transplantation

Referral for liver transplantation was made on the established criteria for transplantation. These would be persistent liver decompensation, a diagnosis of an HCC that is within the criteria for transplantation or on quality of life issues. The main contraindications for referral were on-going substance abuse (alcohol or drugs) and HIV infection. In 2004 HIV infection was no longer considered a contraindication for assessment.

2.2 Combination interferon and ribavirin treatment in hospital practice in United Kingdom study.

2.2.1 Patients

All centres in the UK that were known to have had patients that were treated with combination viraferon (IFN alpha-2b) and ribavirin from Schering-Plough between 1997 and 2000 were invited to participate in the study. All adult patients were eligible for treatment if they were seropositive for HCV RNA on testing with the PCR. Patients with decompensated cirrhosis, major psychiatric disorders, seizure disorders, cardiovascular disease and those unable to practice contraception were

excluded. All patients that were suitable from the Royal Infirmary of Edinburgh were excluded. These patients were analysed separately and are described under the Edinburgh cohort studies.

2.2.2 Data collection

Those centres that agreed to participate were supplied with a specially designed database application that standardised and facilitated data collection.

Each patient's medical record was analysed for: Preliminary demographic data including risk factors for HCV infection, alcohol history, known genotype and coexisting diseases. Pre-treatment clinical details such as the results of liver biopsy, including a modified Ishak score if available and a Childs-Pugh score. Monthly dosing schedule and PCR results. Adverse events with treatment and whether these triggered dose reduction or withdrawal. All data was anonymised and forwarded to a national database for analysis.

2.3 Statistical analysis

2.3.1 Characteristics of study populations

A description of each study population analysed was made using SPSS and Excel software. Chi-squared test or Fisher's exact test were used where appropriate to establish significant associations between categorical covariates. Pearson's correlation coefficient was used for continuous variables. T tests were performed to establish significant differences in mean of continuous variables for different categorical variables. All P values are two-tailed.

2.3.2 Natural history survival analysis

2.3.2.1 Kaplan-Meier analysis

This was used to describe and illustrate the number of patients progressing to defined end-points over time. The analysis for the time from infection to various end-points was performed using either left truncation of the risk set six months prior to the date of the first appointment or no left truncation. The former method takes account of the fact that those referred to hospital are only a sample of the whole population that have had the infection for a similar period of time. The latter method has been used most frequently in other similar retrospective cohort studies in the literature (Poynard et al. 1997). A median time to the end point was assessed where possible. The log rank test was used to perform univariate analysis to investigate the influence of the covariates above on the defined end-point. This analysis was performed using the statistical package R.

2.3.2.2 Cox (proportional hazards) regression analysis

This was used to construct a model and perform multivariate analysis to investigate the influence of covariates on the defined end-point. Again left truncation of the risk set was performed where appropriate using the method described above. Independent predictor variables (covariates) within the model were identified by a stepwise forward conditional selection method, based on the significance of the score statistic. The regression coefficients (b) for these covariates were calculated. For categorical covariates, Exp(b) is equivalent to the increase in hazard (relative risk) in comparison to the reference category. For continuous covariates, it is the increase in hazard for an increase of 1 in the value of the covariate. This analysis was performed using the statistical package R.

2.3.3 Treatment for Hepatitis C analysis

2.3.3.1 Prediction of Sustained viral response

2.3.3.1.1 Univariate analysis

Chi-squared test or Fisher's exact test were used where appropriate to perform univariate significance testing on categorical covariates. All P values are two-tailed.

2.3.3.1.2 Multivariate analysis

Binary logistic regression analysis was used to perform multivariate significance testing on covariates.

2.3.3.2 Survival analysis on late relapse

Kaplan-Meier analysis was performed to demonstrate the occurrence of late relapse in sustained responders using SPSS software.

Chapter 3 The Royal Infirmary of Edinburgh Hepatitis C cohort

3.1 Introduction

This chapter describes the HCV RNA PCR positive cohort in the Royal Infirmary of Edinburgh. To try and reduce the effect of referral bias to a tertiary centre only those patients that live within an Edinburgh 'EH' postcode were included. The time to referral, the basic demographics of the referred patients, the risk categories for infection, the important cofactors for progression, the length of follow up and the outcome are all analysed. The results of this analysis give indications of the likely future health burden of this cohort and the suitability and likely response to treatment.

3.2 Methods

3.2.1 Patients

Inclusion criteria

- HCV antibody positive.
- Attended Royal Infirmary of Edinburgh between January 1991 and December 2004.

Exclusion criteria

- Tertiary referrals from hospitals outside Edinburgh.
- Home address outwith the EH postcode.

3.3 Results

Of the 1007 patients assessed for HCV infection in the Royal Infirmary and on the database, 794 met the criteria for inclusion into this cohort. Of these, 87 were HCV

RNA PCR negative without treatment. A further 13 had never had an HCV RNA PCR measured, all of which had normal ALT measurements. Therefore there were 694 patients in the cohort that were HCV RNA PCR positive.

The characteristics of this HCV RNA PCR positive cohort are analysed in detail in this chapter. Table 1 gives a summary of the basic population.

Table 1 Population characteristics of HCV RNA PCR positive Royal Infirmary cohort.

	Number of patients (n=694)	Percentage
Men	479	69.0%
Women	215	31.0%
Previous hepatitis B infection	211	30.4%
Anti HIV positive	47	6.8%
Patients biopsied	396	57.1%
Treatment after assessment	273	39.3%
Sustained responder after treatment	92	13.3%

3.3.1 Referral to the Royal Infirmary of Edinburgh

3.3.1.1 Source

The majority of patients were referred from General Practice within Edinburgh (41.1%). Genitary Urinary Medicine (9.1%) and the Blood Transfusion service (6.6%) were the other major sources of referrals (Table 2).

3.3.1.2 Type of referral

The majority of patients were first seen in the Royal Infirmary of Edinburgh as an outpatient (Table 3).

3.3.1.3 Age at referral

The mean age at referral was 39.9 years old with a median of 38.7 years old (Table 4).

Table 2 Source of referrals for HCV RNA PCR positive cohort.

Type of source	Specific source	Number	Percent
General Practice	GP	285	41.1
Other units within Royal Infirmary	A&E	5	0.7
	Combined Assessment	11	1.6
	Cardiology	3	0.4
	Dermatology	4	0.6
	Diabetes	2	0.3
	General Medicine	1	0.1
	Genitary Urinary Medicine	63	9.1
	Gynaecology	1	0.1
	Haemaology	31	4.5
	Renal	3	0.4
	Maternity	1	0.1
	Surgical	1	0.1
Other Hospital units in Edinburgh	Blood Transfusion Service	46	6.6
	Regional Infectious Disease Unit	37	5.3
	West General Hospital	32	4.6
	Royal Edinburgh Hospital	4	0.6
	East General Hospital	3	0.4
	Murrayfield Hospital	3	0.4
	Leith Hospital	1	0.1
	Royal Edinburgh Sick Childrens Hospital	1	0.1
	Roodlands Hospital	1	0.1
	Unknown	146	21.0
External	Transfer from other liver unit due to relocation of patient	9	1.3
	Total	694	100.0

Table 3 Type of referral when patient was first seen in Royal Infirmary of Edinburgh.

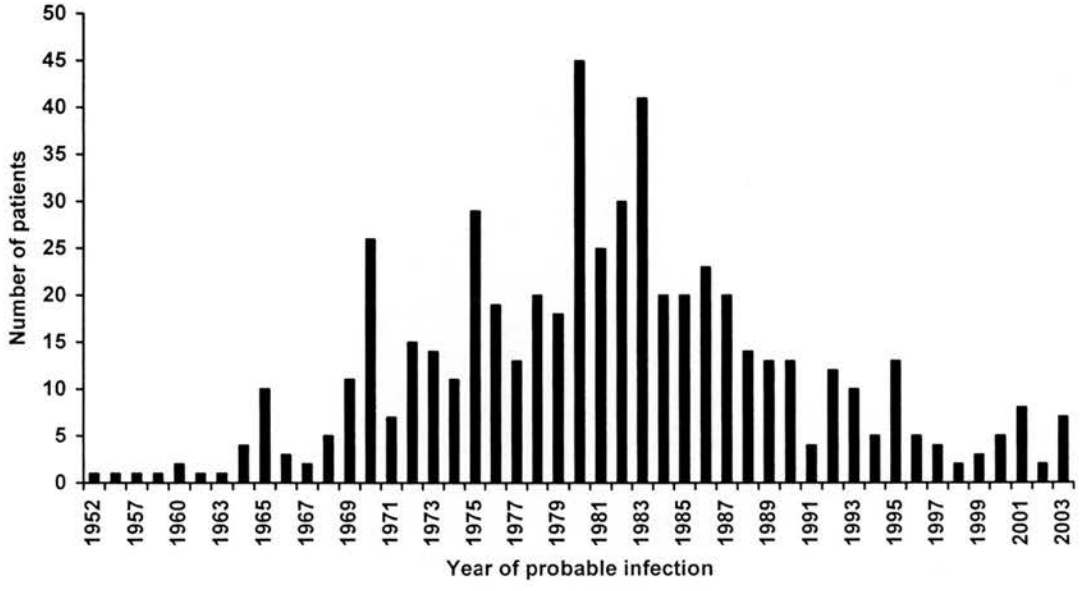
Site of recruitment	Number	Percent
Inpatient	30	4.3
Outpatient	558	80.4
Unknown	106	15.3
Total	694	100.0

3.3.2 Variables recorded at first appointment or first admission

3.3.2.1 Probable date of HCV infection

The date of probable infection could be estimated in 559 patients and is plotted as a histogram in Figure 2.

Figure 2 A histogram of the year of probable HCV infection for all HCV RNA PCR positive.



3.3.2.2 Age at HCV infection

In patients that had a probable date of infection, calculation of the patient's age at infection was possible (Table 4). The mean was 22.1 years, but the patients with an IDU risk category it was 20.3 years compared with 26.4 years for those that did not. The median age at infection is 19.6 years for the cohort and 19.2 years for the patients with an IDU risk category. The minimum infection age in the cohort was 0.1 years (vertical transmission) and the maximum is 70.2 years. The age at infection distribution is positively skewed (1.83 with a standard error of skewedness of 0.10).

Table 4 Descriptive statistics of the continuous variables age at infection, HCV diagnosis and at first appointment or admission, weight, height and Body Mass Index.

	Age at infection	Age at HCV Diagnosis	Age at first appointment	Weight (kg)	Height (m)	BMI
Valid	458	635	694	326	559	317
Missing	236	59	0	368	135	377
Mean	22.07	38.63	39.93	72.67	1.71	24.60
Standard Error of Mean	0.43	0.45	0.42	0.73	0.005	0.25
Median	19.57	37.12	38.72	70.00	1.72	23.81
Standard Deviation	10.07	11.26	11.01	15.63	0.09	4.45
Skewness	1.83	0.88	0.89	0.85	-0.12	1.15
Standard Error of skewness	0.10	0.10	0.09	0.11	0.13	0.14
Minimum	0.01	14.11	14.70	39.00	1.50	15.78
Maximum	70.19	81.36	81.67	147.00	1.93	43.28

kg: kilograms, m: metres, BMI: Body Mass Index

The only significant correlation between the age at HCV infection and the other continuous variables was a negative one with the height of the patient (Table 5).

Table 5 Correlation between age at infection and the continuous variables.

		Weight	Height	BMI
Age at HCV infection	Pearson Correlation	-0.015	-0.135	0.090
	p-value (2-tailed)	0.771	0.022	0.134
	Number	394	286	277

BMI: Body Mass Index

In Table 6a the age at HCV infection is categorised into three groups and cross-tabulated with the patient's sex. Chi-squared testing approaches a significant difference (p=0.082) between male and females in their age at infection distribution. A higher proportion of males (56.0%) being infected at age under 20 years compared with females (46.2%). For the whole HCV PCR positive cohort the modal age group is 'Under 20 years' (52.8%).

Table 6b is a cross-tabulation between the age at infection categories and whether patients have IDU as their risk category for infection. Chi-squared testing reveals a significant difference between those that have IDU as their risk category for infection and those that do not ($p<0.0001$) in their age at infection distributions. Only 5.5% of patients with IDU as their risk category were greater than 30 years at HCV infection compared to 34.6% of those that did not.

Table 6 (a) Age at HCV infection by gender.

			Sex		Total
			Female	Male	
Age at HCV infection	Under 20 years	Number	85	210	295
		%	28.8%	71.2%	
	20 to 30 years	Number	68	118	186
		%	36.6%	63.4%	
	More than 30 years	Number	31	47	78
		%	39.7%	60.3%	
Total		Number	184	375	559
		%	32.7%	67.1%	

Pearson Chi-square (2df) =5.01, p-value =0.082

(b) Age at HCV infection by IDU risk category patients.

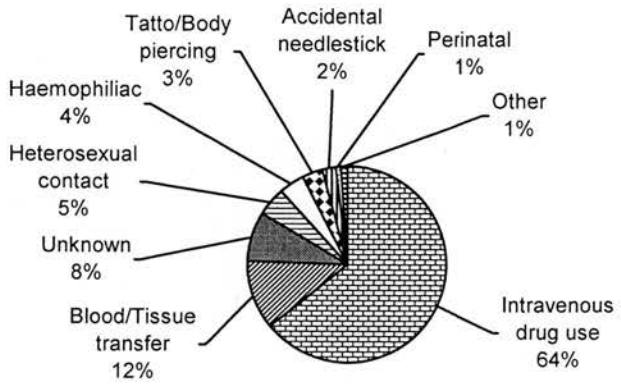
			IDU		Total
			No	Yes	
Age at HCV infection	Under 20 years	Number	66	229	295
		%	22.4%	77.6%	
	20 to 30 years	Number	40	146	186
		%	21.5%	78.5%	
	More than 30 years	Number	56	22	78
		%	71.8%	28.2%	
Total		Number	162	397	559
		%	29.0%	71.0%	

Pearson Chi-square (2df) = 80.78, p-value < 0.0001

3.3.2.3 Risk category for infection

64.1% had intravenous drug use as the probable source of infection (Figure 3).

Figure 3 Pie chart risk category for infection.



Chi-square testing shows a significant difference in the sex ratios of patients again categorised by whether their risk category is IDU and non-IDU (Table 7).

Table 7 IDU risk category by gender.

Risk category			Sex		Total
			Female	Male	
IDU	No	Number	90	159	249
		%	36.1%	63.9%	
	Yes	Number	125	320	445
		%	28.1%	71.9%	
Total		Number	215	479	694
		%	31.0%	69.0%	

Pearson Chi-square (1df) = 4.84, p-value = 0.03

3.3.2.4 Country in which HCV infection occurred

In the overwhelming majority of patients, Scotland was thought to have been the country in the HCV infection was acquired (Table 8).

Table 8 Country or continent in which HCV thought to have been acquired.

Country/Continent	Number	Percent
Africa	5	0.7
Indian subcontinent	13	1.9
Middle-East	2	0.3
North America	10	1.4
Northern Europe	13	1.9
Oceania	6	0.9
Rest of Europe	10	1.4
Rest of UK	17	2.4
Scotland	503	72.5
South-East Asia	6	0.9
South America	1	0.1
Unknown	108	15.6
Total	694	100.0

3.3.2.5 Ethnicity of patient

Most patients were white (96.7%) with the next commonest racial group being Pakistani (Table 9).

Table 9 The ethnicity of patients that are HCV RNA PCR positive.

Ethnicity	Number	Percent
White	671	96.7
Pakistani	12	1.7
Any other	5	0.7
Black African	2	0.3
Bangladeshi	1	0.1
Black Caribbean	1	0.1
Chinese	1	0.1
Indian	1	0.1
Total	694	100.0

3.3.2.6 Genotype of the virus

Four hundred and eighty-two patients had their genotype determined. 51.7% of these had a genotype 1 virus (Figure 4).

Figure 4 Pie charts of virus genotype.

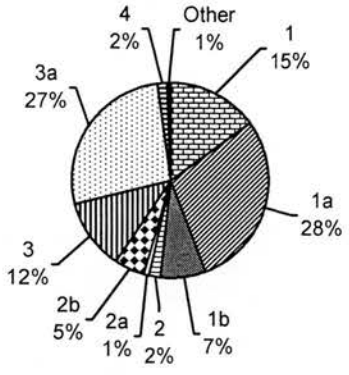


Table 10 shows that the patients genotype is significantly less likely to have been ascertained for patients whose risk category for infection is IDU, but when known, the genotype differs neither by sex nor by IDU / non-IDU status (data not shown).

Table 10 Genotype ascertainment by risk category.

Risk category			Genotyped		Total
			No	Yes	
IDU	No	Number	62	187	249
		%	24.9%	75.1%	100.0%
	Yes	Number	150	295	445
		%	33.7%	66.3%	100.0%
Total		Number	212	482	694
		%	30.5%	69.5%	100.0%

Chi-square(1df)=5.84, p-value = 0.016

3.3.2.7 HIV status

Table 11 shows there is a significant association between HIV infection status and IDU risk category status within this HCV PCR positive cohort.

Table 11 Risk category by HIV status.

Risk category			HIV		Total
			No	Yes	
IDU	No	Number	243	6	249
		%	97.6%	2.4%	
	Yes	Number	404	41	445
		%	90.8%	9.2%	
Total		Number	647	47	694
		%	93.2%	6.8%	

Pearson Chi-square (1df) =11.71, P-value =0.001

3.3.2.8 HBV infection status

Two hundred and eleven patients have had previous HBV infection defined as being HBcAb positive, at their first assessment (Table 12). However, only 8 had HBsAg suggesting lack of clearance of the virus. Vaccination has only been performed in 35 of the 343 (10.2%) patients that had not previously had HBV infection.

Table 12 HBV infection status of HCV PCR cohort.

HBV status	Number	Percent
HBcAb negative, HBsAb negative (non immunised)	308	44.4
HBcAb negative, HBsAb positive (immunised)	35	5.0
HBcAb positive, HBeAg negative, HBsAg negative	201	29.0
HBsAg positive, HBeAg positive	2	0.3
HBsAg positive, HBV DNA negative	3	0.4
HBsAg positive, HBV DNA unknown	5	0.7
Unknown	140	20.2
Total	694	100.0

Table 13 shows there is a significant association between previous HBV infection status and IDU risk category status within this HCV PCR positive cohort.

Table 13 IDU Risk category by HBV status.

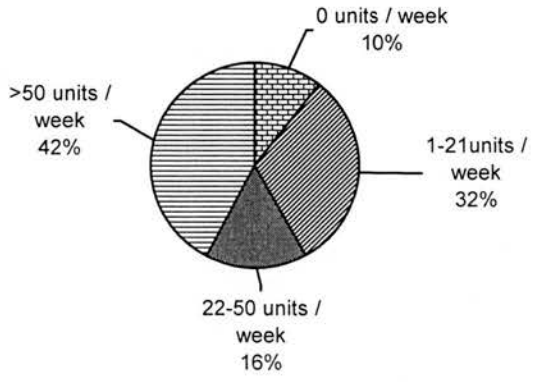
Risk category			Previous HBV		Total
			No	Yes	
IDU	No	Number	202	47	249
		%	81.1%	18.9%	
	Yes	Number	281	164	445
		%	63.1%	36.9%	
Total		Number	483	211	694
		%	69.6%	30.4%	

Pearson Chi-square (1df) =24.39, P-value <0.0001

3.3.2.9 Previous maximum alcohol intake

The previous maximum alcohol intake, which was maintained for at least 5 years, was obtained in 565 patients. 42.1% of these had consumed more than 50 units of alcohol per week (Figure 5).

Figure 5 Pie chart of previous maximum alcohol intake.



Previous maximum alcohol consumption varies between men and women (Table 14a). In this cohort 46.4 % of women drink alcohol modestly (1 to 21 units per week). The other three alcohol consumption categories are equally frequent among women. In contrast, for men, consumption of more than 50 units per week is the modal category (51.8%) and very few men don't drink (7.0%). Chi-square testing confirms that alcohol consumption distribution depends on sex of the patient ($p<0.0001$).

Table 14b shows that 64.5% of patients with an IDU risk category status compared with only 45.3% of those that do not, have a previous maximum consumption of more than 21 units per week. Chi-square test confirms that the consumption distribution depends also on IDU risk category status ($p<0.0001$).

Table 14c, suggests that the association of previous maximum alcohol consumption with IDU risk category status is not accounted for purely by sex differences.

Table 14 (a) Previous maximum alcohol consumption by sex.

			Sex		Total
			Female	Male	
Previous maximum alcohol intake	0 units/week	Number	32	27	59
		%	54.2%	45.8%	
	1-21 units/week	Number	83	95	178
		%	46.6%	53.4%	
	22-50 units/week	Number	26	64	90
		%	28.9%	71.1%	
	>50 units/week	Number	38	200	238
		%	16.0%	84.0%	
Total		Number	179	386	565
		%	31.7%	68.3%	

Pearson Chi-square (3df) =59.72, P-value <0.0001

(b) Previous maximum alcohol consumption by IDU risk category status.

			IDU		Total
			No	Yes	
Previous maximum alcohol intake	0 units/week	Number	29	30	59
		%	49.2%	50.8%	
	1-21 units/week	Number	75	103	178
		%	42.1%	57.9%	
	22-50 units/week	Number	29	61	90
		%	32.2%	67.8%	
	>50 units/week	Number	57	181	238
		%	23.9%	76.1%	
Total		Number	190	375	565
		%	33.6%	66.4%	

Pearson Chi-square (3df) =22.21, P-value <0.0001

(c) Previous maximum alcohol consumption by IDU status in men.

			IDU		Total
			No	Yes	
Previous maximum alcohol intake	0 units/week	Number	12	15	27
		%	44.4%	55.6%	
	1-21 units/week	Number	33	62	95
		%	34.7%	65.3%	
	22-50 units/week	Number	23	41	64
		%	35.9%	64.1%	
	>50 units/week	Number	46	154	200
		%	23.0%	77.0%	
Total		Number	114	272	386
		%	29.5%	70.5%	

Pearson Chi-square (3df) =9.48, P-value =0.024

3.3.2.10 Intravenous drug use status

At least 8.4% of patients had been injecting intravenous drugs within a year of their last appointment or admission (Table 15). Of the 103 'Unknowns', 80 have intravenous drug use as their risk category for infection but it is unknown whether they are still injecting drugs or not.

Table 15 Intravenous drug use status of HCV PCR positive patients.

Intravenous drug status	Number	Percent
Injected in last 12 months	58	8.4
Last injected greater than 12months ago	307	44.2
Never injected	226	32.6
Unknown	103	14.8
Total	694	100.0

3.3.2.10.1 Methadone use

At least 27.0% (120 out of 445) of HCV RNA PCR positive patients that have used intravenous drugs were on a methadone maintenance program (Table 16).

Table 16 Methadone use in HCV RNA PCR positive cohort

Methadone use	Number	Percent
Yes	120	17.3
No	237	34.1
Unknown	88	12.7
Not applicable as no previous Intravenous drug use	249	35.9
Total	694	100.0

3.3.2.11 Smoking status

At least 49.7% of patients are currently smoking or have previously smoked cigarettes (Table 17).

Table 17 Smoking status in HCV RNA PCR positive patients.

Smoking status	Number	Percent
Current	309	44.5
Ex smoker	36	5.2
Never	38	5.5
Unknown	311	44.8
Total	694	100.0

3.3.2.12 Weight, Height and Body Mass Index

The mean weight at first appointment or admission was 72.7 kilograms and the mean height 1.71 metres (Table 4). The Body Mass Index was calculated in those patients that had both weight and height measurements available.

3.3.3 Time from infection to first appointment or admission

Using the probable date of HCV infection, a mean and median time from infection to first appointment or admission can be calculated (Table 18).

Table 18 Descriptive statistics for time from infection to first appointment or admission and length of follow up.

	Time from infection to first appointment or admission (years)	Length of follow up from first appointment or admission (years)
Mean	16.84	3.90
Standard Error of Mean	0.38	0.14
Median	17.02	2.85
Standard Deviation	8.97	3.75
Skewness	0.33	1.19
Standard Error of skewness	0.10	0.09
Minimum	0	0
Maximum	49.60	26.91

There is a significant positive correlation between the time from infection to first appointment or admission with the patient's weight and Body Mass Index (BMI) and significant negative correlation with patient's age at HCV infection (Table 19).

Table 19 Correlation for time from infection to first appointment or admission and length of follow up with continuous variables recorded at first appointment or admission

		Time from infection to first appointment or admission (years)	Length of follow up from first appointment or admission (years)
Age at HCV infection	Pearson Correlation	-0.385	-0.004
	p-value (2-tailed)	<0.0001	0.933
	Number	556	553
Weight	Pearson Correlation	0.176	0.010
	p-value (2-tailed)	<0.0001	0.824
	Number	394	456
Height	Pearson Correlation	0.047	0.062
	p-value (2-tailed)	0.429	0.263
	Number	286	325
BMI	Pearson Correlation	0.216	0.013
	p-value (2-tailed)	<0.0001	0.824
	Number	277	317

The mean time from infection to first appointment or admission was calculated for each category within the categorical variables; age at HCV infection, sex of the patient, whether the risk factor for infection was IDU or not, previous maximum alcohol intake, HIV status, genotype of the virus and whether the patient was cirrhotic. T testing revealed a significant difference in the means for the categories; age at HCV infection, sex of the patient, previous maximum alcohol in take, HIV co-infection, previous HBV infection and whether the patient was cirrhotic (Table 20).

Table 20 Categorical covariates in which the mean time from first infection to first appointment or admission is significantly different on T testing.

		n	Mean	Standard Deviation	Standard Error Mean	p value
Age at HCV infection	**Under 20 years**	294	19.74	8.18	0.48	<0.0001
	20 to 30 years	186	14.75	8.71	0.64	<0.0001
	More than 30 years	76	10.75	8.11	0.93	0.001
Sex	**Female**	183	15.73	8.70	0.64	0.041
	Male	373	17.39	9.07	0.47	
Previous maximum alcohol intake	**0 units/week**	42	16.56	9.92	1.53	0.302
	1 to 21 units/week	158	14.98	8.51	0.68	0.002
	22 to 50 units/week	78	18.53	8.06	0.91	<0.0001
	More than 50 units/week	193	18.52	9.12	0.66	
HIV	**No**	516	17.11	9.05	0.40	0.012
	Yes	40	13.43	7.26	1.15	
Previous HBV	**No**	384	15.57	9.07	0.46	<0.0001
	Yes	172	19.68	8.09	0.62	
Cirrhosis	**No**	442	15.99	8.62	0.41	<0.0001
	Yes	114	20.16	9.59	0.90	

3.3.4 Length of follow up from first appointment or admission

The length of follow up was the years between date of the patient's first appointment or admission to the date of last follow up.

There were no significant correlation between the length of follow up and age at HCV infection, weight, height and BMI of patient (Table 19)

The mean length of follow up was significantly shorter in those that previously drank more than 50 units/week and those with no previous HBV infection This was also the case in non-cirrhotics compared with cirrhotics (Table 21).

Table 21 Categorical covariates in which the mean length of follow up from first appointment or admission is significant different on T testing.

		n	Mean	Standard Deviation	Standard Error Mean	p value
Previous maximum alcohol intake	**Less than 50 units/week**	326	4.39	4.06	0.22	0.038
	More than 50 units/week	237	3.72	3.37	0.22	
Previous HBV	**No**	475	3.70	3.58	0.16	0.043
	Yes	211	4.36	4.07	0.28	
Cirrhosis	**No**	530	3.73	3.62	0.16	0.030
	Yes	156	4.47	4.10	0.33	

3.3.5 Outcome

The number of patients progressing to each end-point is recorded in Table 22. Any complication of cirrhosis was defined as the presence of ascites, encephalopathy, HCC or grade 2 oesophageal varices. Major complications of cirrhosis were the same except the varices had to be bleeding.

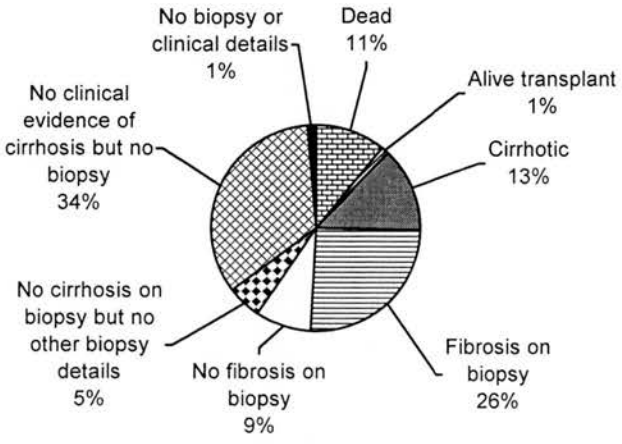
Table 22 Number of HCV RNA PCR positive patients progressing to the end-points.

End-point	Number	Percent
Cirrhosis on biopsy	87	12.5
Cirrhosis clinically or on biopsy	157	22.6
Any complication of cirrhosis	83	12.0
A major complication of cirrhosis	73	10.5
Complications of cirrhosis		
Grade II oesophageal varices	48	6.9
Bleeding oesophageal varices	25	3.6
Ascites	55	7.9
Encephalopathy	25	3.6
Hepatocellular carcinoma	23	3.3
Liver transplantation	13	1.9
Death	75	10.8
Causes of death		
Liver related deaths	36	5.2
Non-liver related deaths	18	2.6
Unknown cause of death (out of hospital)	21	3.0

3.3.6 Stage of disease at the end of follow-up

In Figure 6 a breakdown of the stage of the disease at the end of 2004 is displayed. The amount of fibrosis on biopsy is based on the patient's last biopsy. This potentially could have taken place years prior to the December 2004 time point. If the patient had subsequently developed clinical evidence of cirrhosis or been transplanted or had died they are classified as this.

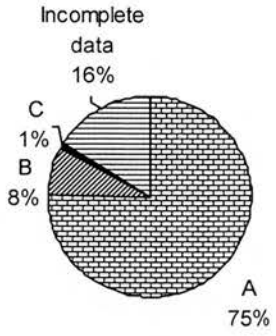
Figure 6 Stage of disease of HCV RNA PCR positive cohort at end of 2004.



3.3.7 Stage of the disease of the alive cirrhotic patients at end of follow-up

Of the 92 patients in the cohort that were cirrhotic, 70 were Childs Pugh A on the latest blood results and clinical examination (Figure 7). Twenty-nine of the alive cirrhotics (31.5%) had had at least one complication of cirrhosis.

Figure 7 Child Pugh score of alive, cirrhotic HCV RNA PCR positive patients at the end of December 2004.



3.4 Discussion

The basic characteristics of the HCV RNA PCR population are very similar those previously described from a tertiary care centre in an area of low prevalence (Foster et al. 1997), (Poynard et al. 1997). Two thirds are male and 64% are likely to have acquired the infection through IDU. In only 8% was it not possible to identify a probable source of infection. Somewhat unusually the cohort is mainly white with 72.5% acquiring the infection in Scotland.

The commonest source of referral was local GPs, with only 6.6% coming from the Blood transfusion service. Attendance as an outpatient was the first point of contact with the service in the vast majority. Patients were presenting around the age of 40 years. It is not possible to be certain about the exact date of acquisition of infection in any of the patients. However a probable date has been estimated from the date of first exposure to a known risk factor for HCV infection. In patients that have IDU as their risk category, the assumption is that in the majority infection is acquired in the first year of use. This is based on the seroprevalence rate of 78% at one year amongst the US IDU community cohort (Thomas et al. 1995a). The 90% prevalence rates

amongst in the 15-19 year old age group in Glasgow IDU cohort in 1990 would also suggest rapid acquisition (Goldberg et al. 1998). However this study also demonstrates that this is not constant over time and therefore the variability in this estimate is unknown. Most comparible studies have made this assumption (Poynard et al. 1997). Using this estimate the majority of patients were infected in the early 1980s reflecting the high prevalence of drug use in this population. As expected those that acquired the infection through drug use did so at a significantly younger age. Men appear to acquire the infection at an earlier age compared with women although this did not quite reach significance. Significantly more men acquired the infection through IDU compared with women.

About half of the population tested for genotype had genotype 1, and so if treated would require a year of treatment and would have a lower SVR rate. No association between genotype and IDU or sex could be found, although IDU patients were significantly less likely to have been tested for genotype which may have masked an association.

Only 6.8% of the cohort was co-infected with HIV as the Regional infectious disease unit in Edinburgh specialises in management of these patients. Those with co-infection were more likely to have acquired the infection through IDU. One third of the cohort had had previous HBV infection, reflecting the significant association of previous HBV and IDU. However, only 1% appeared to have evidence of ongoing infection. At present only 10.2% have been vaccinated against HBV despite the recommendations of the World Health Organisation (World Health Organization 1998) and the National Institute of Health (National Institutes of Health 2002).

The covariate used to categorise previous alcohol intake was previous maximum alcohol intake maintained for greater than five years. This was acquired from review of the patient's notes. The accuracy of this will have varied depending on the thoroughness of the patient's initial assessment by different clinicians and the patient's accuracy in their replies. As there has been no assessment of the accuracy of this estimation, conclusions may be susceptible to error. However irrespective of these caveats it is of great concern that 42% of all patients and 52% of men appear to have drunk in excess of 50 units of alcohol for more than five years prior to presentation. There was a significant difference between men and women in this covariate, and those that acquired the infection through IDU and those that did not. At least 8% of the cohort and potentially up to 20% had recently used intravenous drugs with a quarter of those that acquired the infection by IDU on a methadone program, while half the cohort has smoked in the past. With a median weight of 72 kg and a BMI of 24.7, suggest that just less than half the cohort is obese.

The median time from infection to first appointment or admission was about 17 years with a median follow up thereafter of about 3 years. Therefore the bulk of the population have not yet reached the point at which the complications of cirrhosis will be seen. Interestingly those that presented a longer time after infection were more likely to have a higher BMI. This may reflect the natural changes in body weight with age, but may contribute to worsening in fibrosis with age via liver steatosis. In this cohort, the older the age at infection, the shorter the time from infection to first presentation. Men presented longer after infection than women, as did those with no HIV co-infection and those with previous HBV. Those that had drunk more than 50

units / week presented later after infection than those that drank less than 21 unit / week, but had less years of follow up after presentation.

At least 22% of the cohort has cirrhosis clinically, although only about half of these have had it confirmed by biopsy or laparoscopy. At least one complication of cirrhosis was seen in 12% of patients, which was most commonly the presence of ascites in 7.9%. Grade 2 oesophageal varices have been found in about 7% of patients overall, but only half of these have bled. Looking at major complications of cirrhosis (excludes varices that have not bled), the rate was 10.5%. HCC has been found in 15% of cirrhotics which reflects HCV cirrhosis being a significant risk factor for this tumour. The concern with the high proportion of cirrhotic patients seen in this cohort is that this simply is a result of referral bias. This undoubtedly is true to a certain extent, however it is interesting to note that only 4.3% first presented as an inpatient and the vast majority were referred by GPs via the outpatient department.

So far only 13 patients have met the criteria for transplantation and have been transplanted. Eleven percent of the cohort has died and in those in whom we know the cause, liver-related death is twice as common as non-liver-related death. However in 25% we do not know the cause as the patient has died out of hospital.

At the end of the period of follow up at least 13% of the cohort were alive and cirrhotic. This may be an underestimate as some patients may have progressed since their last biopsy and a third of the cohort have not had a biopsy, although at the time of clinical assessment there was no evidence of cirrhosis. Of the cirrhotics that were alive at the end of follow-up, three-quarters were Childs A.

Overall this cohort is made up of a significant proportion of middle-aged men who acquired the infection less than 20 years ago, principally through IDU, and have a

significant history of alcohol abuse. The covariates that may influence progression are often significantly correlated and also influence the time to presentation. A significant proportion are already are presenting with cirrhosis and its complications, the rate of which is likely to rise significantly in the next 10 years.

Chapter 4 The natural history from infection to cirrhosis

4.1 Introduction

Numerous retrospective / cross-sectional studies have been performed in various parts of the world, recruiting patients with established chronic liver disease due to HCV (Freeman et al. 2003a). Most have concentrated on the time to develop biopsy-proven cirrhosis from the probable infection date. There has been considerable variation in the results depending on which country or community the research was performed in and whether the research was community or hospital based. These differences may reflect the differing frequencies of the known risk factors in each patient cohort and referral bias. The variation between countries has implications for estimating the health impact of the infection and the provision of resources required.

Many of the retrospective studies do not take into consideration that the patients analysed are a sample of whole HCV RNA PCR positive population and have simply performed Kaplan–Meier survival analysis without left truncation of the risk set at the time of presentation. This study will look at the progression to cirrhosis analysed with and without this left truncation.

There are problems with the date of diagnosis of cirrhosis as an end-point. As there is no definitive clinical method to diagnose its onset accurately, the time to biopsy-proven cirrhosis will usually be a considerable overestimate. This is compounded by many of the studies including those that had already developed cirrhosis complications (clearly a later stage of the disease) at the date of diagnosis cirrhosis end-point. In this study only the time to uncomplicated cirrhosis is analysed. The time at which a biopsy is taken is also very susceptible to referral bias. Therefore in this study the end-point of clinically diagnosed cirrhosis, as defined in the methods

section, is also analysed. Clearly this may include some patients that do not actually have cirrhosis due the lack of sensitivity and specificity of these clinical methods.

In this study to reduce the effect of referral bias to a tertiary centre, only those patients that live within an Edinburgh 'EH' postcode were included. The time to referral, the basic demographics of the referred patients, the risk categories for infection, the important cofactors for progression, the length of follow up and the outcome are all analysed. The influences of various host and viral factors on the progression to cirrhosis and its complications were also assessed.

4.2 Methods

4.2.1 Patients

4.2.1.1 The time from infection to clinically diagnosed uncomplicated Childs A cirrhosis study.

Inclusion criteria

- A probable date of infection that could be estimated.
- Recorded date of first appointment or admission.
- Qualitative HCV RNA PCR positive, six months after the presumed time of infection.

Exclusion criteria

- Tertiary referrals from hospitals outside Edinburgh.
- Home address outwith the EH postcode.
- Evidence of Autoimmune, Primary Biliary Cirrhosis, Primary Sclerosing Cholangitis, Haemochromatosis or Wilson's disease.

- The presence, at the time of diagnosis of cirrhosis, of a complication of cirrhosis including oesophageal varices (grade 2 or more), a variceal bleed, ascites, encephalopathy, bilirubin greater than 35, albumen less than 35 or a prothrombin time greater than 4 seconds prolonged.
- The presence, at the time of diagnosis of cirrhosis, of an HCC.

4.2.1.2 The time from infection to biopsy- or laparoscopy-proven uncomplicated Childs A cirrhosis study.

Inclusion criteria

- As above.
- A liver biopsy or laparoscopy

Exclusion criteria

- As above

4.2.2 End-points

The time from infection to clinically diagnosed uncomplicated Childs A cirrhosis study.

The date of first diagnosis of cirrhosis was determined either by biopsy, laparoscopy, a hyaluronic acid greater than 100 or ultrasonographic evidence of cirrhosis (course or nodular echotexture). In those that were non-cirrhotic, the date of last follow-up was used as the censor point.

The time from infection to biopsy-proven uncomplicated Childs A cirrhosis study.

The date of first diagnosis of cirrhosis was determined only by biopsy or at laparoscopy. In those that were non-cirrhotic, the date of the last biopsy was used as the censor point.

4.2.3 Covariates analysed to predict occurrence of end-points

Age at infection, previous maximum alcohol intake, smoking, methadone use, previous HBV, HIV co-infection, sex, previous IFN treatment, probable source of infection, genotype of the virus, BMI and weight.

4.3 Results

4.3.1 The time from infection to clinically diagnosed uncomplicated Childs A cirrhosis study

4.3.1.1 Cohort demographics

Of the 694 HCV RNA PCR positive patients discussed in chapter 3, 500 met the inclusion and exclusion criteria for this study. The characteristics of this study population are summarised in Table 23.

Table 23 Population characteristics.

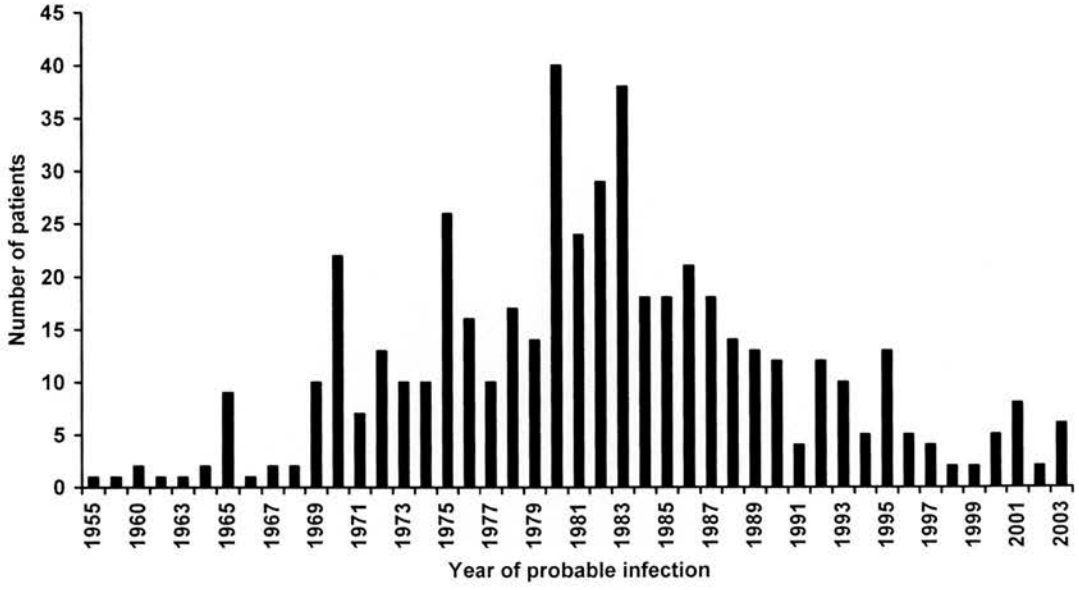
	Number	Percent
Men	333	66.4
Women	167	33.4
Previous hepatitis B infection	145	29.0
Anti HIV positive	32	6.4
Treatment following initial assessment	201	40.2
Sustained responders to treatment	68	13.6
Previous /current smoker at first assessment	271	54.2
Methadone use at first assessment	97	19.4
Patients biopsied	290	58.0

4.3.1.2 Variables recorded at first appointment or first admission

4.3.1.2.1 Probable date of HCV infection

The date of probable infection is plotted as a histogram in Figure 8. The mode date of probable infection is 1980.

Figure 8 A histogram of the year of probable HCV infection.



4.3.1.2.2 Age at HCV infection

The median age at infection is 19.4 years for the cohort and 19.1 years for the patients with an IDU risk category (Table 24). The age at infection distribution is positively skewed.

Table 24 Descriptive statistics of the continuous variables age at infection, HCV diagnosis and at first appointment or admission, weight, height and Body Mass Index.

	Age at infection	Age at Diagnosis	Age at first appointment	Weight (Kg)	Height (m)	BMI
Valid	500	470	500	360	263	256
Missing	0	30	0	140	237	244
Mean	21.56	37.03	38.53	71.75	1.71	24.14
Standard Error of Mean	0.43	0.47	0.45	0.79	0.01	0.261
Median	19.42	35.75	37.40	70.00	1.71	23.51
Standard Deviation	9.62	10.20	10.10	14.92	0.094	4.18
Skewness	1.95	0.84	0.82	0.64	-0.09	1.20
Standard Error of skewness	0.11	0.11	0.11	0.13	0.15	0.15
Minimum	0.01	14.11	14.70	39.00	1.50	15.78
Maximum	70.19	81.36	81.67	131.00	1.93	43.28

Kg: Kilogram, m: Metres, BMI: Body Mass Index

There were no significant correlations between the age at HCV infection and the other continuous variables.

If the age at HCV infection is categorised into three groups and cross-tabulated with the patient's sex, there is no significant difference between males and females in their age at infection distribution on Chi-square testing. There is a higher proportion of males (57.4%) being infected at age under 20 years compared to females (48.5%). For the whole HCV PCR positive cohort the modal age group is 'Under 20 years' (54.4%).

Table 25 is a cross-tabulation between the age at infection categories and whether patients have IDU as their risk category for infection. Chi-squared testing reveals a significant difference between those that have IDU as their risk category for infection and those that do not ($p<0.0001$) in their age at infection distributions. Only 5.2% of patients with IDU as their risk category were greater than 30 years at HCV infection compared to 29.9% of those that did not.

Table 25 Age at HCV infection by IDU risk category patients.

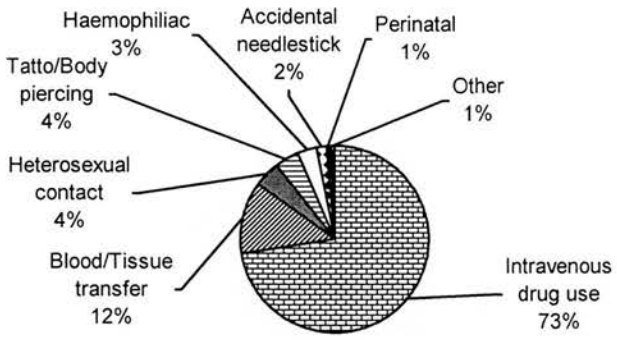
			IDU		Total
			No	Yes	
Age at HCV infection	Under 20 years	Number	61	211	272
		%	22.4%	77.6%	
	20 to 30 years	Number	35	133	168
		%	20.8%	79.2%	
	More than 30 years	Number	41	19	60
		%	68.3%	31.7%	
Total		Number	137	363	500
		%	27.4%	72.6%	

Pearson Chi-square (2df) = 57.56, p-value < 0.0001

4.3.1.2.3 Risk category for infection

72.6% had intravenous drug use as the probable source of infection (Figure 9).

Figure 9 Pie chart risk category for infection.



Chi-square testing does not show a significant difference in the sex ratios of patients categorised by whether their risk category is IDU or non-IDU.

4.3.1.2.4 Ethnicity of patient

Most patients were white (97.4%) with the next commonest racial group being Pakistani (1.2%).

4.3.1.2.5 Genotype of the virus

Three hundred and fifty-seven patients had their genotype determined of which 54.8% of these had a genotype 1 virus (Figure 10).

Figure 10 Pie charts of virus genotype.

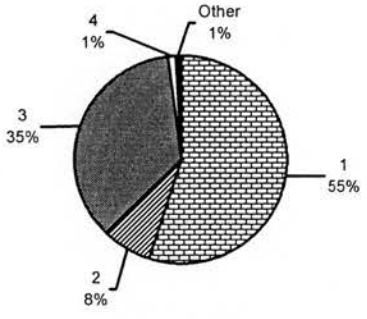


Table 26 shows that the patient's genotype is significantly less likely to have been ascertained for patients whose risk category for infection is IDU, but when known, the genotype differs neither by sex nor by IDU / non-IDU status (data not shown).

Table 26 Genotype ascertainment by risk category

			Genotyped		Total
			No	Yes	
IDU	No	Number	24	113	137
		%	17.5%	82.5%	
	Yes	Number	119	244	363
		%	32.8%	67.2%	
Total		Number	143	357	500
		%	28.6%	71.4%	

Pearson Chi-square (1df)=11.35, p-value = 0.01

4.3.1.2.6 HIV status

There is no significant association between HIV infection status and IDU risk category status within this cohort.

4.3.1.2.7 HBV infection status

One hundred and forty-five patients have had previous HBV infection, defined as being HBcAb positive, at their first assessment (Table 27). However only 5 had ongoing infection suggested by the presence of HBsAg.

Table 27 HBV infection status of HCV PCR cohort.

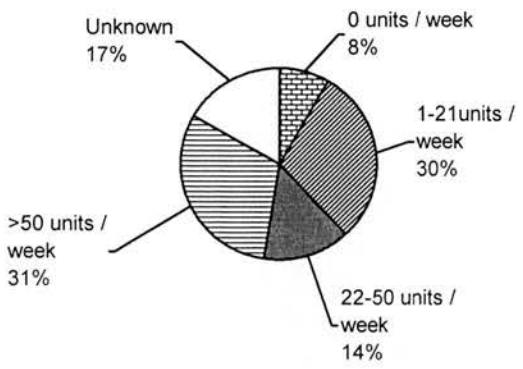
HBV status	Number	Percent
HBcAb negative, HBsAb negative (non immunised)	229	45.8
HBcAb negative, HBsAb positive (immunised)	27	5.4
HBcAb positive, HBeAg negative, HBsAg negative	140	28.0
HBsAg positive, HBeAg positive	1	0.2
HBsAg positive, HBV DNA negative	1	0.2
HBsAg positive, HBV DNA unknown	3	0.6
Unknown	99	19.8
Total	500	100.0

There is a significant association between previous HBV infection status and IDU risk category status within this cohort (data not shown).

4.3.1.2.8 Previous maximum alcohol intake

The previous maximum alcohol intake, which was maintained for at least 5 years, was obtained in 417 patients. Overall 31.0% had consumed more than 50 units of alcohol per week (Figure 11).

Figure 11 Pie chart of previous maximum alcohol intake.



Previous maximum alcohol consumption varies between men and women (Table 28a). In this cohort 50.0% of women drink alcohol modestly (1 to 21 units per week). The other three alcohol consumption categories are equally frequent among women. In contrast, for men, consumption of more than 50 units per week is the modal category (47.3%) and very few men don't drink (6.2%). Chi-square testing confirms that alcohol consumption distribution depends on sex of the patient ($p<0.0001$).

Table 28b shows that 68.8% of patients with an IDU risk category status compared with only 36.9% of those that do not, have a previous maximum consumption of more than 21 units per week. Chi-square test confirms that the consumption distribution depends also on IDU risk category status ($p<0.0001$).

Table 28 (a) Previous maximum alcohol consumption by sex.

			Sex		Total
			Female	Male	
Previous maximum alcohol intake	0 units/week	Number	24	17	41
		%	58.5%	41.5%	
	1-21 units/week	Number	71	78	149
		%	47.7%	52.3%	
	22-50 units/week	Number	22	50	72
		%	30.6%	69.4%	
	>50 units/week	Number	25	130	155
		%	16.1%	83.9%	
Total		Number	142	275	417
		%	34.1%	65.9%	

Pearson Chi-square (3df) =45.78, P-value <0.0001

(b) Previous maximum alcohol consumption by IDU risk category status.

			IDU		Total
			No	Yes	
Previous maximum alcohol intake	0 units/week	Number	17	24	41
		%	41.5%	58.5%	
	1-21 units/week	Number	53	96	149
		%	35.6%	64.4%	
	22-50 units/week	Number	16	56	72
		%	22.2%	77.8%	
	>50 units/week	Number	25	130	155
		%	16.1%	83.9%	
Total		Number	111	306	417
		%	26.6%	73.4%	

Pearson Chi-square (3df) =20.18, P-value <0.0001

4.3.1.2.9 Weight, Height and Body Mass Index

The mean weight at first appointment or admission was 71.7 kilograms and the mean height 1.71 metres (Table 24). The Body Mass Index was calculated in those patients that had both weight and height measurements available.

4.3.1.3 Time from infection to first appointment or admission

Using the probable date of HCV infection, a mean and median time from infection to first appointment or admission can be calculated (Table 29).

Table 29 Descriptive statistics for time from infection to first appointment or admission and length of follow up.

	Time from infection to first appointment or admission (years)	Length of follow up from first appointment or admission (years)
Mean	16.47	4.03
Standard Error of Mean	0.40	0.17
Median	16.38	3.01
Standard Deviation	8.85	3.78
Skewness	0.35	1.20
Standard Error of skewness	0.11	0.11
Minimum	0.03	0.00
Maximum	49.60	26.91

There is a significant positive correlation between the time from infection to first appointment or admission with the patient's weight and BMI and significant negative correlation with patient's age at HCV infection.

The mean time from infection to first appointment or admission was calculated for each category within the categorical variables. T testing revealed a significant difference in the means for the categories; age at HCV infection, previous maximum alcohol intake, HIV status, previous HBV infection and cirrhosis status (Table 30).

4.3.1.4 Length of follow up from first appointment or admission

The length of follow up was the years between date of the patient's first appointment or admission to the date of last follow up.

There were no significant correlation between the length of follow up and age at HCV infection, weight, height and BMI of patient.

The mean length of follow up was significantly shorter in those that previously drank more than 50 units/week compared with those that did not. This was also the case in non-cirrhotics compared with cirrhotics (Table 31).

Table 30 Categorical covariates in which the mean time from first infection to first appointment or admission is significantly different on T testing.

		Number	Mean	Standard Deviation	Standard Error Mean	p value
Age at HCV infection	Under 20 years	272	19.34	8.10	0.49	<0.0001
	20 to 30 years	168	14.23	8.61	0.66	<0.0001
	More than 30 years	60	9.74	7.33	0.95	<0.0001
Previous maximum alcohol intake	0 units/week	41	16.19	9.74	1.52	0.252
	1 to 21 units/week	149	14.48	8.09	0.66	0.003
	22 to 50 units/week	72	17.89	7.66	0.90	
	More than 50 units/week	155	18.49	9.28	0.75	<0.0001
HIV	No	468	16.73	8.89	0.41	0.013
	Yes	32	12.73	7.37	1.30	
Previous HBV	No	15.36	8.92	0.47	15.36	<0.0001
	Yes	19.19	8.09	0.67	19.19	
Cirrhosis	No	440	16.03	8.60	0.41	0.003
	Yes	60	19.70	10.01	1.29	

Table 31 Categorical covariates in which the mean length of follow up from first appointment or admission is significant different on T testing.

		Number	Mean	Standard Deviation	Standard Error Mean	p value
Previous maximum alcohol intake	Less than 50 units/week	262	4.51	3.94	0.24	0.008
	More than 50 units/week	155	3.53	3.08	0.25	
Cirrhosis	No	440	3.87	3.73	0.18	0.011
	Yes	60	5.19	4.01	0.52	

4.3.1.5 Outcome

Sixty patients (12.0%) progressed to cirrhosis. Table 32 demonstrates how the diagnosis of cirrhosis was made.

Table 32 Number of patients progressing to cirrhosis and the mode of first diagnosis of cirrhosis.

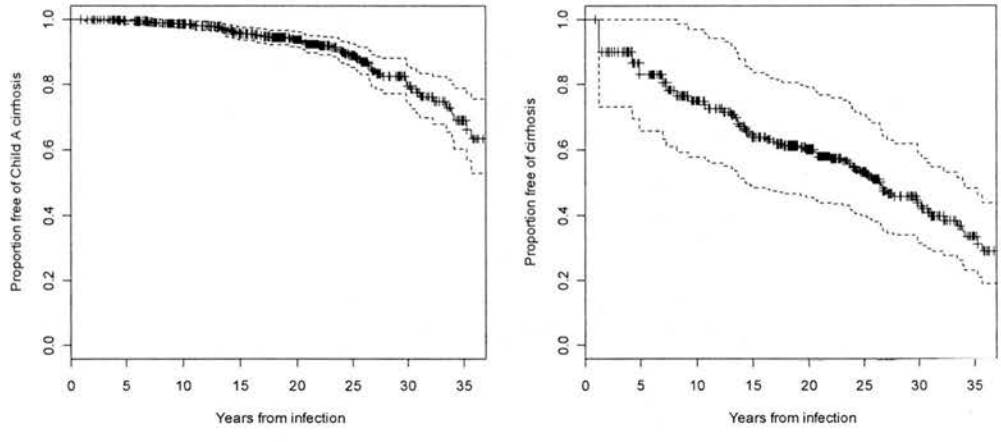
Outcome	Mode of first diagnosis	Number	Percent
Cirrhotic	Biopsy	33	6.6
	Hyaluronic acid (>100)	20	4.0
	Radiology	7	1.4
Non-cirrhotic		440	88.0
	Total	500	100.0

4.3.1.6 Kaplan-Meier analysis

The Kaplan-Meier graph for the time from infection to uncomplicated Child A cirrhosis is plotted in Figure 12.

Figure 12 Kaplan-Meier graphs for the proportion of patients free of Childs A cirrhosis (a) without left truncation at date of first appointment or admission (b) with left truncation at date of first appointment or admission. Dotted lines represent 95% confidence intervals.

(a) **(b)**



4.3.1.7 Cox regression analysis

The independent risk factors identified for progression to cirrhosis were a previous maximum alcohol intake of greater than 50 units per week, an older age at infection and HIV coinfection. The relative effect these factors exert (Exp(b)) within the constructed model are documented in Table 33.

Table 33 Results of Cox regression analysis for independent risk factors for progression to cirrhosis.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.000044	1.06	1.03	1.09
Previous maximum alcohol intake of greater than 50u/wk	0.0011	2.06	1.46	4.64
HIV coinfection	0.00039	6.09	2.25	16.52
Rsquare= 0.068	Maximum possible= 0.626			

4.3.2 The time from infection to biopsy or laparoscopy proven uncomplicated Childs A cirrhosis

4.3.2.1 Cohort demographics

Of the 694 HCV RNA PCR positive patients, 287 met the inclusion and exclusion criteria for this study. The characteristics of this study population are summarised in Table 34.

Table 34 Population characteristics.

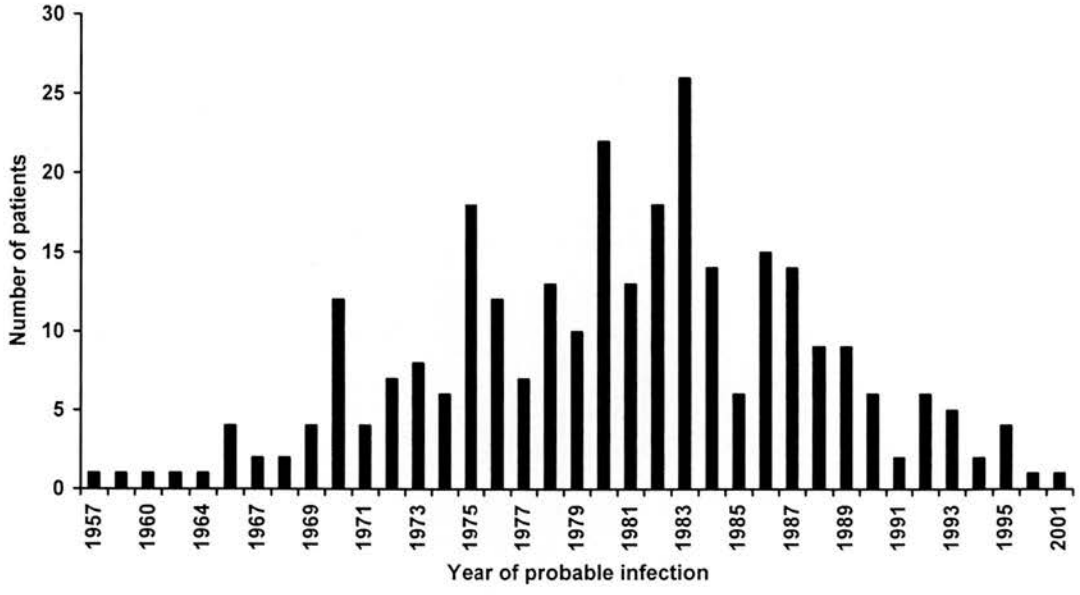
	Number	Percent
Men	202	70.4
Women	85	29.6
Anti HIV positive	31	10.8
Previous hepatitis B infection	93	32.4
Treatment following initial assessment	157	54.7
Sustained responders to treatment	54	18.8
Previous /current smoker at first assessment	135	47.0
Methadone use at first assessment	43	15.0

4.3.2.2 Variables recorded at first appointment or first admission

4.3.2.2.1 Probable date of HCV infection

The date of probable infection is plotted as a histogram in Figure 13. The modal date of probable infection was 1983.

Figure 13 A histogram of the year of probable HCV infection.



4.3.2.2.2 Age at HCV infection

The median age at infection is 19.2 years for the cohort (Table 35). The age at infection distribution is positively skewed.

Table 35 Descriptive statistics of the continuous variables age at infection, HCV diagnosis and at first appointment or admission, weight, height and Body Mass Index.

	Age at infection	Age at diagnosis	Age at first appointment	Weight (Kg)	Height (m)	BMI
Valid	287	272	287	174	126	122
Missing	0	15	0	113	161	165
Mean	21.05	36.68	37.41	72.49	1.72	24.29
Standard Error of Mean	0.54	0.57	0.56	1.15	0.01	0.34
Median	19.17	35.75	36.45	70.00	1.74	23.69
Standard Deviation	9.18	9.33	9.41	15.13	0.09	3.75
Skewness	1.87	0.77	0.72	1.01	-0.38	0.88
Standard Error of skewness	0.14	0.15	0.14	0.18	0.22	0.22
Minimum	0.01	14.29	17.53	43.00	1.52	17.86
Maximum	63.68	74.44	74.74	147.00	1.93	37.87

Kg: Kilograms, m: Metres, BMI: Body Mass Index

There was a significant positive correlation between the age at HCV infection and the BMI of the patient (Pearson correlation 0.184, p=0.042).

There is a significant difference between males and females in their age at infection distribution (Table 36a). This is also demonstrated for those that have IDU as their risk category for infection, compared with those that did not (Table 36b).

Table 36 (a) Age at HCV infection by gender.

			Sex		Total
			Female	Male	
Age at HCV infection	Under 20 years	Number	39	128	167
		%	23.4%	76.6%	
	20 to 30 years	Number	33	58	91
		%	36.3%	63.7%	
	More than 30 years	Number	13	16	29
		%	44.8%	55.2%	
Total		Number	85	202	287
		%	29.6%	70.4%	

Pearson Chi-square (2df) =8.29, p-value =0.016

(b) Age at HCV infection by IDU risk category patients.

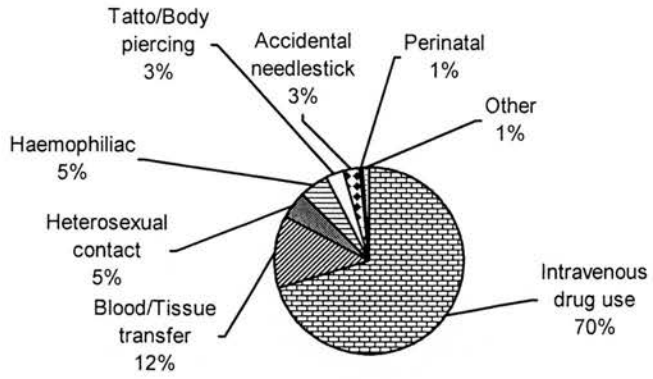
			IDU		Total
			No	Yes	
Age at HCV infection	Under 20 years	Number	41	126	167
		%	24.6%	75.4%	
	20 to 30 years	Number	22	69	91
		%	24.2%	75.8%	
	More than 30 years	Number	23	6	29
		%	79.3%	20.7%	
Total		Number	86	201	287
		%	30.0%	70.0%	

Pearson Chi-square (2df) = 37.43, p-value < 0.0001

4.3.2.2.3 Risk category for infection

70.0% had intravenous drug use as the probable source of infection (Figure 14)

Figure 14 Pie chart risk category for infection.



4.3.2.2.4 Ethnicity of patient

Most patients were white (95.8%) with the next commonest racial group being Pakistani (1.7%).

4.3.2.2.5 Genotype of the virus

One hundred and eighty-eight patients had their genotype determined, of which 54.3% had a genotype 1 virus (Figure 15).

Figure 15 Pie charts of virus genotype, previous maximum alcohol intake and probable source of infection.

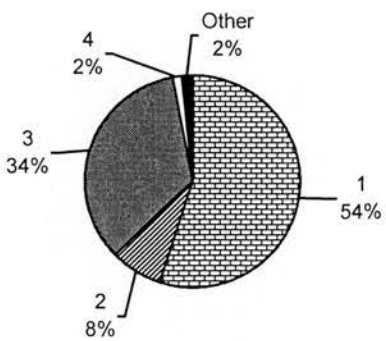


Table 37 shows that the patient's genotype is significantly less likely to have been ascertained for patients whose risk category for infection is IDU, but when known, the genotype differs neither by sex nor by IDU / non-IDU status (data not shown).

Table 37 Genotype ascertainment by risk category

			Genotyped		Total
			No	Yes	
IDU	No	Number	17	69	86
		%	19.8%	80.2%	
	Yes	Number	82	119	201
		%	40.8%	59.2%	
Total		Number	99	188	287
		%	34.5%	65.5%	

Pearson Chi-square(1df)=11.79, p-value = 0.001

4.3.2.2.6 HIV status

There is no significant association between HIV infection status and IDU risk category status within this cohort.

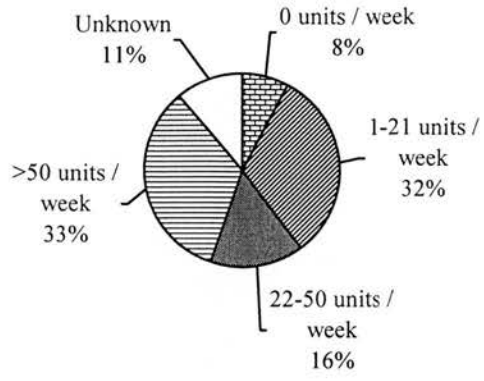
4.3.2.2.7 HBV infection status

One hundred patients have had previous HBV infection, defined as being HBcAb positive, at their first assessment. However only 5 had evidence of ongoing infection, suggested by the presence of HBsAg. There is a significant association between previous HBV infection status and an IDU risk category status (p=0.007).

4.3.2.2.8 Previous maximum alcohol intake

The previous maximum alcohol intake, which was maintained for at least 5 years, was obtained in 255 patients. Overall 33.4% had consumed more than 50 units of alcohol per week (Figure 16).

Figure 16 Pie chart of previous maximum alcohol intake.



Previous maximum alcohol consumption varies between men and women (Table 38a). In this cohort 51.3 % of women drink alcohol modestly (1 to 21 units per week). The other three alcohol consumption categories are equally frequent among women. In contrast, for men, consumption of more than 50 units per week is the modal category (45.3%) and very few men don't drink (5.6%). Chi-square testing confirms that alcohol consumption distribution depends on sex of the patient ($p<0.0001$), and an IDU risk category (Table 38b).

Table 38 (a) Previous maximum alcohol consumption by sex

			Sex		Total
			Female	Male	
Previous maximum alcohol intake	0 units/week	Number	12	10	22
		%	54.5%	45.5%	
	1-21 units/week	Number	39	53	92
		%	42.4%	57.6%	
	22-50 units/week	Number	10	35	45
		%	22.2%	77.8%	
	>50 units/week	Number	15	81	96
		%	15.6%	84.4%	
Total		Number	76	179	255
		%	29.8%	70.2%	

Pearson Chi-square (3df) =23.87, P-value <0.0001

(b) Previous maximum alcohol consumption by IDU risk category status

			IDU		Total
			No	Yes	
Previous maximum alcohol intake	0 units/week	Number	8	14	22
		%	36.4%	63.6%	
	1-21 units/week	Number	37	55	92
		%	40.2%	59.8%	
	22-50 units/week	Number	11	34	45
		%	24.4%	75.6%	
	>50 units/week	Number	16	80	96
		%	16.7%	83.3%	
Total		Number	72	183	255
		%	28.2%	71.8%	

Pearson Chi-square (3df) =13.90, P-value =0.003

4.3.2.2.9 Weight, Height and Body Mass Index

The mean weight at first appointment or admission was 72.5 kilograms and the mean height 1.72 metres (Table 35). The BMI was calculated in those patients that had both weight and height measurements available.

4.3.2.3 Time from infection to first appointment or admission

Using the probable date of HCV infection, a mean and median time from infection to first appointment / admission or to biopsy can be calculated (Table 39). The mean and median time from infection to the biopsy that was used as the censor point in the survival analysis is also displayed. The mean time from first appointment or admission to biopsy was 1.38 years. To demonstrate the full length of follow up after the first appointment or admission, the mean and median time to the last follow up is displayed. The date of the last follow up was on average 3.75 years after the last biopsy.

Table 39 Descriptive statistics for time from infection to first appointment or admission, to biopsy and length of follow up irrespective of biopsy.

	Time from infection to first appointment or admission (years)	Time from infection to first cirrhotic biopsy or last biopsy if non cirrhotic (years)	Time from first appointment or admission to last follow up irrespective of biopsy (years)
Mean	15.87	17.25	5.13
Standard Error of Mean	0.45	0.45	0.23
Median	15.61	16.60	4.99
Standard Deviation	7.60	7.56	3.98
Skewness	0.36	0.33	0.55
Standard Error of skewness	0.14	0.14	0.14
Minimum	0.00	1.41	0.00
Maximum	41.83	42.42	20.05

There is a significant positive correlation between the time from infection to first appointment or admission with the patient's weight and BMI and significant negative correlation with patient's age at HCV infection. The same was true of the time from infection to biopsy.

The mean time from infection to first appointment or admission was calculated for each category within the categorical variables. T testing revealed a significantly greater mean time with a younger age at infection, a higher previous alcohol intake, negative HIV status, previous HBV infection and being cirrhotic (Data not shown). The same analysis was performed looking at the mean time to biopsy. It revealed a significantly greater mean time with a younger age at infection, a higher previous alcohol intake, negative HIV status, and being cirrhotic (Table 40)

Table 40 Categorical covariates in which the mean time from first infection to first cirrhotic biopsy or last biopsy if non-cirrhotic, is significantly different on T testing.

		Number	Mean	Standard Deviation	Standard Error Mean	p value
Age at HCV infection	Under 20 years	167	19.22	7.53	0.58	<0.0001
	20 to 30 years	91	15.21	6.93	0.72	<0.0001
	More than 30 years	29	12.23	5.52	1.02	0.021
Previous maximum alcohol intake	0 units/week	22	16.00	10.41	2.22	0.012
	1 to 21 units/week	92	15.80	7.39	0.77	
	22 to 50 units/week	45	18.20	7.66	1.14	
	More than 50 units/week	96	18.44	6.84	0.70	
HIV	No	256	17.55	7.55	0.47	0.050
	Yes	31	14.72	7.33	1.32	
Cirrhosis	No	237	16.20	7.05	0.46	<0.0001
	Yes	50	22.18	8.01	1.13	

4.3.2.4 Length of follow up from first appointment or admission

The length of follow up was the years between the date of the patient's first appointment or admission to the date of last follow up, irrespective of when the biopsy was taken that was used for censoring. There were no significant correlations between the length of follow up and age at HCV infection, weight, height and BMI of patient. The mean length of follow up was significantly shorter in those previously drinking more than 50 units/week compared with those that did not (Table 41).

Table 41 Categorical covariates in which the mean length of follow up from first appointment or admission is significant different on T testing

		Number	Mean	Standard Deviation	Standard Error Mean	p value
Previous maximum alcohol intake	< 50 units/week	159	5.70	4.29	0.34	0.013
	> 50 units/week	96	4.45	3.10	0.32	

4.3.2.5 Outcome

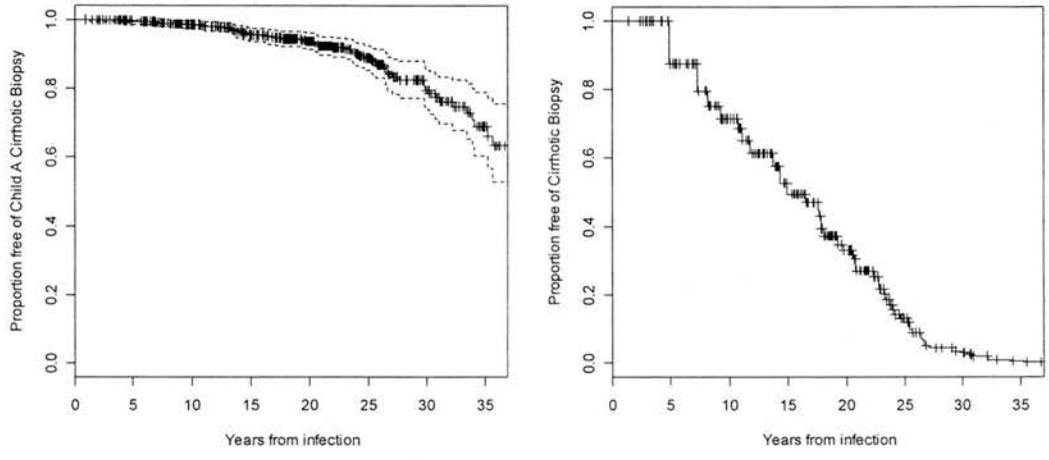
Fifty patients (17.4%) were cirrhotic on liver biopsy.

4.3.2.6 Kaplan-Meier analysis

The Kaplan-Meier graph for the time from infection to a biopsy of uncomplicated Child A cirrhosis is plotted in Figure 17.

Figure 17 Kaplan-Meier graphs for the proportion of patients free of Childs A cirrhosis on biopsy. (a) without left truncation at date of first appointment or admission (b) with left truncation at date of first appointment or admission. Dotted lines represent 95% confidence intervals, however they are too wide to be drawn on graph (b).

(a) **(b)**



4.3.2.7 Cox regression analysis

The independent risk factors identified for progression to cirrhosis on biopsy were a previous maximum alcohol intake of greater than 50 units per week, an older age at infection and previous HBV infection. The relative effect these factors exert (Exp(b)) within the constructed model are documented in Table 42.

Table 42 Results of Cox regression analysis for independent risk factors for progression to cirrhosis on biopsy.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.000043	1.07	1.03	1.10
Previous maximum alcohol intake of greater than 50u/wk	0.0025	2.80	1.44	5.46
Previous HBV infection	0.013	2.46	1.21	5.00
R square= 0.09	Maximum possible= 0.57			

4.4 Discussion

This study attempts to define the progression to cirrhosis in HCV RNA PCR positive patients that have a probable date of infection presenting to a regional centre in Scotland.

4.4.1 Comparison between the two cohorts

The two cohorts are subsets of the whole population of HCV RNA PCR positive patients. Therefore for this analysis to be applicable to this whole population, the two cohorts described in this chapter have to be reasonable matched to it. To make comparisons in the progression to cirrhosis between the two cohorts, they also have to be similarly matched.

In the two cohorts about 70% of patients were likely to have acquired the infection through IDU. This is slightly higher than the PCR positive cohort. This reflects the inclusion criteria of a probable date of infection, which excludes those in whom it was not possible to identify a probable source of infection. The ethnicity was similar in both cohorts and was mainly white.

In both cohorts, patients were presenting around the age of 38 years, with a similar median age of infection at around 19 years. As with the HCV RNA PCR positive population, those that acquired the infection through IDU did so at a significantly

younger age. Men appeared to acquire the infection at an earlier age compared with women although this did not quite reach significance in clinical diagnosis of cirrhosis cohort but did in the biopsy cohort.

The infecting virus was genotype 1 in about 54% of the both cohorts, which was slightly more than the HCV RNA PCR positive population (51.7%). Only 6.4% of the clinical diagnosis of cirrhosis cohort was co-infected with HIV, which was similar to the HCV RNA PCR positive population, while this was higher at 10.8% in the biopsy study. One third of both cohorts had had previous HBV infection. However, only 1% appeared to have evidence of ongoing infection in each cohort.

Around 32% of all patients in both cohorts had drunk in excess of 50 units of alcohol for more than five years, prior to presentation. This was less than that seen in the HCV RNA PCR positive cohort (42%). Proportionately fewer men drank at this level in these cohorts with 47% in the clinical diagnosis of cirrhosis cohort and 45% in the biopsy cohort. Men drank significantly more than women, as did those that acquired the infection through IDU compared with those that did not. The median weight and BMI was the same in both cohorts and similar to the HCV RNA PCR positive population.

The median time from infection to first appointment or admission was about 16.4 years with a median follow up thereafter to the censoring point of about 3.1 years in the clinical diagnosis cohort, but 15.6 years and 1.4 years in the biopsy cohort respectively. Therefore censoring in the biopsy study was occurring on average 2.5 years earlier in the course of the infection.

In the clinical diagnosis of cirrhosis cohort 12% were cirrhotic, of which 55% were first diagnosed on biopsy, while in the biopsy cohort 17.4% were cirrhotic. This

suggests a significant bias in the selection of those biopsied towards patients with more advanced disease.

The survival curves were created in two ways to allow comparison with previous studies (without left-truncation) and to use a more statistically accurate method (with left-truncation).

In both cohorts the Kaplan-Meier graph, without left truncation at the time of presentation, for the proportion free of cirrhosis were very similar. In neither was it possible to estimate the median time to cirrhosis as it did not cross the 0.5 proportion line, but it is likely to be in excess of 35 years. However when Kaplan-Meier analysis is performed without left-truncation, it assumes that all patients that are HCV RNA PCR positive in the South-east of Scotland have been censored and are presenting at random times from infection to the Royal Infirmary. Clearly this is not the case as only a proportion are being referred and this referral process is significantly affected by the vast majority of those that have been found to have advanced disease in the cohort, first presenting at or just before the time of this diagnosis of advanced disease (data not shown).

When left truncation is used, it takes into account that this is a sample of the whole population. The problem with the randomness of presentation still remains however. In both cohorts the survival line, falls much more rapidly as at each census point there are fewer patients. In the clinical diagnosis of cirrhosis cohort the median time to cirrhosis is 26 years (CI 16-36 years) while in the biopsy cohort it is 16 years (CI are too wide to be calculated due to the small number of patients at each census-point). These wide CIs clearly demonstrate that for this analysis to be attempted a considerably larger cohort of patients is required, for it to be interpretable.

When Cox regression analysis with left-truncation was performed in the clinically diagnosed cirrhosis cohort, the best model establishes an older age at infection, a previous alcohol intake of greater than 50 units / week and HIV co-infection as independent predictors of progression to cirrhosis. While for the biopsy cohort the same factors were found except that it includes previous HBV infection rather than HIV co-infection. However neither of these two models had a high R square score compared with the maximum possible, suggesting that other covariates, not tested in this analysis, are required to more accurately predict progression to cirrhosis.

However the covariates also significantly affect the mean time from infection to presentation and to biopsy. This may lead to distortion in the apparent effect a covariate is having on the progression to cirrhosis. The older the age at infection, the shorter the time from infection to first presentation was true of both cohorts. This may lead to an underestimation of the effect of age on progression. In both cohorts those that had drunk more than 50 units / week presented longer after infection compared with those that had drunk less than 21 unit / week. This may lead to an overestimation of the effect of alcohol on progression.

4.4.2 Comparison with previous literature

The first large retrospective series published analysed the biopsies of 1157 French patients with chronic HCV infection who had a probable date of infection and excluded HIV positive patients (Poynard et al. 1997). This cohort of patients has subsequently been expanded to include 2313 liver biopsies but drew similar conclusions (Poynard et al. 2001). The median time from infection to biopsy in the French cohort was 12.4 years. The median time to biopsy confirmed cirrhosis was 30 years estimated by Kaplan-Meier analysis without left-truncation, with 17.9% of the

cohort being cirrhotic. Cox regression analysis identified the age at infection of greater than 40 years, male sex and a daily alcohol intake of greater than 50 grams (equivalent to approximately 40 units / week) predicted early cirrhosis development. In neither Edinburgh cohort was the sex of the patient identified as an independent risk factor. However male sex is significantly associated with heavier drinking, an earlier age at infection and therefore this may overestimate the effect of male sex on progression in a cohort.

As alcohol can cause cirrhosis alone it has been important to establish if its effect in patients with HCV is purely additive or synergistic. The Italian study looked at lifetime daily alcohol intake in both anti-HCV positive and negative patients admitted for the first time to a district hospital for liver decompensation (Corrao & Arico 1998). It found there was a dose-effect relationship with the risk of liver cirrhosis, and below 50 grams / day there was an additive structure and a multiplicative structure for consumption greater than 125 grams / day. Another important question is whether there is a safe level of alcohol intake for people with HCV? The more recent study looking at lifetime daily alcohol intake suggested that this was less than 50 grams per day (Monto et al. 2004). In this Edinburgh study it was only when the previous alcohol intake was greater than 50 units per day did it become an independent predictor of progression to cirrhosis, lower cut offs were not significant (data not shown).

HIV infection has been identified as an independent risk factor for progression to cirrhosis in the clinical diagnosis cohort but not in the biopsy cohort. This is despite those with HIV co-infection presenting sooner after infection, which would underestimate its influence. This is in accordance with the meta-analysis to quantify

the effect of HIV co-infection on progressive liver disease in persons with HCV (Graham et al. 2001). The prevalence in each of the cohorts is different with co-infection being commoner in the biopsy cohort, but how this influenced the analysis is not at present known. If all HIV positive patients were excluded from analysis then no new independent risk factor could be identified (data not shown).

Previous HBV infection was an independent predictor of progression to cirrhosis in the biopsy cohort but not the clinical diagnosis of cirrhosis cohort, despite of the prevalence being much the same in both cohorts. This is unusual as only a very small proportion of these patients were HBsAg positive. This result corresponds to the study of HBcAb positive but HBsAg negative patients, in which 46% of patients HBV DNA was present in the liver and this correlated with increased risk of cirrhosis compared with those in whom it was not detected (Cacciola et al. 1999). However in the biopsy cohorts those with previous HBV infection had their biopsy significantly longer after infection than those that did not.

In the large retrospective studies, the genotype of the virus and the absolute level of the viraemia were not shown to be independent predictors of progression in Western populations (Poynard et al. 1997). Although the level of viraemia was not assessed in the Edinburgh cohorts, no particular genotype was identified as an independent covariate for progression to cirrhosis.

The weight and BMI of the patient have not been shown to influence the progression to cirrhosis in the Edinburgh cohorts, despite this being shown in other studies via liver steatosis (Hourigan et al. 1999). Both variables are subject to change over time and therefore their influence may not be constant. In both cohorts those that presented a longer time after infection were more likely to have a higher BMI. As

suggested in chapter 3 this may reflect the natural changes in body weight with age. It may however lead to an overestimation of its effect on progression that is being demonstrated in these other studies.

4.4.3 Limitations of the study

The major disadvantage of this form of retrospective study is the effect of referral bias. This cannot be avoided in any study that is hospital based and there is little doubt that this has occurred with these cohorts. Selection of those requiring liver biopsy introduces a further bias present in the biopsy cohort and to a lesser degree in the clinical diagnosis of cirrhosis cohort study. In previous studies large numbers of patients recruited were actually being entered into treatment trials, which again increase the selection bias of the cohort. In this study all patients were included whether being considered for treatment or not.

Another disadvantage is that during follow-up some patients were undergoing treatment. However the presence or absence of treatment was not identified as an independent risk factor for progression to cirrhosis. But in other studies there is now good evidence that treatment prior to cirrhosis can affect fibrosis progression (Poynard et al. 2002b).

The entry requirement for the study of having a probable date of infection, has led to a slight overrepresentation of patients thought to have acquired the infection through IDU. In these patients the duration of infection has to be an estimate as it is based on the assumption that infection is acquired in the first year of use. As discussed in chapter 3 the variability in this estimate is unknown.

The covariate used to assess the influence of alcohol on progression was previous maximum alcohol intake maintained for greater than five years. As discussed in

chapter 3, there are clear limitations to the accuracy of this to quantify the level of alcohol exposure, it was felt that it would provide a clinically applicable categorisation.

Finally a proportion of the cohort may have acquired their cirrhosis and its complications predominantly from the effect of alcohol, as no patients were excluded on the basis of their alcohol intake. However in clinical practise it is usually not possible to differentiate which is the more important factor. Also to ignore those that consume large amounts of alcohol will distort the clinical relevance of the study, as such a high proportion of the patients that are referred are in this group.

4.4.4 Conclusion

Despite the acknowledged limitations of the study, it does provide further evidence of the key roles of the age of the patient at infection and the level of alcohol consumption in the progression to cirrhosis and its complications. It shows that previously established risk factors for progression, such as the sex of a patient, are not the same in all cohorts of patients. This raises some concerns about applying the results of previous cohort studies to other populations, when trying to make accurate predictions of health impact of HCV infection for a local cohort. It also demonstrates how significantly linked the covariates that are considered to influence the progression to cirrhosis are, and therefore the difficulty in statistically demonstrating their effect. It shows the time to presentation significantly varies according to each covariate and how this might confound the results of the analysis. Finally the importance of left truncation of the risk set at the time of presentation has been emphasised and shown to influence the outcome of analysis. However for this approach to be adopted, much larger cohorts are required.

Chapter 5 The natural history to the complications of cirrhosis

5.1 Introduction

Accurate prediction of the onset of the complications of cirrhosis is necessary to ensure appropriate surveillance procedures are in place to detect them, as well as allocation of sufficient health resources to manage these patients.

The studies described in chapter 4 concentrate on the length of time from infection to progression to cirrhosis, however it can be argued that the most important end-points are the onset of cirrhosis complications including the development of HCC, liver-related death or transplantation, as these trigger and necessitate medical intervention. They also have the added advantage that, by their nature, their onset should be easier to define accurately. In the large retrospective, hospital based studies the percentage of patients that progress to decompensation (Makris et al. 1996) or HCC (Tong et al. 1995), (Roudot-Thoraval et al. 1997) is often recorded. However no Kaplan-Meier survival analysis has been performed to demonstrate the length of time from infection to these end-points.

An alternative approach that has been taken is to follow-up a relatively homogeneous starting population of uncomplicated Childs A cirrhotics and document the onset of these end-points. A number of studies with this design have been performed (Hu & Tong 1999), (Fattovich et al. 1997), (Serfaty et al. 1998), (Benvegnu et al. 2004). The inclusion criteria have varied between these studies making direct comparison difficult. The inclusion of varying proportions of patients that have undergone treatment may also have influenced outcome.

In the studies described in this chapter both approaches are taken in a well-defined and relatively static hospital based population in which the consumption of alcohol was high. In the study from infection to the complications of cirrhosis, the analysis

was performed with and without left-truncation at the time of first appointment or admission to analyse its effect. In this study to reduce the effect of referral bias to a tertiary centre, only those patients that live within an Edinburgh 'EH' postcode were included. For the study looking at the progression from a diagnosis of cirrhosis, two cohorts were examined. In one the diagnosis of cirrhosis could be made clinically as in chapter 4, in the other the diagnosis was only biopsy- or laparoscopy proven. The influences of various host and viral factors on the progression to the complications of cirrhosis were also assessed.

5.2 Methods

5.2.1 Patients

5.2.1.1 The time from infection to the complications of cirrhosis study

Inclusion criteria

- A probable date of infection that could be estimated.
- Recorded date of first appointment or admission.
- Qualitative HCV RNA PCR positive, six months after the presumed time of infection

Exclusion criteria

- Tertiary referrals from hospitals outside Edinburgh.
- Home address out with the EH postcode.
- Evidence of Autoimmune, Primary Biliary Cirrhosis, Primary Sclerosing Cholangitis, Haemochromatosis or Wilson's disease.

5.2.1.2 The time from clinically diagnosed uncomplicated Childs A cirrhosis to the complications of cirrhosis study

Inclusion criteria

- A date of diagnosis of cirrhosis determined either by biopsy, laparoscopy, a hyaluronic acid greater than 100 or ultrasonographic evidence of cirrhosis (course or nodular echotexture).
- Qualitative HCV RNA PCR positive, six months after the presumed time of infection

Exclusion criteria

- The presence, at the time of diagnosis of cirrhosis, of a complication of cirrhosis including oesophageal varices (grade 2 or more), a variceal bleed, ascites, encephalopathy, bilirubin greater than 35, albumen less than 35 or a prothrombin time greater than 4 seconds prolonged.
- The presence, at the time of diagnosis of cirrhosis of an HCC.
- Evidence of Autoimmune, Primary Biliary Cirrhosis, Primary Sclerosing Cholangitis, Haemochromatosis or Wilson's disease.

5.2.1.3 The time from biopsy or laparoscopy proven uncomplicated Childs A cirrhosis to the complications of cirrhosis study

Inclusion criteria

- A date of diagnosis of cirrhosis determined by liver biopsy or laparoscopy.
- Qualitative HCV RNA PCR positive, six months after the presumed time of infection

Exclusion criteria

- The presence, at the time of diagnosis of cirrhosis, of a complication of cirrhosis including oesophageal varices (grade 2 or more), a variceal bleed, ascites, encephalopathy, bilirubin greater than 35, albumen less than 35 or a prothrombin time greater than 4 seconds prolonged.
- The presence, at the time of diagnosis of cirrhosis of an HCC.
- Evidence of Autoimmune, Primary Biliary Cirrhosis, Primary Sclerosing Cholangitis, Haemochromatosis or Wilson's disease.

5.2.2 End-points

- Any complication of cirrhosis: presence of ascites, encephalopathy, oesophageal varices (grade 2 and above), and HCC.
- A major complication of cirrhosis: ascites or encephalopathy, an oesophageal variceal bleed or the diagnosis of HCC.
- Oesophageal variceal bleed.
- HCC.
- Liver-related death or transplantation.

If none of the end-points occurred, the censor point for the patient was the date of last follow-up.

5.2.3 Covariates analysed to predict occurrence of end-points

Age at infection, sex of patient, previous maximum alcohol intake, previous smoking, previous methadone use, weight, BMI, previous HBV infection, HIV co-infection, previous IFN treatment, probable source of infection and genotype of the virus.

For the uncomplicated Childs A cirrhosis cohorts, age at diagnosis of cirrhosis was also analysed.

5.3 Results

5.3.1 The time from infection to the complications of cirrhosis study

5.3.1.1 Cohort demographics

Of the 694 HCV RNA PCR positive patients, 551 met the inclusion and exclusion criteria for this study. The characteristics of this study population are summarised in Table 43.

Table 43 Population characteristics.

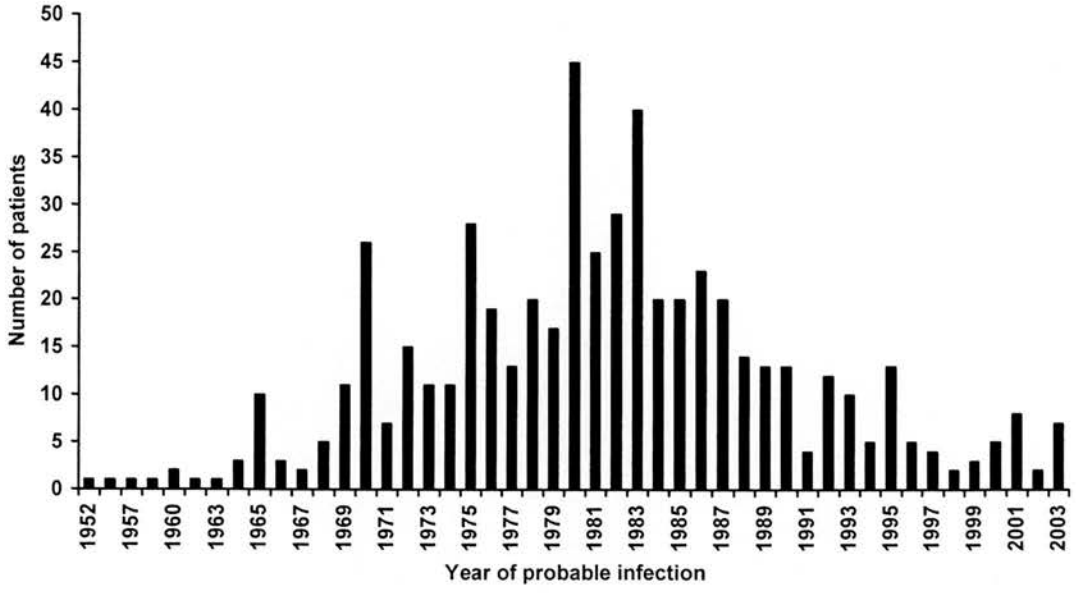
	Number	Percent
Men	371	67.3
Women	180	32.7
Anti HIV positive	40	7.3
Previous hepatitis B infection	170	30.9
Treatment following initial assessment	222	40.2
Sustained responders to treatment	75	13.6
Previous /current smoker at first assessment	295	53.5
Methadone use at first assessment	106	19.2

5.3.1.2 Variables recorded at first appointment or first admission

5.3.1.2.1 Probable date of HCV infection

The date of probable infection is plotted as a histogram in Figure 18. The modal date of probable infection was 1980.

Figure 18 A histogram of the year of probable HCV infection.



5.3.1.2.2 Age at HCV infection

The median age at infection is 19.6 years for the cohort (Table 44). The age at infection distribution is positively skewed.

Table 44 Descriptive statistics of the continuous variables age at infection, HCV diagnosis and diagnosis of cirrhosis, years from infection to cirrhosis, weight, height and Body Mass Index.

	Age at infection	Age at HCV diagnosis	Age at diagnosis of cirrhosis	Years from infection to cirrhosis	Weight (kg)	Height (m)	BMI
Valid	551	515	109	109	393	286	277
Missing	0	36	442	442	158	265	274
Mean	21.94	37.93	47.32	21.91	72.32	1.71	24.43
Standard Error of Mean	0.42	0.47	1.02	0.88	0.77	0.005	0.264
Median	19.57	36.78	46.01	21.39	70.00	1.72	23.72
Standard Deviation	9.93	10.69	10.68	9.22	15.32	0.09	4.39
Skewness	1.86	0.88	0.93	0.20	0.67	-0.11	1.14
Minimum	0.01	14.11	27.93	1.19	39	1.50	15.78
Maximum	70.19	81.36	81.35	44.73	131	1.93	43.28

Kg: kilograms, m: metres, BMI: Body Mass Index

There was a significant negative correlation between the age at HCV infection and the height of the patient (Pearson correlation -0.135, p=0.022).

There is no significant difference between males and females in their age at infection distribution on Chi-square testing. But there was between those that have IDU as their risk category for infection and those that do not (p<0.0001) (Table 45).

Table 45 Age at HCV infection by IDU risk category patients.

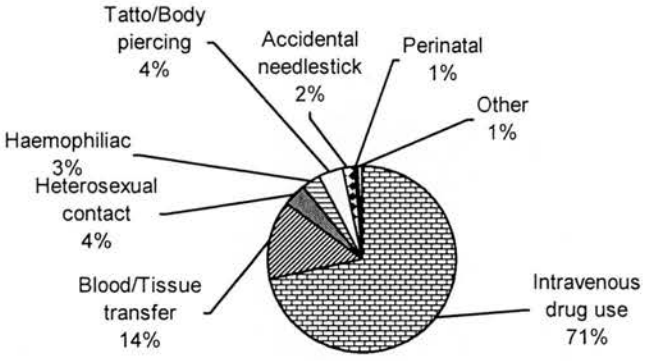
			IDU		Total
			No	Yes	
Age at HCV infection	Under 20 years	Number	65	227	292
		%	22.3%	77.7%	
	20 to 30 years	Number	39	146	185
		%	21.1%	78.9%	
	More than 30 years	Number	52	22	74
		%	70.3%	29.7%	
Total		Number	156	395	551
		%	28.3%	71.7%	

Pearson Chi-square (2df) = 74.22, p-value < 0.0001

5.3.1.2.3 Risk category for infection

70.0% had intravenous drug use as the probable source of infection (Figure 19)

Figure 19 Pie chart risk category for infection



On Chi-square testing the difference in the sex ratios of patients categorised by whether their risk category is IDU and non-IDU does approach significance (p= 0.068).

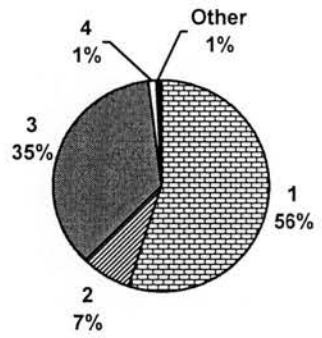
5.3.1.2.4 Ethnicity of patient

Most patients were white (97.3%) with the next commonest racial group being Pakistani (1.3%).

5.3.1.2.5 Genotype of the virus

Three hundred and ninety patients had their genotype determined, of these 56.0% had a genotype 1 virus (Figure 20).

Figure 20 Pie charts of virus genotype.



The patients genotype is significantly (p= 0.006) less likely to have been ascertained for patients whose risk category for infection is IDU, but when known, the genotype differs neither by sex nor by IDU / non-IDU status (data not shown).

5.3.1.2.6 HIV status

There is an association between HIV infection status and IDU risk category status within this cohort that approaches significance (p=0.067).

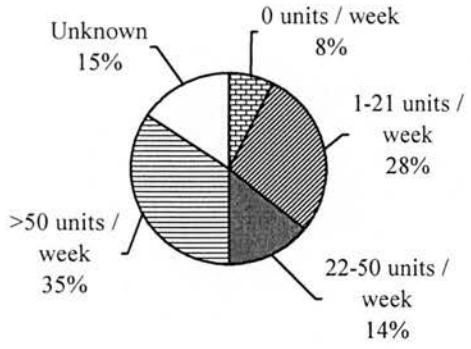
5.3.1.2.7 HBV infection status

One hundred and seventy patients have had previous HBV infection, defined as being HBcAb positive, at their first assessment. However, only 6 had HBsAg suggesting a lack of viral clearance. There is a significant association between previous HBV infection status and IDU risk category status within this cohort ($p<0.0001$).

5.3.1.2.8 Previous maximum alcohol intake

The previous maximum alcohol intake, which was maintained for at least 5 years, was obtained in 466 patients. Overall 34.5% had consumed more than 50 units of alcohol per week (Figure 21).

Figure 21 Pie chart of previous maximum alcohol intake.



Previous maximum alcohol consumption varies between men and women (Table 46 a). In this cohort 48.4 % of women drink alcohol modestly (1 to 21 units per week). In contrast, for men, consumption of more than 50 units per week is the modal category (50.5%) and very few men don't drink (5.4%). Chi-square testing confirms that alcohol consumption distribution depends on sex of the patient ($p<0.0001$).

Table 46b shows that 64.0% of patients with an IDU risk category status compared with only 40.8% of those that do not, have a previous maximum consumption of

more than 21 units per week. Chi-square test confirms that the consumption distribution depends also on IDU risk category status ($p<0.0001$).

Table 46c, suggests that the association of previous maximum alcohol consumption with IDU risk category status is not accounted for purely by sex differences.

Table 46 (a) Previous maximum alcohol consumption by sex.

			Sex		Total
			Female	Male	
Previous maximum alcohol intake	0 units/week	Number	25	17	42
		%	59.5%	40.5%	
	1-21 units/week	Number	74	82	156
		%	47.4%	52.6%	
	22-50 units/week	Number	22	56	78
		%	28.2%	71.8%	
	>50 units/week	Number	32	158	190
		%	16.8%	83.2%	
Total		Number	153	313	466
		%	32.8%	67.2%	

Pearson Chi-square (3df) =51.4, P-value <0.0001

(b) Previous maximum alcohol consumption by IDU risk category status

			IDU		Total
			No	Yes	
Previous maximum alcohol intake	0 units/week	Number	18	24	42
		%	42.9%	57.1%	
	1-21 units/week	Number	59	97	156
		%	37.8%	62.2%	
	22-50 units/week	Number	19	59	78
		%	24.4%	75.6%	
	>50 units/week	Number	34	156	190
		%	17.9%	82.1%	
Total		Number	130	336	466
		%	27.9%	72.1%	

Pearson Chi-square (3df) =22.2, P-value <0.0001

(c) Previous maximum alcohol consumption by IDU status in men.

			IDU		Total
			No	Yes	
Previous maximum alcohol intake	0 units/week	Number	7	10	17
		%	41.2%	58.8%	100.0%
	1-21 units/week	Number	25	57	82
		%	30.5%	69.5%	100.0%
	22-50 units/week	Number	16	40	56
		%	28.6%	71.4%	100.0%
	>50 units/week	Number	28	130	158
		%	17.7%	82.3%	100.0%
Total		Number	76	237	313
		%	24.3%	75.7%	

Pearson Chi-square (3df) =8.62, P-value =0.035

5.3.1.2.9 Weight, Height and Body Mass Index

The mean weight at first appointment or admission was 72.3 kilograms and the mean height 1.71 metres (Table 44). The Body Mass Index was calculated in those patients that had both weight and height measurements available.

5.3.1.3 Time from infection to first appointment or admission or cirrhosis complication end points

Using the probable date of HCV infection, a mean and median time from infection to first appointment or admission can be calculated (Table 47). There is a significant positive correlation between the time from infection to first appointment or admission with the patient's weight and Body Mass Index (BMI) and significant negative correlation with patient's age at HCV infection.

The mean time from infection to first appointment or admission was calculated for each category within the categorical variables. A significant difference was found in the means for the categories; age at HCV infection, sex of the patient, previous maximum alcohol intake, previous HBV infection and HIV status (Table 48).

Table 47 Descriptive statistics for the time from infection to first appointment or admission and from infection to the cirrhosis complication end points or last follow up, if they did not occur.

	Years from infection to end point or last follow up (for complication end points)								
End point	**First appointment or admission**	**Grade 2 oesophageal varices**	**Bleeding oesophageal varices**	**Ascites**	**Encephalopathy**	**Hepatocellular carcinoma**	**Any complication of cirrhosis**	**Major complication of cirrhosis**	**Liver related death or transplantaion**
Mean	16.83	21.23	21.33	21.29	21.35	21.35	21.18	21.26	21.39
Standard Error	0.38	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Median	17.00	21.60	21.63	21.60	21.63	21.67	21.56	21.60	21.62
Standard Deviation	8.96	9.34	9.42	9.41	9.43	9.43	9.31	9.38	9.40
Skewness	0.342	0.02	0.04	0.04	0.03	0.02	0.02	0.03	0.03
Standard Error of skewness	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Minimum	0.03	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Maximum	49.60	50.22	50.22	50.22	50.22	50.22	50.22	50.22	50.22

Table 48 Categorical covariates in which the mean time from first infection to first appointment or admission is significantly different on T testing

		N	Mean	Standard Deviation	Standard Error Mean	p value
Age at HCV infection	**Under 20 years**	292	19.75	8.21	0.48	<0.0001 (vs 20-30 years); <0.0001 (vs More than 30 years)
	20-30 years	185	14.72	8.72	0.64	0.001 (vs More than 30 years)
	More than 30 years	74	10.63	7.76	0.90	
Previous maximum alcohol intake	**0 units/week**	42	16.56	9.92	1.53	0.296 (vs 1 to 21 units/week)
	1 to 21 units/week	156	14.98	8.38	0.67	0.002 (vs 22 to 50 units/week); <0.0001 (vs More than 50 units/week)
	22 to 50 units/week	78	18.53	8.06	0.91	
	More than 50 units/week	190	18.50	9.19	0.67	
Sex of patient	**Female**	180	15.72	8.61	0.64	0.038
	Male	371	17.38	9.09	0.47	
Previous HBV	**No**	15.56	9.03	0.46	15.56	<0.0001
	Yes	19.68	8.13	0.62	19.68	
HIV	**No**	511	17.10	9.03	0.40	0.012
	Yes	40	13.43	7.26	1.15	

5.3.1.4 Outcome

The number of patients progressing to each end-point is recorded in Table 49. Liver related deaths include 3 deaths post liver transplant. In four patients the cause of death was unknown.

Table 49 Number of patients progressing to the end-points.

End point	Number	Percentage
Grade 2 oesophageal varices	28	5.1
Bleeding oesophageal varices	14	2.5
Ascites	32	5.8
Encephalopathy	12	2.2
Hepatocellular carcinoma	15	2.7
Any cirrhosis complication	49	8.9
Major cirrhosis complication	40	7.3
Liver transplantation	10	1.8
Liver related deaths	26	4.7
Non-liver related deaths	23	4.2
Unknown deaths	4	0.01

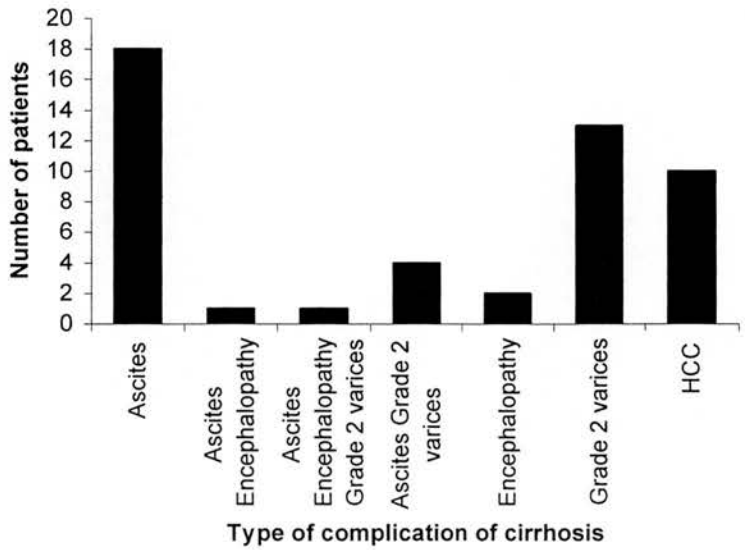
The complication or combinations of complications present at the first diagnosis of any complication of cirrhosis and at the first episode of a major complication of cirrhosis are recorded in figure 22a and 22b respectively.

5.3.1.4.1 Kaplan-Meier analysis

The Kaplan-Meier graph for each of the end-points is plotted with (Figure 22) and without (Figure 23) left truncation at date of first appointment or admission.

Figure 22 The complication or combinations of complications present at the time of (a) the first diagnosis of any complication of cirrhosis, and (b) the first episode of major complication of cirrhosis.

(a)



(b)

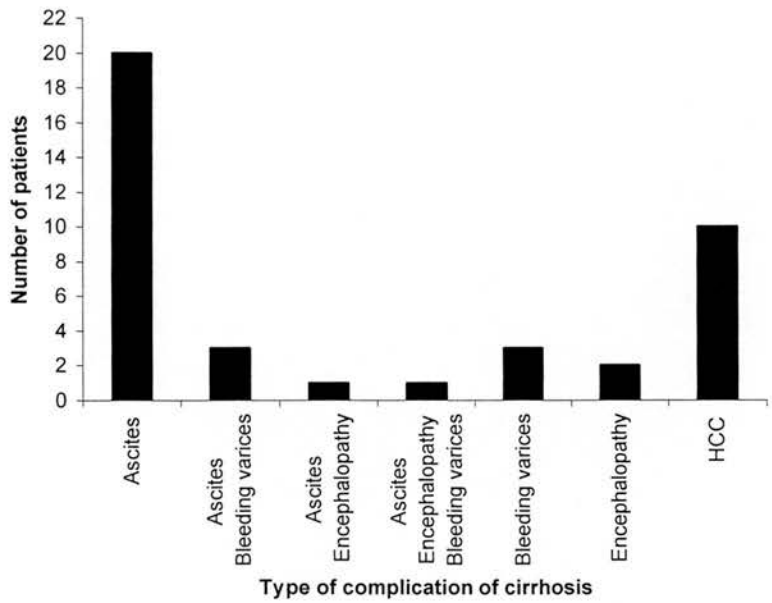
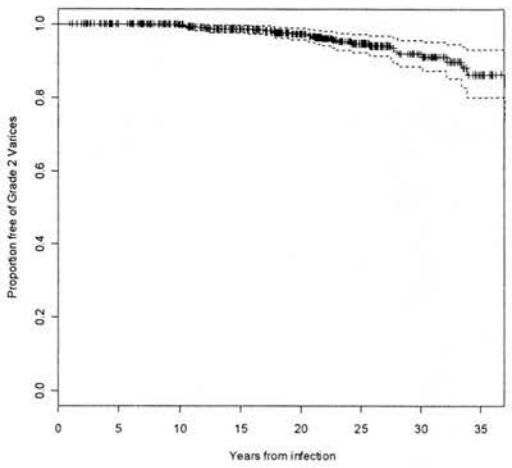
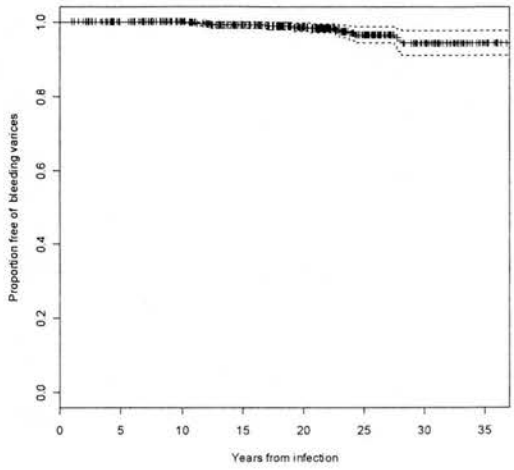


Figure 23 Kaplan-Meier graphs, without left truncation at date of first appointment or admission, for the proportion of patients free of (a) grade 2 oesophageal varices (b) bleeding oesophageal varices (c) ascites (d) encephalopathy (e) hepatocellular carcinoma (f) any complication of cirrhosis, (g) a major complication of cirrhosis (h) liver related death or liver transplantation. The survival curve was curtailed when five patients are left at risk. Where present the dotted lines represent 95% confidence intervals.

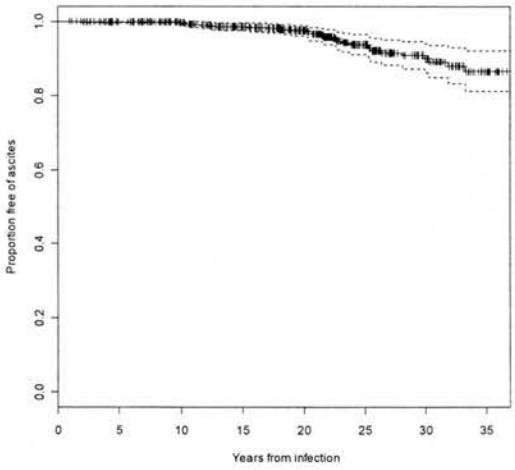
(a)



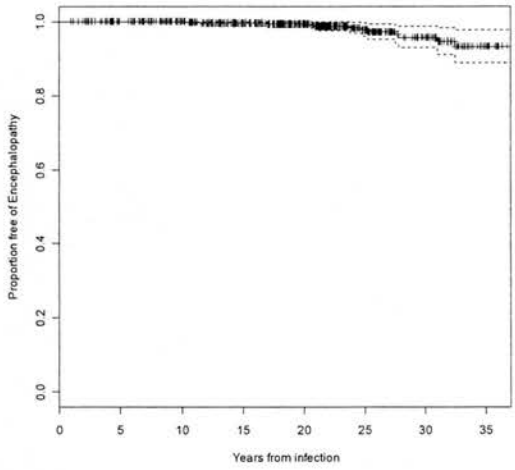
(b)



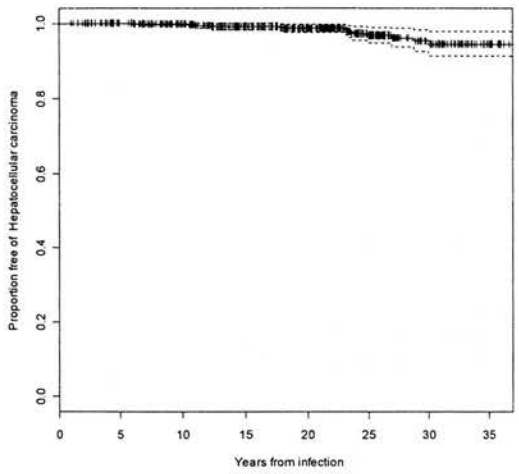
(c)



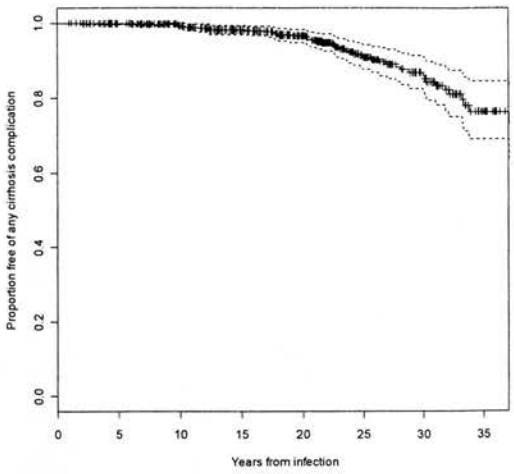
(d)



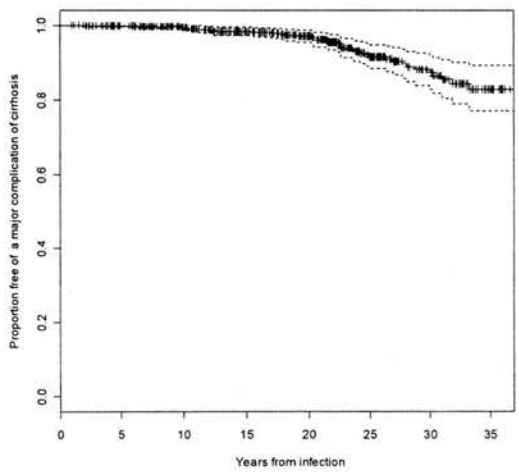
(e)


Proportion free of Hepatocellular carcinoma
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
35
Years from infection

(f)


Proportion free of any cirrhosis complication
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
35
Years from infection

(g)


Proportion free of a major complication of cirrhosis
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
35
Years from infection

(h)

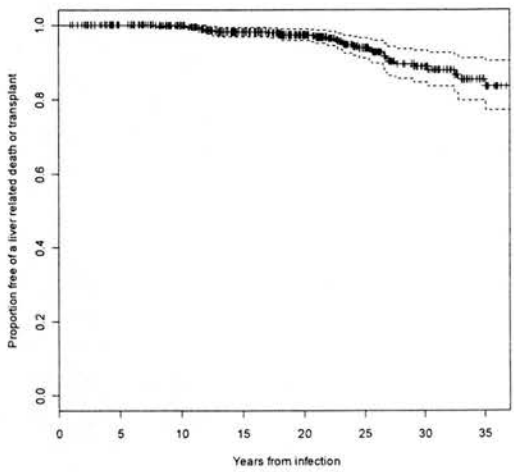
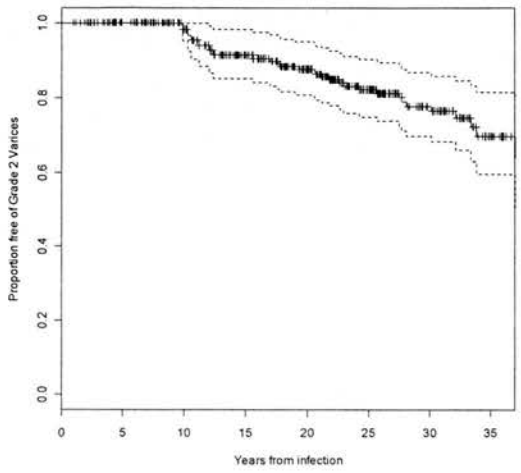
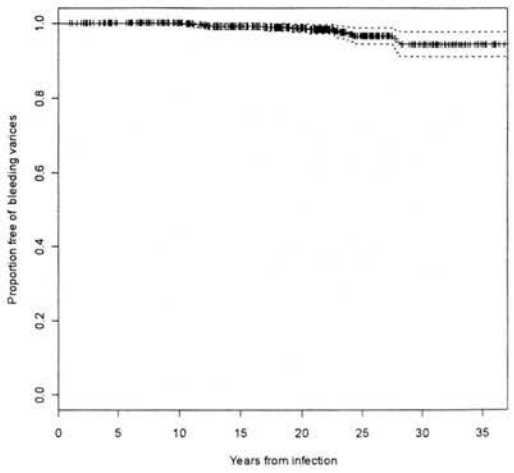

Proportion free of a liver related death or transplant
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
35
Years from infection

Figure 24 Kaplan-Meier graphs, with left truncation at date of first appointment or admission, for the proportion of patients free of (a) grade 2 oesophageal varices (b) bleeding oesophageal varices (c) ascites (d) encephalopathy (e) hepatocellular carcinoma (f) any complication of cirrhosis, (g) a major complication of cirrhosis (h) liver related death or liver transplantation. The survival curve was curtailed when five patients are left at risk. Dotted lines represent 95% confidence intervals.

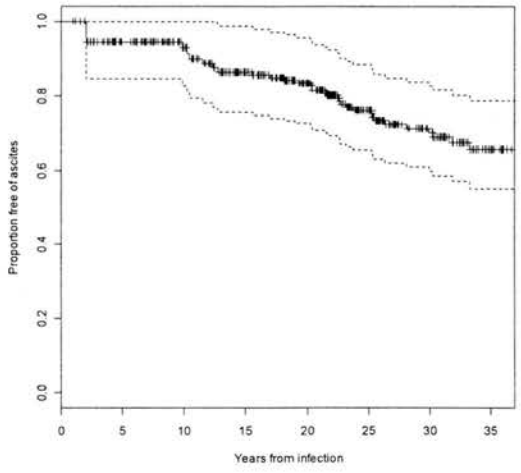
(a)



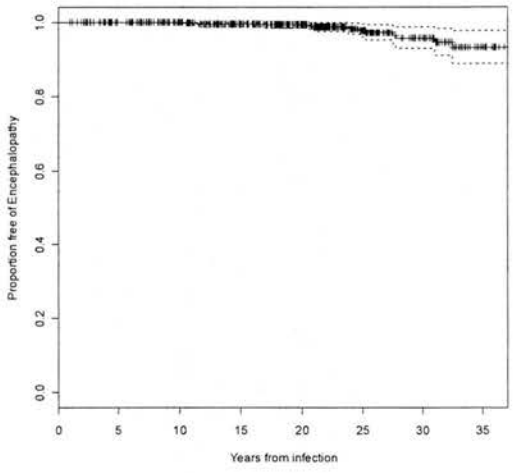
(b)



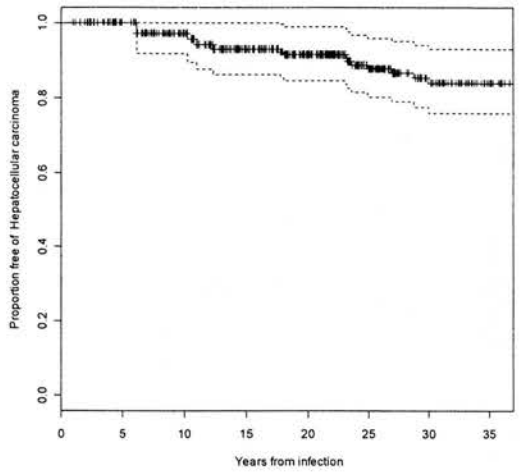
(c)



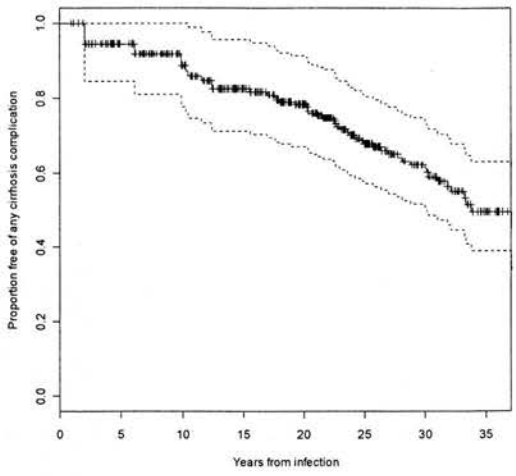
(d)



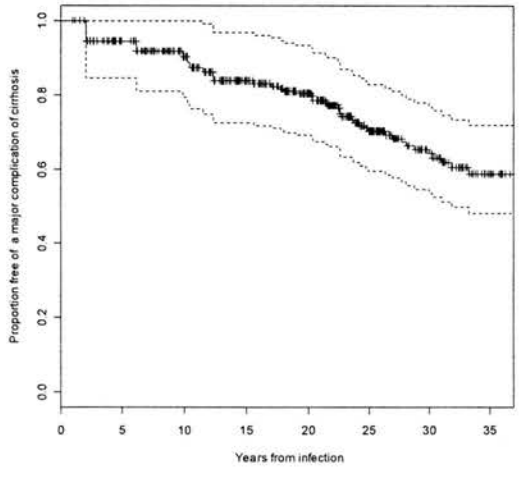
(e)



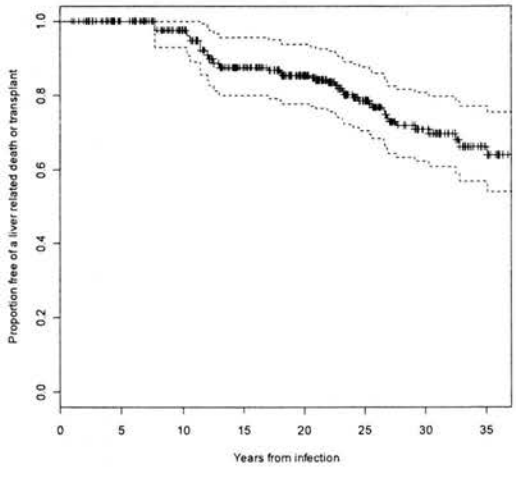
(f)



(g)



(h)



5.3.1.4.2 Median time to complications of cirrhosis

As none of the survival curve for the end-points passed below 0.5, a median could not be calculated.

5.3.1.4.3 Cox regression analysis

5.3.1.4.3.1 Oesophageal varices

The independent risk factors identified for progression to grade 2 oesophageal varices were a previous maximum alcohol intake of greater than 50 units per week,

an older age at infection and the risk category not being intravenous drug use. For first bleeding oesophageal varices they were a previous maximum alcohol intake of greater than 50 units per week and an older age at infection. The relative effect these factors exert (Exp(b)) within the constructed model are documented in Table 50.

Table 50 Results of Cox regression analysis for independent risk factors for progression to (a) Grade 2 oesophageal varices (b) Bleeding oesophageal varices.

(a)

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.026	1.05	1.01	1.09
Previous maximum alcohol intake of greater than 50u/wk	0.0000087	7.77	3.15	19.18
Intravenous drug risk category	0.0078	0.31	0.13	0.74

R square= 0.067 Maximum possible= 0.39

(b)

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.0033	1.10	1.03	1.17
Previous maximum alcohol intake of greater than 50u/wk	0.0010	43.6	4.58	415.07

R square= 0.063 Maximum possible= 0.229

5.3.1.4.3.2 Ascites

The independent risk factors identified for progression to ascites were a previous maximum alcohol intake of greater than 50 units per week, an older age at infection and no previous treatment with an IFN-based regime. The relative effects these factors exert within the constructed model are documented in Table 51.

Table 51 Results of Cox regression analysis for independent risk factors for progression to ascites.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.0000023	1.10	1.06	1.15
Previous maximum alcohol intake of greater than 50u/wk	0.000024	8.42	3.13	22.64
Treated with interferon based regime	0.0029	0.28	0.12	0.65

R square= 0.125 Maximum possible= 0.433

5.3.1.4.3.3 Encephalopathy

The independent risk factors identified for progression to encephalopathy were a previous maximum alcohol intake of greater than 50 units per week and an older age at infection. The relative effects these factors exert within the constructed model are documented in Table 52.

Table 52 Results of Cox regression analysis for independent risk factors for progression to encephalopathy.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.0130	1.08	1.02	1.15
Previous maximum alcohol intake of greater than 50u/wk	0.0053	9.61	1.96	47.08

R square= 0.034 Maximum possible= 0.197

5.3.1.4.3.4 Hepatocellular carcinoma

The independent risk factors identified for progression to HCC were a previous maximum alcohol intake of greater than 50 units per week, an older age at infection and and no previous treatment with an IFN-based regime. The relative effects these factors exert within the constructed model are documented in Table 53.

Table 53 Results of Cox regression analysis for independent risk factors for progression to hepatocellular carcinoma.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.00000065	1.17	1.10	1.24
Previous maximum alcohol intake of greater than 50u/wk	0.0073	8.83	1.80	43.33
Treated with interferon based regime	0.028	0.74	0.05	0.84

R square= 0.1 Maximum possible= 0.225

5.3.1.4.3.5 Any complication of cirrhosis

The independent risk factors identified for progression to any complication of cirrhosis were a previous maximum alcohol intake of greater than 50 units per week and an older age at infection. The relative effects these factors exert within the constructed model are documented in Table 54.

Table 54 Results of Cox regression analysis for independent risk factors for progression to any complication of cirrhosis.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.000000043	1.09	1.05	1.12
Previous maximum alcohol intake of greater than 50u/wk	0.0000034	4.51	2.39	8.51

R square= 0.1 Maximum possible= 0.578

5.3.1.4.3.6 Major complication of cirrhosis

The independent risk factors identified for progression to a major complication of cirrhosis were a previous maximum alcohol intake of greater than 50 units per week, an older age at infection and no previous treatment with an IFN-based regime. The relative effects these factors exert within the constructed model are documented in Table 55.

Table 55 Results of Cox regression analysis for independent risk factors for progression to major complication of cirrhosis.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.000000058	1.01	1.06	1.14
Previous maximum alcohol intake of greater than 50u/wk	0.000012	6.27	2.76	14.23
Treatment with interferon based regime	0.0061	0.36	0.18	0.75

R square= 0.133 Maximum possible= 0.504

5.3.1.4.3.7 Liver transplantation or death

The independent risk factors identified for progression to liver transplantation or liver-related death, were an older age at infection, a previous maximum alcohol intake of greater than 50 units per week, no previous treatment with an IFN-based regime and previous HBV infection. The relative effects these factors exert within the constructed model are documented in Table 56.

Table 56 Results of Cox regression analysis for independent risk factors for progression to liver transplantation or death.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at infection	0.0000000062	1.11	1.07	1.15
Previous maximum alcohol intake of greater than 50u/wk	0.00013	5.98	2.40	14.93
Treatment with an interferon-based regime	0.000056	0.15	0.06	0.38
Previous HBV infection	0.03	2.33	1.08	5.02

R square= 0.164 Maximum possible= 0.459

5.3.2 The time from clinically diagnosed uncomplicated Childs A cirrhosis to the complications of cirrhosis study

5.3.2.1 Cohort demographics

Of the 694 HCV RNA PCR positive patients, 102 met the inclusion and exclusion criteria for this study. The characteristics of this study population are summarised in Table 57.

Table 57 Population characteristics

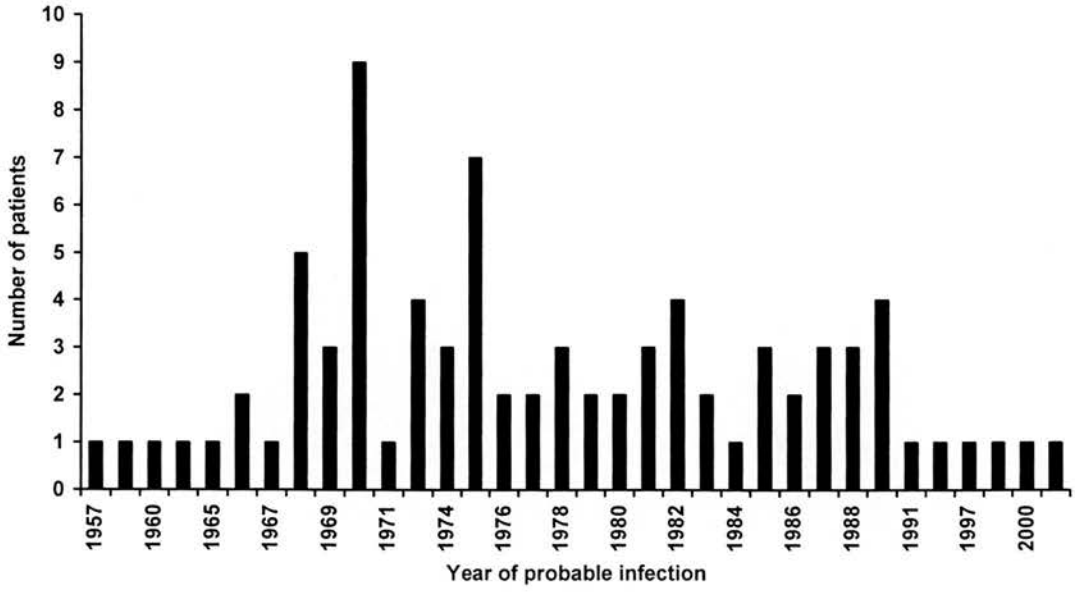
	Number	Percent
Men	80	78.4
Women	22	21.6
Anti HIV positive	9	8.8
Previous hepatitis B infection	35	34.3
Treatment following diagnosis of cirrhosis	61	59.8
Sustained responders to treatment	17	16.7
Previous /current smoker at first assessment	44	33.1
Methadone use at first assessment	15	14.7

5.3.2.2 Variables recorded at first appointment or first admission

5.3.2.2.1 Probable date of HCV infection

The date of probable infection is plotted as a histogram in Figure 25. The modal date of probable infection was 1970.

Figure 25 A histogram of the year of probable HCV infection.



5.3.2.2.2 Age at HCV infection

The median age at infection is 21.9 years for the cohort. The age at infection distribution is positively skewed (Table 58).

Table 58 Descriptive statistics of the continuous variables age at infection, HCV diagnosis and diagnosis of cirrhosis, years from infection to cirrhosis, weight, height and Body Mass Index.

	Age at infection	Age at HCV Diagnosis	Age at diagnosis of cirrhosis	Years from infection to cirrhosis	Weight (kg)	Height (m)	BMI
Valid	83	94	102	83	65	46	42
Missing	19	8	0	19	37	56	60
Mean	25.38	44.32	46.74	21.13	78.21	1.71	26.25
Standard Error	1.30	1.01	0.97	1.01	1.93	0.01	0.74
Median	21.92	43.45	46.39	20.49	76.00	1.72	25.33
Standard Deviation	11.81	9.76	9.77	9.23	15.54	0.08	4.79
Skewness	1.18	0.49	0.65	0.29	0.45	-0.55	0.56
Standard Error of skewness	0.26	0.25	0.24	0.26	0.30	0.35	0.36
Minimum	5.47	22.71	26.03	1.19	52.00	1.52	18.34
Maximum	63.68	74.44	78.01	44.73	120.00	1.89	37.87

Kg: Kilograms, m: metres, BMI: Body Mass Index

There is no significant difference between males and females in their age at infection distribution on Chi-square testing, but there is between those that have IDU as their risk category for infection and those that do not Table 59.

Table 59 Age at HCV infection by IDU risk category patients

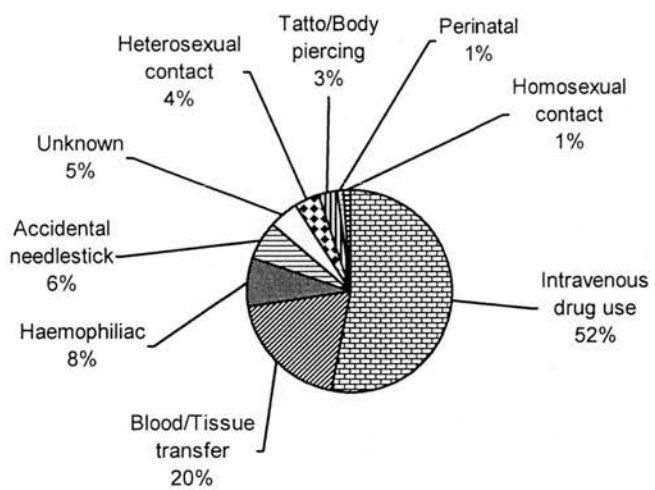
			IDU		Total
			No	Yes	
Age at HCV infection	Under 20 years	Number	8	27	35
		%	22.9%	77.1%	
	20 to 30 years	Number	7	16	23
		%	30.4%	69.6%	
	More than 30 years	Number	20	5	25
		%	80.0%	20.0%	
Total		Number	35	48	83
		%	42.2%	57.8%	

Pearson Chi-square (2df) = 21.32, p-value < 0.0001

5.3.2.2.3 Risk category for infection

52.0% had intravenous drug use as the probable source of infection (Figure 26)

Figure 26 Pie chart risk category for infection



On Chi-square testing there was no difference in the sex ratios of patients categorised by whether their risk category is IDU and non-IDU.

5.3.2.2.4 Ethnicity of patient

Most patients were white (94.0%) with the next commonest racial group being Pakistani (3.9%).

5.3.2.2.5 Genotype of the virus

Seventy-seven patients had their genotype determined of these 40.0% had a genotype 1 virus (Figure 27).

Figure 27 Pie charts of virus genotype.

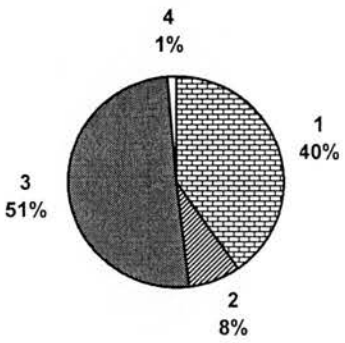


Table 60 shows that the patients genotype is significantly less likely to have been ascertained for patients whose risk category for infection is IDU, but when known, the genotype differs neither by sex nor by IDU / non-IDU status (data not shown).

Table 60 Genotype ascertainment by risk category

Risk category			Genotyped		Total
			No	Yes	
IDU	No	Number	7	41	48
		%	14.6%	85.4%	
	Yes	Number	18	36	54
		%	33.3%	66.7%	
Total		Number	25	77	102
		%	24.5%	75.5%	

Fisher's Exact test P-value = 0.038

5.3.2.2.6 HIV status

Table 61 shows that there is an significant association between HIV infection status and IDU risk category status within this cohort.

Table 61 Risk category by HIV status.

Risk category			HIV		Total
			No	Yes	
IDU	No	Number	48	0	48
		%	100.0%	0%	
	Yes	Number	45	9	54
		%	83.3%	16.7%	
Total		Number	93	9	102
		%	91.2%	8.8%	

Fisher's Exact test P-value =0.003

5.3.2.2.7 HBV infection status

Thirty-five patients have had previous HBV infection, defined as being HBcAb positive, at their first assessment. However, only 7 had HBsAg suggesting ongoing infection. Table 62 shows there is a significant association between previous HBV infection status and IDU risk category status within this cohort.

Table 62 IDU Risk category by HBV status.

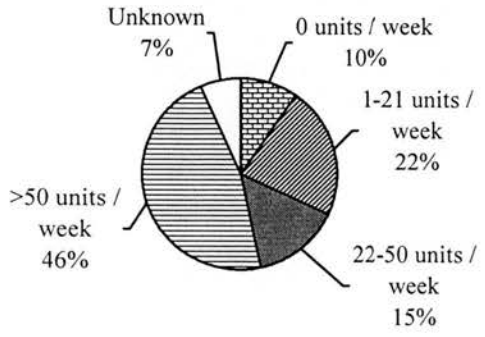
Risk category			Previous HBV		Total
			No	Yes	
IDU	No	Number	38	10	48
		%	79.2%	20.8%	
	Yes	Number	29	25	54
		%	53.7%	46.3%	
Total		Number	67	35	102
		%	65.7%	34.3%	

Fisher's Exact test P-value =0.012

5.3.2.2.8 Previous maximum alcohol intake

The previous maximum alcohol intake, which was maintained for at least 5 years, was obtained in 95 patients. Overall 46.1% had consumed more than 50 units of alcohol per week (Figure 28).

Figure 28 Pie chart of previous maximum alcohol intake.



Previous maximum alcohol consumption does not vary significantly between men and women (Table 63a). For men and women, consumption of more than 50 units per week is the modal category.

Table 63b shows that 76.9.0% of patients with an IDU risk category status compared with only 51.2% of those that do not, have a previous maximum consumption of more than 21 units per week. Chi-square test confirms that the consumption distribution depends also on IDU risk category status (p=0.008).

Table 63 (a) Previous maximum alcohol consumption by sex

			Sex		Total
			Female	Male	
Previous maximum alcohol intake	0 units/week	Number	5	5	10
		%	50.0%	50.0%	
	1-21 units/week	Number	6	17	23
		%	26.1%	73.9%	
	22-50 units/week	Number	2	13	15
		%	13.3%	86.7%	
	>50 units/week	Number	9	38	47
		%	19.1%	80.9%	
Total		Number	22	73	95
		%	23.2%	76.8%	

Pearson Chi-square (3df) =5.40, P-value =0.145

(b) Previous maximum alcohol consumption by IDU risk category status

			IDU		Total
			No	Yes	
Previous maximum alcohol intake	0 units/week	Number	7	3	10
		%	70.0%	30.0%	
	1-21 units/week	Number	14	9	23
		%	60.9%	39.1%	
	22-50 units/week	Number	9	6	15
		%	60.0%	40.0%	
	>50 units/week	Number	13	34	47
		%	27.7%	72.3%	
Total		Number	43	52	95
		%	45.3%	54.7%	

Pearson Chi-square (3df) =11.92, P-value =0.008

5.3.2.2.9 Weight, Height and Body Mass Index

The mean weight at first appointment or admission was 78.2 kilograms and the mean height 1.71 metres (Table 58). The Body Mass Index was calculated in those patients that had both weight and height measurements available.

5.3.2.3 Time from the diagnosis of cirrhosis to the cirrhosis complication end points or last follow up.

The mean and median time from the diagnosis of cirrhosis to the cirrhosis complication or last follow up if the complication did not occur is displayed in Table 64.

Table 64 Descriptive statistics for the time from the diagnosis of cirrhosis to the cirrhosis complication end points or last follow up, if they did not occur.

	Years from diagnosis of cirrhosis to end point or last follow up							
End point	**Grade 2 oesophageal varices**	**Bleeding oesophageal varices**	**Ascites**	**Encephalopathy**	**Hepatocellular carcinoma**	**Any complication of cirrhosis**	**Major complication of cirrhosis**	**Liver related death or transplantaion**
Mean	4.24	4.87	4.84	4.81	4.82	4.06	4.77	4.90
Standard Error of Mean	0.34	0.38	0.37	0.37	0.38	0.33	0.37	0.37
Median	3.39	4.06	4.06	4.03	4.06	3.39	4.06	4.07
Standard Deviation	3.45	3.83	3.74	3.72	3.80	3.33	3.74	3.77
Skewness	0.902	1.048	0.824	1.021	0.968	0.931	0.838	1.02
Standard Error of skewness	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Minimum	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Maximum	15.14	20.27	17.18	19.48	19.30	15.14	17.18	19.79

5.3.2.4 Outcome

The number of patients progressing to each end-point is recorded in Table 65. Two of the liver related deaths occurred in patients that had had a transplant. In one patient the cause of death was not known.

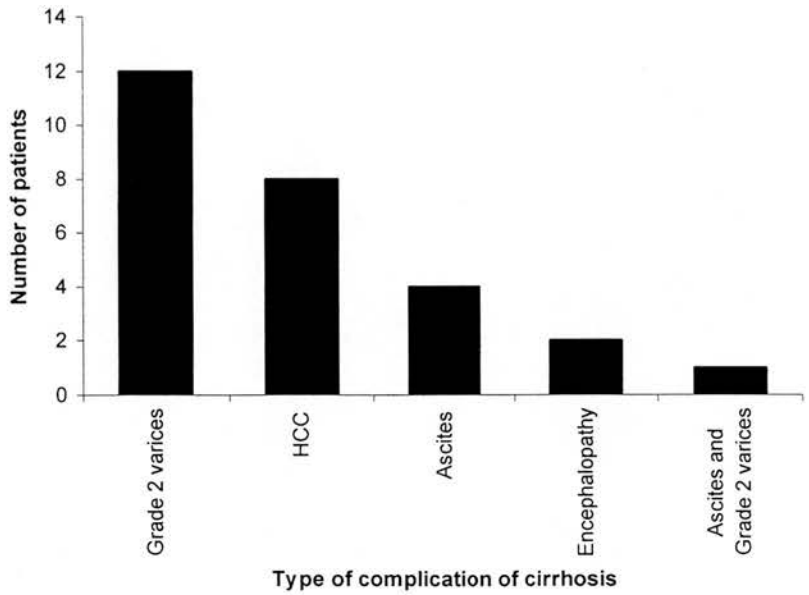
Table 65 Number of patients progressing to the end-points.

End point	Number	Percentage
Grade 2 oesophageal varices	13	12.7
Bleeding oesophageal varices	3	2.9
Ascites	10	9.8
Encephalopathy	5	4.9
Hepatocellular carcinoma	12	11.8
Any cirrhosis complication	27	26.5
Major cirrhosis complication	17	16.7
Liver transplantation	5	4.9
Liver related deaths	13	12.7
Non-liver related deaths	4	3.9
Unknown deaths	1	1.0

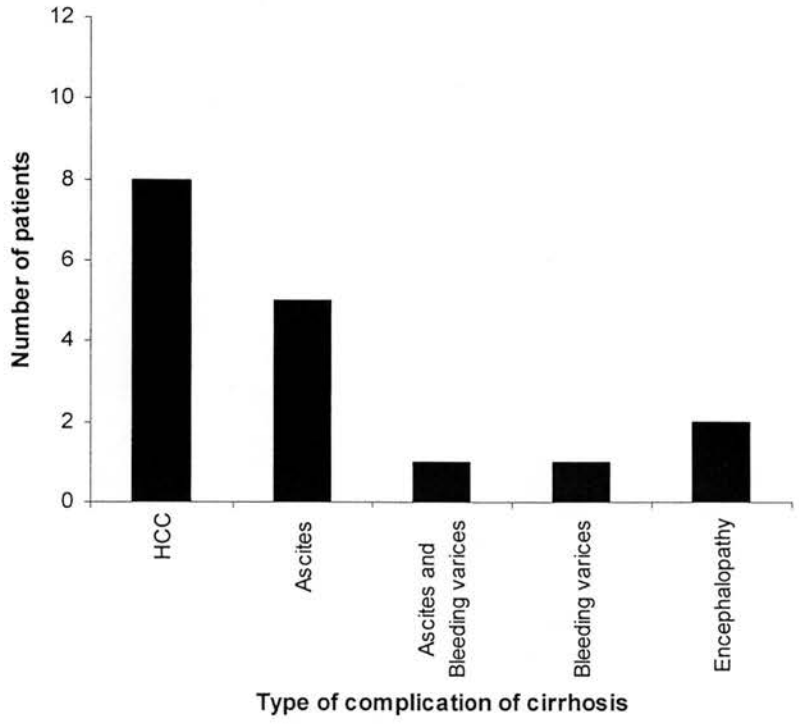
The complication or combinations of complications present at the first diagnosis of any complication of cirrhosis and at the first major complication of cirrhosis are recorded in figure 29a and 29b respectively.

Figure 29 The complication or combinations of complications present at the time of (a) the first diagnosis of any complication of cirrhosis, and (b) the first episode of major complication of cirrhosis.

(a)



(b)

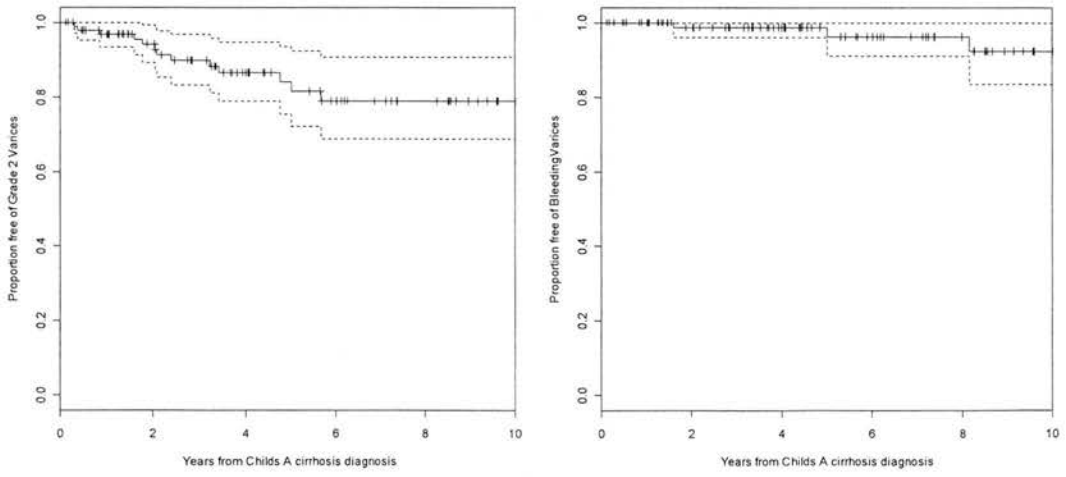


5.3.2.4.1 Kaplan-Meier analysis

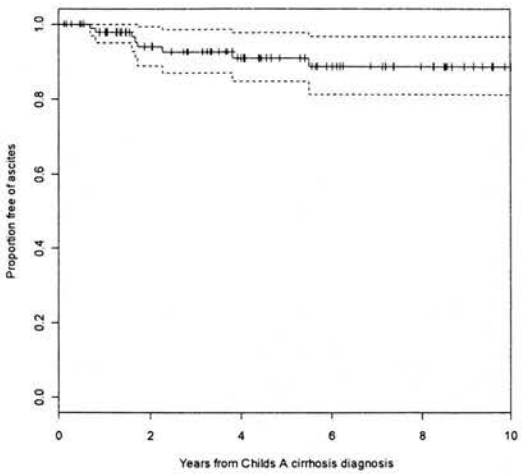
The Kaplan-Meier graph for each of the end-points is plotted with (Figure 30).

Figure 30 Kaplan-Meier graphs, without left truncation at date of first appointment or admission, for the proportion of patients free of (a) grade 2 oesophageal varices (b) bleeding oesophageal varices (c) ascites (d) encephalopathy (e) hepatocellular carcinoma (f) any complication of cirrhosis, (g) a major complication of cirrhosis (h) liver related death or liver transplantation. The survival curve was curtailed when five patients are left at risk. Dotted lines represent 95% confidence intervals.

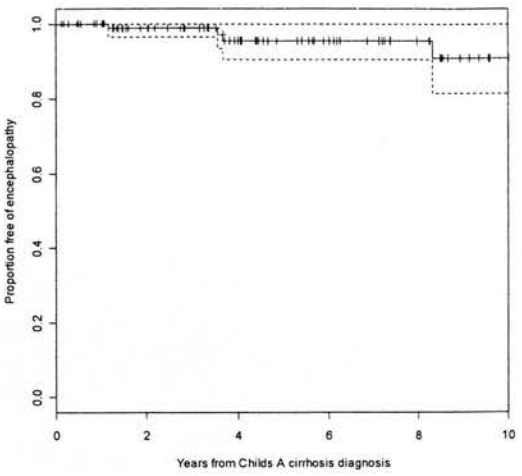
(a) (b)



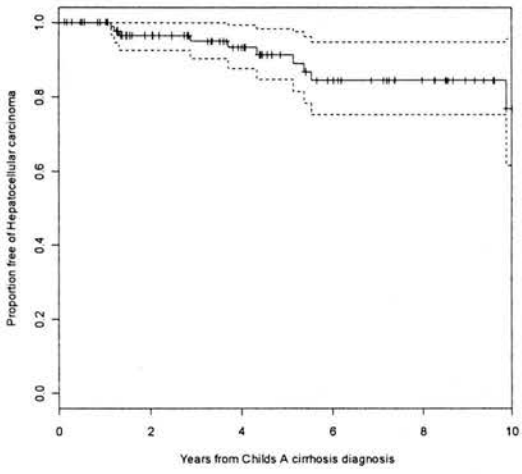
(c)


Proportion free of ascites
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from Childs A cirrhosis diagnosis

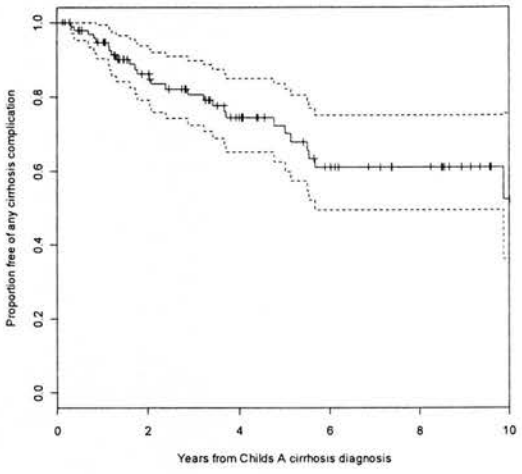
(d)


Proportion free of encephalopathy
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from Childs A cirrhosis diagnosis

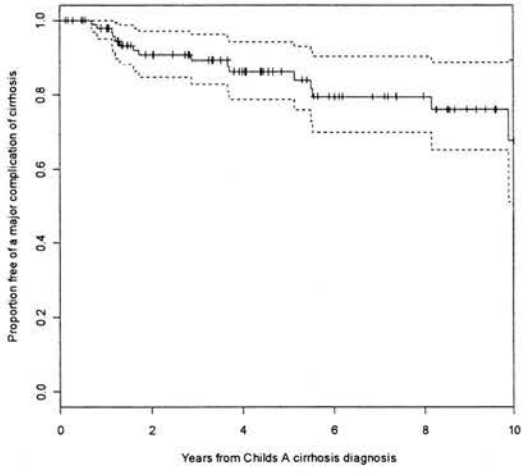
(e)


Proportion free of Hepatocellular carcinoma
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from Childs A cirrhosis diagnosis

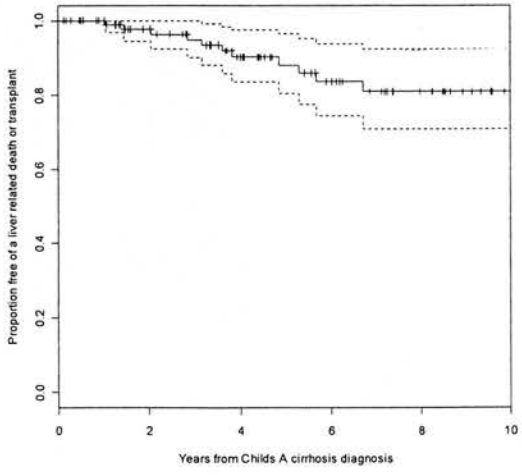
(f)


Proportion free of any cirrhosis complication
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from Childs A cirrhosis diagnosis

(g)


Proportion free of a major complication of cirrhosis
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from Childs A cirrhosis diagnosis

(h)


Proportion free of a liver related death or transplant
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from Childs A cirrhosis diagnosis

5.3.2.4.2 Median time to endpoint

As none of the survival curve for the end-points passed below 0.5, a median could not be calculated.

5.3.2.4.3 Cox regression analysis

5.3.2.4.3.1 Oesophageal varices

No independent risk factors identified for progression to grade 2 oesophageal varices. Previous HBV infection was the closest to approach significance with a p value of 0.065. For first bleeding oesophageal varices no independent risk factors could be identified due to the low number of events.

5.3.2.4.3.2 Ascites

The independent risk factor identified for progression to ascites no previous treatment with an IFN-based regime. The relative effect that this factor exerts (Exp(b)) within the constructed model is documented in Table 66.

Table 66 Results of Cox regression analysis for independent risk factors for progression to ascites.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Previous treatment with interferon-based regime	0.023	0.16	0.032	0.77

R square= 0.061 Maximum possible= 0.496

5.3.2.4.3.3 Encephalopathy

No independent risk factors could be identified due to the low number of events.

5.3.2.4.3.4 Hepatocellular carcinoma

The independent risk factor identified for progression to HCC was an older age at diagnosis of Childs A cirrhosis. The relative effect this factor exerts within the constructed model are documented in Table 67.

Table 67 Results of Cox regression analysis for independent risk factors for progression to hepatocellular carcinoma.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at diagnosis of Childs A cirrhosis	0.00023	1.10	1.05	1.16

R square= 0.116 Maximum possible= 0.546

5.3.2.4.3.5 Any complication of cirrhosis

The independent risk factors identified for progression to any complication of cirrhosis were an older age at diagnosis of Childs A cirrhosis and previous HBV infection. The relative effects these factors exert within the constructed model are documented in Table 68.

Table 68 Results of Cox regression analysis for independent risk factors for progression to any complication of cirrhosis.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at diagnosis of cirrhosis	0.0320	1.09	1.00	1.09
Previous HBV infection	0.0061	4.87	1.39	7.34

R square= 0.089 Maximum possible= 0.878

5.3.2.4.3.6 Major complication of cirrhosis

The independent risk factors identified for progression to a major complication of cirrhosis were an older age at diagnosis of cirrhosis and a previous maximum alcohol intake of greater than 50 units per week. The relative effects these factors exert within the constructed model are documented in Table 69.

Table 69 Results of Cox regression analysis for independent risk factors for progression to major complication of cirrhosis.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at diagnosis of cirrhosis	0.0079	1.08	1.02	1.15
Previous maximum alcohol intake of greater than 50u/wk	0.0140	4.97	1.38	17.88

R square= 0.104 Maximum possible= 0.732

5.3.2.4.3.7 Liver transplantation or death

The independent risk factors identified for progression to liver transplantation or death were an older age at diagnosis of cirrhosis and no previous treatment with an IFN based regime. The relative effects these factors exert within the constructed model are documented in Table 70.

Table 70 Results of Cox regression analysis for independent risk factors for progression to liver transplantation or death.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at diagnosis of cirrhosis	0.030	1.06	1.01	1.11
Previous treatment with interferon-based regime	0.028	0.21	0.05	0.84

R square= 0.13 Maximum possible= 0.588

5.3.3 The time from biopsy or laparoscopy proven uncomplicated Childs A cirrhosis to the complications of cirrhosis study

5.3.3.1 Cohort demographics

Of the 694 HCV RNA PCR positive patients, 41 met the inclusion and exclusion criteria for this study. The characteristics of this study population are summarised in Table 71.

Table 71 Population characteristics.

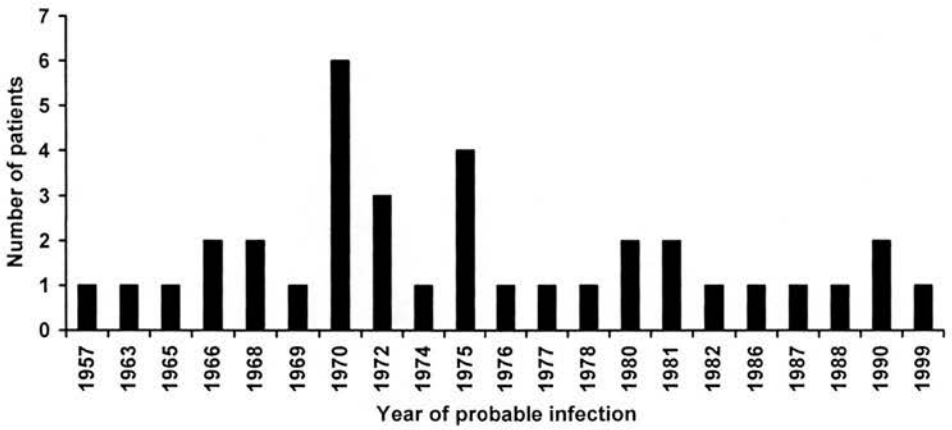
	Number	Percent
Men	33	80.5
Women	8	19.5
Anti HIV positive	6	14.6
Previous hepatitis B infection	17	41.5
Treatment following initial assessment	24	58.5
Sustained responders to treatment	6	14.6
Previous /current smoker at first assessment	20	48.8
Methadone use at first assessment	7	17.1

5.3.3.2 Variables recorded at first appointment or first admission

5.3.3.2.1 Probable date of HCV infection

The date of probable infection is plotted as a histogram in Figure 31.

Figure 31 A histogram of the year of probable HCV infection.



5.3.3.2.2 Age at HCV infection

The median age at infection are 18.3 years for the cohort. The age at infection distribution is positively skewed (Table 30).

Table 72 Descriptive statistics of the continuous variables age at infection, HCV diagnosis and diagnosis of cirrhosis, years from infection to cirrhosis, weight, height and Body Mass Index.

	Age at infection	Age at HCV Diagnosis	Age at diagnosis of cirrhosis	Years from infection to cirrhosis	Weight (kg)	Height (m)	BMI
Valid	36	39	41	36	25	17	15
Missing	5	2	0	5	16	24	26
Mean	22.23	43.90	45.03	22.31	78.52	1.74	26.64
Standard Error of Mean	1.99	1.34	1.36	1.49	3.49	0.01	1.465 80
Median	18.27	43.41	43.86	22.21	75.00	1.74	25.10
Standard Deviation	11.94	8.38	8.70	8.94	17.46	0.05	5.68
Skewness	1.78	0.56	0.59	0.03	0.79	0.51	0.63
Standard Error of skewness	0.39	0.38	0.37	0.39	0.46	0.55	0.58
Minimum	5.47	28.90	29.56	4.08	52.00	1.66	19.38
Maximum	63.66	68.35	68.56	42.42	120.00	1.86	37.87

Kg: Kilograms, m: metres, BMI: Body Mass Index

There is no significant difference between males and females in their age at infection distribution on Chi-square testing, but there is between those that have IDU as their risk category for infection and those that do not (Table 73).

Table 73 Age at HCV infection by IDU risk category patients.

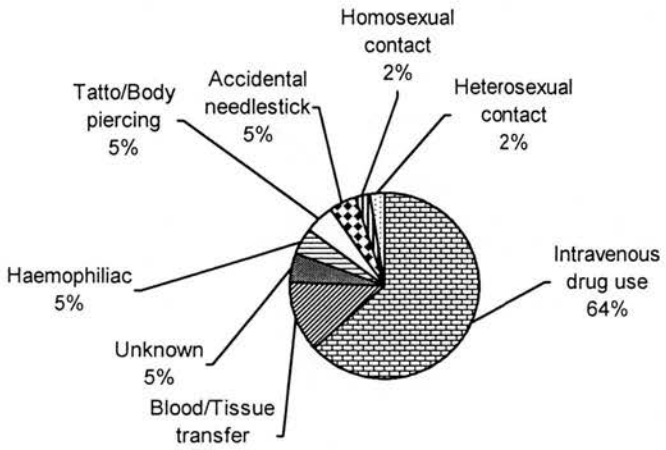
			IDU		Total
			No	Yes	
Age at HCV infection	**Under 20 years**	**Number**	5	18	23
		%	21.7%	78.3%	
	20 to 30 years	**Number**	0	6	6
		%	.0%	100.0%	
	More than 30 years	**Number**	5	2	7
		%	71.4%	28.6%	
Total		**Number**	10	26	36
		%	27.8%	72.2%	

Pearson Chi-square (2df) = 9.374, p-value = 0.009

5.3.3.2.3 Risk category for infection

70.0% had intravenous drug use as the probable source of infection (Figure 32)

Figure 32 Pie chart risk category for infection



On Chi-square testing there is no difference in the sex ratios of patients categorised by whether their risk category is IDU and non-IDU.

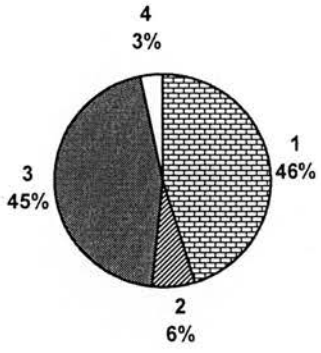
5.3.3.2.4 Ethnicity of patient

Most patients were white (95.1%).

5.3.3.2.5 Genotype of the virus

Thirty-one patients had their genotype determined, of these 46.0% had a genotype 1 virus (Figure 33).

Figure 33 Pie charts of virus genotype.



The patient's genotype is equally likely to have been ascertained for patients whose risk category for infection is IDU.

5.3.3.2.6 HIV status

Table 74 shows that like the HCV RNA PCR positive cohort there is an association that approaches significance between HIV infection status and IDU risk category status.

Table 74 Risk category by HIV status.

			HIV		Total
			No	Yes	
IDU	No	Number	15	0	15
		%	100.0%	.0%	
	Yes	Number	20	6	26
		%	76.9%	23.1%	
Total		Number	35	6	41
		%	85.4%	14.6%	

Fisher's Exact test P-value =0.070

5.3.3.2.7 HBV infection status

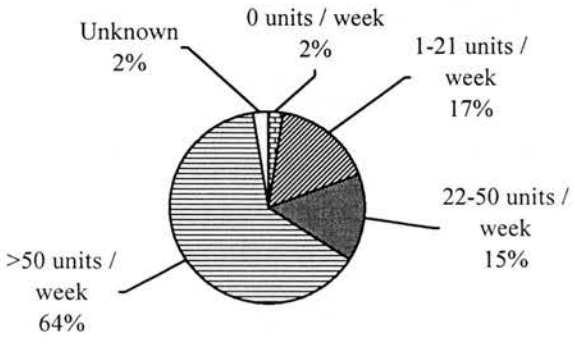
Seventeen patients have had previous HBV infection, defined as being HBcAb positive, at their first assessment. However, only 1 had HBsAg suggesting lack of

clearance of the virus. Previous HBV infection status is not significantly associated with IDU risk category status within this cohort.

5.3.3.2.8 Previous maximum alcohol intake

The previous maximum alcohol intake, which was maintained for at least 5 years, was obtained in 40 patients. Overall 63.5% had consumed more than 50 units of alcohol per week (Figure 34).

Figure 34 Pie chart of previous maximum alcohol intake.



Previous maximum alcohol consumption does not vary significantly between men and women in this cohort. For men and women, consumption of more than 50 units per week is still the modal category. The consumption distribution does not depend on IDU risk category status in this cohort either.

5.3.3.2.9 Weight, Height and Body Mass Index

The mean weight at first appointment or admission was 78.5 kilograms and the mean height 1.74 metres (Table 72). The Body Mass Index was calculated in those patients that had both weight and height measurements available.

5.3.3.3 Time from infection to first appointment or admission or cirrhosis complication end points

The mean and median time from the diagnosis of cirrhosis to the cirrhosis complication or last follow up if the complication did not occur is displayed in Table 75.

Table 75 Descriptive statistics for the time from the diagnosis of biopsy proven cirrhosis to the cirrhosis complication end points or last follow up, if they did not occur.

	Years from diagnosis of biopsy proven cirrhosis to end point or last follow up							
End point	**Grade 2 oesophageal varices**	**Bleeding oesophageal varices**	**Ascites**	**Encephalopathy**	**Hepatocellular carcinoma**	**Any complication of cirrhosis**	**Major complication of cirrhosis**	**Liver related death or transplantaion**
Mean	4.57	5.16	5.06	5.15	5.09	4.47	4.99	5.07
Standard Error of Mean	0.59	0.70	0.65	0.69	0.69	0.59	0.66	0.68
Median	3.22	4.08	4.08	4.08	4.08	3.22	4.08	4.08
Standard Deviation	3.75	4.48	4.21	4.41	4.42	3.75	4.25	4.38
Skewness	0.91	1.27	1.01	1.18	1.17	0.97	1.01	1.29
Standard Error of skewness	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Minimum	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Maximum	15.14	20.27	17.18	19.48	19.30	15.14	17.18	19.79

5.3.3.4 Outcome

The number of patients progressing to each end-point is recorded in Table 76. One of the liver related deaths occurred after liver transplantation.

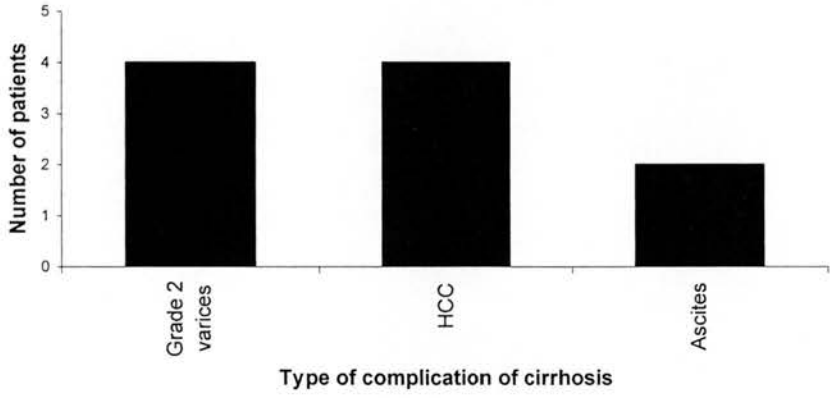
Table 76 Number of patients progressing to the end-points.

End point	Number	Percentage
Grade 2 oesophageal varices	4	9.8
Bleeding oesophageal varices	0	0
Ascites	4	9.8
Encephalopathy	1	2.4
Hepatocellular carcinoma	5	12.2
Any cirrhosis complication	10	24.4
Major cirrhosis complication	7	17.1
Liver transplantation	3	7.3
Liver related deaths	4	9.8
Non-liver related deaths	3	7.3

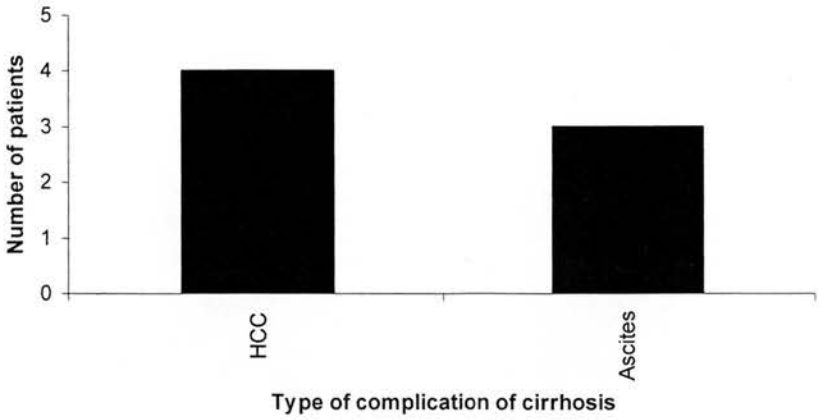
The complication or combinations of complications present at the first diagnosis of any complication of cirrhosis and at the first major complication of cirrhosis are recorded in figure 35a and 35b respectively.

Figure 35 The complication or combinations of complications present at the time of (a) the first diagnosis of any complication of cirrhosis, and (b) the first major complication of cirrhosis.

(a)



(b)

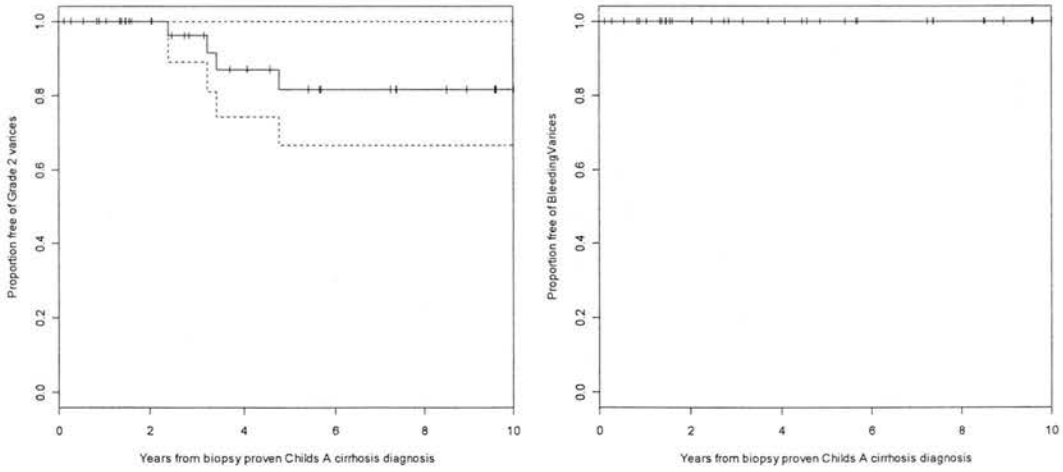


5.3.3.4.1 Kaplan-Meier analysis

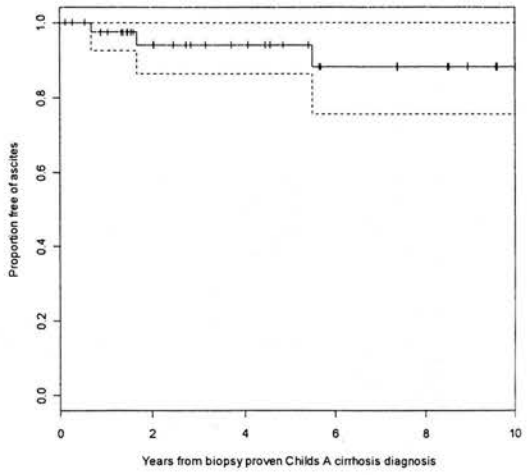
The Kaplan-Meier graph for each of the end-points is plotted with (Figure 19) and without (Figure 36) left truncation at date of first appointment or admission.

Figure 36 Kaplan-Meier graphs, without left truncation at date of first appointment or admission, for the proportion of patients free of (a) grade 2 oesophageal varices (b) bleeding oesophageal varices (c) ascites (d) encephalopathy (e) hepatocellular carcinoma (f) any complication of cirrhosis, (g) a major complication of cirrhosis (h) liver related death or liver transplantation. The survival curve was curtailed when five patients are left at risk. Dotted lines represent 95% confidence intervals.

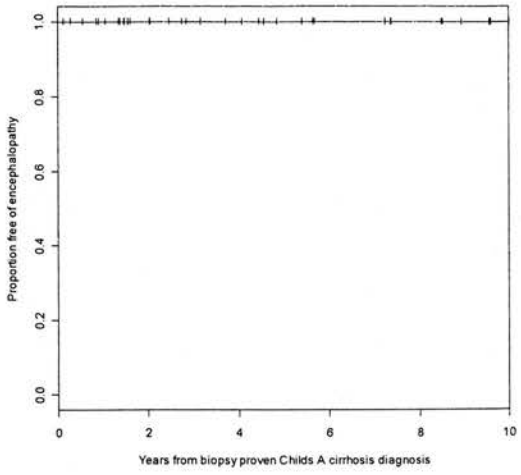
(a) (b)



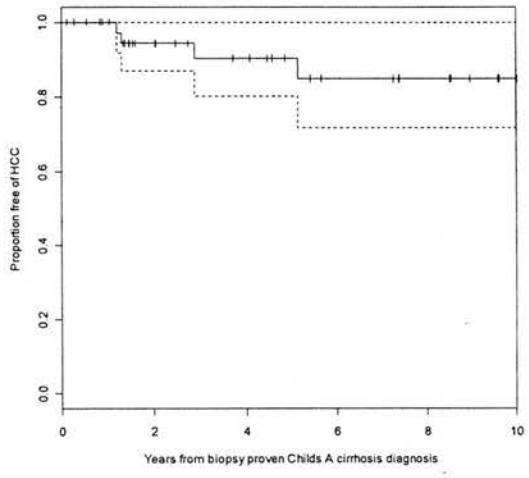
(c)


Proportion free of ascites
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from biopsy proven Childs A cirrhosis diagnosis

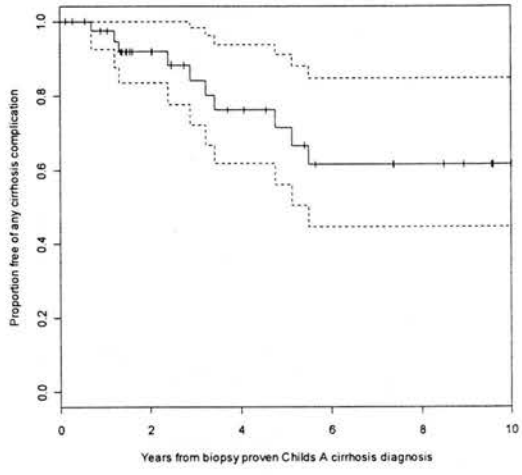
(d)


Proportion free of encephalopathy
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from biopsy proven Childs A cirrhosis diagnosis

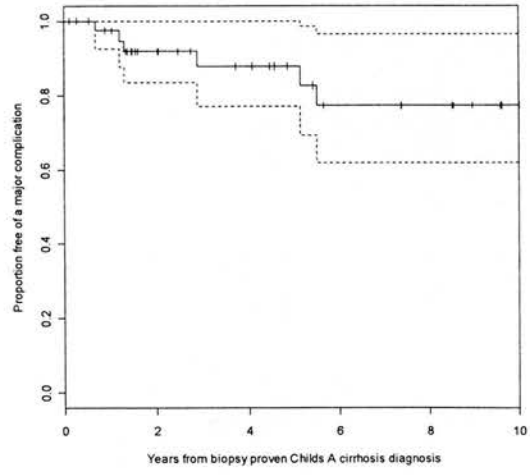
(e)


Proportion free of HCC
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from biopsy proven Childs A cirrhosis diagnosis

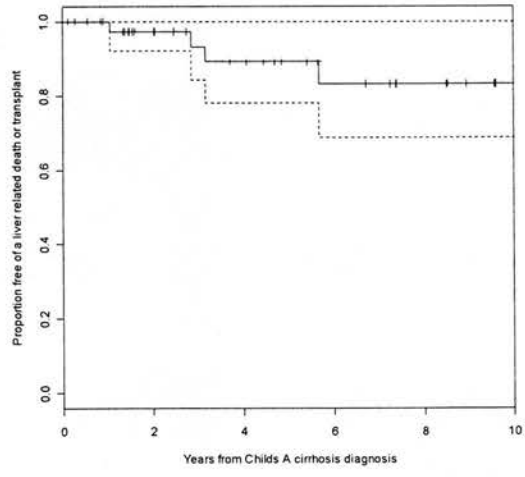
(f)


Proportion free of any cirrhosis complication
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from biopsy proven Childs A cirrhosis diagnosis

(g)


Proportion free of a major complication
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from biopsy proven Childs A cirrhosis diagnosis

(h)


Proportion free of a liver related death or transplant
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Years from Childs A cirrhosis diagnosis

5.3.3.4.2 Median time to endpoints

As none of the survival curve for the end-points passed below 0.5, a median could not be calculated.

5.3.3.4.3 Cox regression analysis

5.3.3.4.3.1 Oesophageal varices

For progression to grade 2 oesophageal varices no independent risk factors could be identified due to the low number of events. For first bleeding oesophageal varices no independent risk factors could be identified, as there were no events.

5.3.3.4.3.2 Ascites

No independent risk factors could be identified due to the low number of events.

5.3.3.4.3.3 Encephalopathy

No independent risk factors could be identified, as there were no events.

5.3.3.4.3.4 Hepatocellular carcinoma

The independent risk factor identified for progression to HCC was an older age at diagnosis of biopsy proven Childs A cirrhosis. The relative effect this factor exerts (Exp(b)) within the constructed model is documented in Table 77.

Table 77 Results of Cox regression analysis for independent risk factors for progression to hepatocellular carcinoma.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at diagnosis of biopsy proven Childs A cirrhosis	0.0096	1.30	1.07	1.58

R square= 0.253 Maximum possible= 0.471

5.3.3.4.3.5 Any complication of cirrhosis

The independent risk factor identified for progression to any complication of cirrhosis was an older age at diagnosis of biopsy proven Childs A cirrhosis. The relative effect this factor exerts within the constructed model is documented in Table 78.

Table 78 Results of Cox regression analysis for independent risk factors for progression to any complication of cirrhosis.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at diagnosis of biopsy proven cirrhosis	0.015	1.11	1.03	1.20

R square= 0.134 Maximum possible= 0.781

5.3.3.4.3.6 Major complication of cirrhosis

The independent risk factors identified for progression to a major complication of cirrhosis was an older age at diagnosis of biopsy proven Childs A cirrhosis. The relative effect this factor exerts within the constructed model is documented in Table 79.

Table 79 Results of Cox regression analysis for independent risk factors for progression to major complication of cirrhosis.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at diagnosis of biopsy proven cirrhosis	0.0092	1.15	1.04	1.29

R square= 0.167 Maximum possible= 0.612

5.3.3.4.3.7 Liver transplantation or death

The independent risk factors identified for progression to liver transplantation or death were an older age at diagnosis of biopsy proven Childs A cirrhosis and no

previous treatment with an IFN-based regime. The relative effects these factors exert within the constructed model are documented in Table 80.

Table 80 Results of Cox regression analysis for independent risk factors for progression to liver transplantation or death.

Variable	Significance (p value)	Exp(B)	95% Confidence Interval	
			Lower	Upper
Age at diagnosis of biopsy proven cirrhosis	0.0095	1.18	1.04	1.34
Previous treatment with interferon-based regime	0.0330	0.12	0.02	0.85

R square= 0.356 Maximum possible= 0.648

5.4 Discussion

This study attempts to define the onset of the complications of cirrhosis in three cohorts of HCV RNA PCR positive patients referred and followed up in a regional centre in Scotland.

5.4.1 The time from infection to the complications of cirrhosis study

The infection to cirrhosis complications cohort is a subset of the whole population of HCV RNA PCR positive patients. Therefore for this analysis to be applicable to this whole population, this cohort has to be reasonable matched to it.

In the cohort, 71% of patients were likely to have acquired the infection through IDU. This is slightly higher than the whole HCV RNA PCR positive population. This reflects the inclusion criteria of a probable date of infection, which excludes those in whom it was not possible to identify a probable source of infection. Proportionately more men acquired the infection through IDU compared with women but it does not quite reach significance. As with the whole population, those that acquired the infection through drug use did so at a significantly younger age. Unlike the whole

population there was no significant difference between men and women in their age at infection. A higher percentage (56%) had genotype 1 infection compared with the whole population. IDU patients were significantly less likely to have been tested for genotype. The cohort had a similar prevalence of HIV co-infection and previous HBV infection at 7.3% and 30.9% respectively. The median weight and BMI were also similar. A lower percentage of all patients (34.5%) but the same percentage of men (50.5%), have drunk in excess of 50 units of alcohol for more than five years prior to presentation. There was a significant difference between men and women in this covariate, and those that acquired the infection through IDU and those that did not.

The median time from infection to first appointment or admission was about 17 years, which is no different from the HCV RNA PCR positive population. Those that presented a longer time after infection were more likely to have a higher BMI. The older the age at infection, the shorter the time from infection to first presentation. Those that had drunk more than 50 units / week presented later after infection than those that drank less than 21 unit / week. Those with HIV co-infection presented significantly earlier after infection than those that did not, while men presented significantly later than women. The median time from infection to the censored time-point was about 21.6 years for all end-points.

The end-point 'Any complication of cirrhosis' occurred in 8.9% of the cohort, while 'A major complication of cirrhosis' occurred in 7.3%. At least Grade 2 oesophageal varices developed in 5.1% and 2.5% had a variceal bleed. HCC occurred in 2.7% and a liver related death or transplantation in 6.5%.

In none of the Kaplan-Meier graphs, without truncation, did the survival line pass below the 0.5 proportion and therefore a median time to the end-point could not be calculated. However from the graphs it can be deduced that it would be in excess of 35 years in all end-points. When left-truncation at the time of presentation was used the survival curves fall more steeply but only in the 'Any complication of cirrhosis' end-point does it appear to approach a median at approximately 35 years.

Cox regression analysis with left-truncation was performed to establish independent predictors of progression to each end-point. For every end-point it was found to be an older age at infection and a previous alcohol intake of greater than 50 units / week. No previous treatment with an IFN-based regime was an independent predictor for the end-points; 'Ascites', 'HCC', 'A major complication of cirrhosis' and 'Liver death or transplantation'. Not having IDU as the risk factor for infection was an independent predictor of progression to grade 2 oesophageal varices. Finally previous HBV infection was an independent predictor of liver-related death or transplantation.

5.4.2 The time from clinically diagnosed uncomplicated Childs A cirrhosis to the complications of cirrhosis study

Not surprisingly the clinically diagnosed cirrhotic cohort was different from the whole HCV RNA PCR positive population, as only a select proportion progress to this starting point. A larger proportion was male (79% versus 69%), a larger proportion had IFN-based treatment (60% versus 39%), fewer were currently smoking or had smoked (33% versus 50%) and a larger proportion had acquired the infection through IDU (52% versus 64%). There was no significant difference in the sex ratios of patients categorised by whether their risk category is IDU and non-IDU.

A smaller proportion had genotype 1 infection (40% versus 52%) and both the median weight and BMI were slightly higher (76kg versus 70kg and 25.3 versus 23.8). A larger percentage of all patients (46% versus 42%) but the same percentage of men had drunk in excess of 50 units of alcohol for more than five years prior to presentation. There was a significant difference between men and women in this covariate, and those that acquired the infection through IDU and those that did not.

The median time to the censored time-point depended on the end-point. It varied from 3.39 years for the 'Grade 2 oesophageal varices' end-point to 4.07 years for 'Liver-related death or transplantation' end-point.

'Any complication of cirrhosis' occurred in 26.5% of the cohort, while 'A major complication of cirrhosis' occurred in 16.7%. Grade 2 oesophageal varices developed in 12.7% and 2.9% had a variceal bleed. HCC occurred in 11.8% and a liver related death or transplantation in 17.6%.

In none of the Kaplan-Meier graphs did the survival line pass below the 0.5 proportion and therefore a median time to the end point could not be calculated. However from the graphs it can be deduced that it would be in excess of 10 years in all end-points.

Cox regression analysis was performed to establish independent predictors of progression to each end-point. For every end-point it was found to be an older age at diagnosis of Childs A cirrhosis except 'Grade 2 oesophageal varices', 'Encephalopathy' and 'Ascites'. No previous treatment with an IFN-based regime was an independent predictor in 'Ascites' and 'Liver death or transplantation'. A previous alcohol intake of greater than 50 unit / week was an independent predictor of the 'A major complication of cirrhosis' end-point. Previous HBV infection was an

independent predictor of the 'Any complication of cirrhosis end-point'. No independent predictors of progression to the end-points 'Grade 2 oesophageal varices' or 'Bleeding varices' or 'Encephalopathy'.

5.4.3 The time from biopsy or laparoscopy proven uncomplicated Childs A cirrhosis to the complications of cirrhosis study

The biopsy- or laparoscopy-proven cirrhotic cohort is a subset of the clinically diagnosed cirrhotic cohort. The inclusion criteria for this cohort produces some differences between it and the larger clinically diagnosed cohort. A larger proportion was HIV co-infected (15% versus 9%), had acquired the infection through IDU (64% versus 52%) and had genotype 1 infection (46% versus 40%). A larger proportion of all patients (64% versus 46%) and of men (66% versus 52%) had drunk in excess of 50 units of alcohol for more than five years prior to presentation. There was a no significant difference between men and women in this covariate, and those that acquired the infection through IDU and those that did not.

The median time to the censored time-point depended on the end-point. It varied from 3.32 years for the 'Grade 2 oesophageal varices' end-point to 4.08 years for 'Liver-related death or transplantation' end-point.

'Any complication of cirrhosis' occurred in 24.4% of the cohort, while 'A major complication of cirrhosis' occurred in 17.1%. Grade 2 oesophageal varices developed in 9.8% and none had a variceal bleed. HCC occurred in 12.2% and a liver related death or transplantation in 17.1%. In none of the Kaplan-Meier graphs did the survival line pass below the 0.5 proportion and therefore a median time to the end point could not be calculated. However from the graphs it can be deduced that it would be in excess of 10 years in all end-points.

Cox regression analysis was performed to establish independent predictors of progression to each end-point. For every end-point it was found to be an older age at diagnosis of Childs A cirrhosis except 'Grade 2 oesophageal varices', 'Encephalopathy' and 'Ascites', where no independent predictors of progression to these end-points could be found. No previous treatment with an IFN-based regime was an independent predictor for 'Liver-related death or transplantation'.

5.4.4 Comparison with previous literature

There have been some previous retrospective studies to establish the progression from infection to cirrhosis complications (Makris et al. 1996) or HCC (Tong et al. 1995), (Roudot-Thoraval et al. 1997), (Kiyosawa et al. 1990). None have used Kaplan-Meier survival analysis. Often misleadingly a mean interval from infection to the end-point, not based on Kaplan-Meier analysis has been quoted (Kiyosawa et al. 1990), (Tong et al. 1995). There are two community-based studies that approximate to the cohort analysed in this chapter in their basic characteristics. An American study drawn from an area of high drug use showed at mean duration of 23 years, 2.4% had developed a complication of cirrhosis but no HCC had been diagnosed. In a multivariate model, risk of a complication of cirrhosis was higher for persons aged 38 years or older at enrollment and who reported ingestion of more than 260 g of alcohol per week (Thomas et al. 2000). In the Australian acute hepatitis study at a mean duration of infection of 23 years, 6% had a complication of cirrhosis but again no HCC had been diagnosed (Rodger et al. 2000). As with all community-based studies the incidence of the complications of cirrhosis are much lower compared with hospital studies. This is the case in the infection to cirrhosis complication cohort.

There have been a number of studies describing the natural history of a cohort of uncomplicated cirrhotics with HCV that are described in the introduction. The Italian study of 254 uncomplicated compensated cirrhotic patients (biopsy-proven and clinically determined) most closely follows the methodology of the clinically diagnosed cirrhotic group used in this chapter. It followed-up patients for a median of 7.6 years and 30.7% developed at least one complication of cirrhosis. HCC developed in 20.5% and was the commonest first complication. 17.7% developed ascites and 4.7% had a variceal bleed and 16.3% died a liver-related death (Benvegnu et al. 2004). During follow-up 45.3% received IFN-based therapy and a lower rate of complications were seen in this group (25.2% versus 35.9%).

The study that most closely resembles the biopsy- or laparoscopy- proven cirrhotic cohort followed 112 patients for a median of 4.5 years. It included 49 patients who were treated with IFN. The cumulative probabilities for decompensation and development of HCC were 22.2% and 10.1% in five years, with an estimated yearly incidence of 4.4% and 2.0% respectively (Hu & Tong 1999). The cumulative survival probability was 82.8% from entry and 51.1% from decompensation in five years. Independent variables predicting decompensation were albumin level and older age at infection. As albumin is a known marker for the severity of cirrhosis, its influence may simply have been due to this (those with lower albumins had more advanced cirrhosis at recruitment for the study and required less progression to get reach decompensation). The largest study to date, in biopsy-proven cirrhotics with no complications of cirrhosis followed 255 for a median period of 7 years (Fattovich et al. 2001). Kaplan-Meier 5-year risk of HCC was 5%, of decompensation was 12%

and of event-free survival was 85%. Genotype 1b did not affect the occurrence of HCC, but did increase the risk of liver decompensation three fold.

To attempt a comparison with the two cirrhotic cohorts described in this chapter, the proportion at five years after the diagnosis of uncomplicated cirrhosis with these reported end-points has been estimated from the Kaplan-Meier graphs. The risk of HCC is about 9-12%, the risk of a major complication of cirrhosis is about 11% and the risk of liver related death is about 8-10%.

5.4.5 Predicting occurrence of complications

Age at the diagnosis of cirrhosis consistently was associated with progression to the complications of cirrhosis in all three cohorts. Presumably this is a similar to the effect of age pre-cirrhosis (Poynard et al. 1997). However this might be confounded if the older the patient the more likely they are to have had the infection for longer and therefore potentially the more advanced the disease, despite the efforts to have a homogeneous starting population.

The role of IFN-based treatment in preventing the complications of cirrhosis is controversial. The concern has always been that the association with a better prognosis reported in studies above (Serfaty et al. 1998) may simply reflect those with less severe disease being more likely to receive treatment. A meta-analysis of similar studies has also suggested the effect is low and the observed benefit might be due to spurious associations (Camma et al. 2001). However, although in the clinical diagnosis of cirrhosis cohort a SVR to treatment was not statistically demonstrated to independently influence progression to HCC, no HCC developed in those that achieved an SVR (data not shown).

As discussed in chapter 4, alcohol can cause cirrhosis and its complications alone, therefore it has been important to establish if its effect in patients with HCV is purely additive or synergistic. There is some evidence that it hastens the development of HCC (Donato et al. 1997), especially in association with Diabetes Mellitus (Hassan et al. 2002) and reduces survival (Wiley et al. 1998),(Fattovich et al. 1997),(Niederau et al. 1998). In this study previous alcohol use was documented to identify a history of alcohol abuse, as discussed in chapter 3 this is an estimate and with a potential for error. There was no measure of the alcohol ingested during the course of the study. So those that may have abused alcohol in the past may have heeded the recommendations to moderate intake at the initial assessment, making this covariate a poor surrogate marker for ongoing excessive alcohol use. Alcohol intake at the time of diagnosis of cirrhosis was also analysed as a covariate to predict progression to end-points but was not found to be a significant in Cox regression (data not shown).

Obesity does not appear to be an additional risk factor for HCC in HCV (Nair et al. 2002). This is confirmed by the studies described in this chapter where BMI was not found to be an independent predictor of any of the end-points.

This study has suggested a number of independent risk factors for progression that are difficult to explain such as a non-IDU risk category for progression to grade 2 oesophageal varices. Whilst these are valid statistically, it is likely they have come about due the significant correlations between the tested covariates despite the use of a multivariate method or potentially with untested ones. The R square scores are all low compared with the maximum possible and therefore the models and covariates used do not explain well the variation in progression seen.

5.4.6 Limitations of the study

Patients diagnosed with asymptomatic, grade 2 and above oesophageal varices were included for the end-point 'Any complication of cirrhosis', as they may require medical intervention. This diagnosis is therefore dependent on an endoscopy being performed, and therefore the accuracy of diagnosing their onset maybe limited. However with the oesophageal varices surveillance program described in the methods section, the time lag from onset to diagnosis is minimised. Primary prophylaxis was also practiced on these patients, which might explain the low incidence of bleeding varices. Secondary prophylaxis may also have reduced deaths from bleeding.

A significant proportion of all cohorts underwent treatment during the follow-up period, and this was an independent risk factor in progression to a number of end-points in Cox regression analysis.

The entry requirement for the infection to cirrhosis complications study, of having a probable date of infection, has led to a slight overrepresentation of patients thought to have acquired the infection through intravenous drug abuse. In these patients the duration of infection has to be an estimate, as it is based on the assumption that infection is acquired in the first year of use. The variability in this estimate is unknown as discussed in chapter 3.

The effect of referral bias has been discussed in detail in chapter 4. Clearly it is likely to have had an influence on outcome in the infection to cirrhosis complications cohort. It may also influence the outcome of the cirrhosis cohorts as those that have been referred into hospital may have faster progressing disease, especially as they are likely to already have advanced disease at the time of referral.

5.4.7 Conclusion

Despite the acknowledged limitations of the study, it does provide evidence the age of the patient does influence progression to cirrhosis complications. The influence of other covariates is less clear due to confounding. It attempts for the first time to estimate the duration of infection to the onset of clinically important complications for a hospital-based cohort. It confirms that at five years after the diagnosis of uncomplicated cirrhosis the risk of HCC is about 9-12%, the risk of a major complication of cirrhosis is about 11% and the risk of liver related death is about 8-10%. Larger cohorts with longer follow up are required to clarify these issues further.

Chapter 6 Treatment for Hepatitis C infection

6.1 Introduction

Treatment for patients with HCV infection has been given in the Royal Infirmary of Edinburgh since the initial reports of the efficacy of IFN monotherapy (Hoofnagle et al. 1986), (Davis et al. 1989). Since that time patients have been treated with a variety of regimes inside and outside of clinical trials. There have been three main treatments (Standard IFN monotherapy, Combination standard IFN and ribavirin and Combination PEG-IFN and ribavirin) and these will be analysed in detail. Although other regimes have been used the numbers involved have been small and therefore these will not be analysed.

Before the Medicines Control Agency granted the license for combination IFN and ribavirin therapy in the UK in 2000, a large number of individuals in the UK were prescribed it on a compassionate basis. Its use was not subject to the rigors of a trial, although strict exclusion criteria were adhered to, based on the known contra-indications to the treatment. Data has been retrospectively collected from centres that used the treatment in this way, to establish its safety and efficacy, in a hospital practice setting. These data are also presented and analysed for comparison with the Edinburgh results.

The changes in clinical practice over the period the study encompasses, has made the data more difficult to analyse and interpret. It has therefore not been possible to divide the patients into 6 months and 12 month treatment cohorts, instead an intention-to-treat analysis of the whole cohort has been carried out and an as-treated analysis of those that have received at least 5.3 months of treatment to see the response rates that are possible if the patient completes this length of treatment. The treatment course for combination PEG-IFN and ribavirin is well established with

those with genotype 1 requiring 48 weeks and non-genotype 1 requiring 24 weeks. The intention at the start of treatment was to adhere to this regime. However, for comparison, the analysis is the same as the other treatment regimes.

6.2 Methods

6.2.1 Patients

Edinburgh cohort

All the patients that have been referred to the Royal Infirmary of Edinburgh between January 1990 and December 2004 and have received treatment have been included in this study.

UK combination interferon and ribavirin cohort

As described in the methods chapter.

6.2.2 Data collection

This has been as previously described in the methods chapter for the Edinburgh and UK cohort.

6.2.3 Treatment analysis subgroups

Whole population

An intention to treat analysis of the whole population.

Patients that had received at least 5.3 months of treatment

An as-treated analysis to demonstrate the response rates in those that completed at least six months of treatment.

Patients that had or had not received previous interferon based treatment

An intention to treat analysis, to avoid introducing bias due to previous treatment failure.

6.2.4 Primary end-point

A sustained virological response, defined as the absence of serum HCV RNA six months after treatment was completed.

6.2.5 Covariates analysed to predict primary end-point

Length of treatment, age, sex, genotype, presence or absence of cirrhosis, previous alcohol abuse, continuing alcohol intake, smoking history, previous HBV, duration of infection, Ishak modified Knodell fibrosis stage, previous IFN treatment and pre-treatment weight.

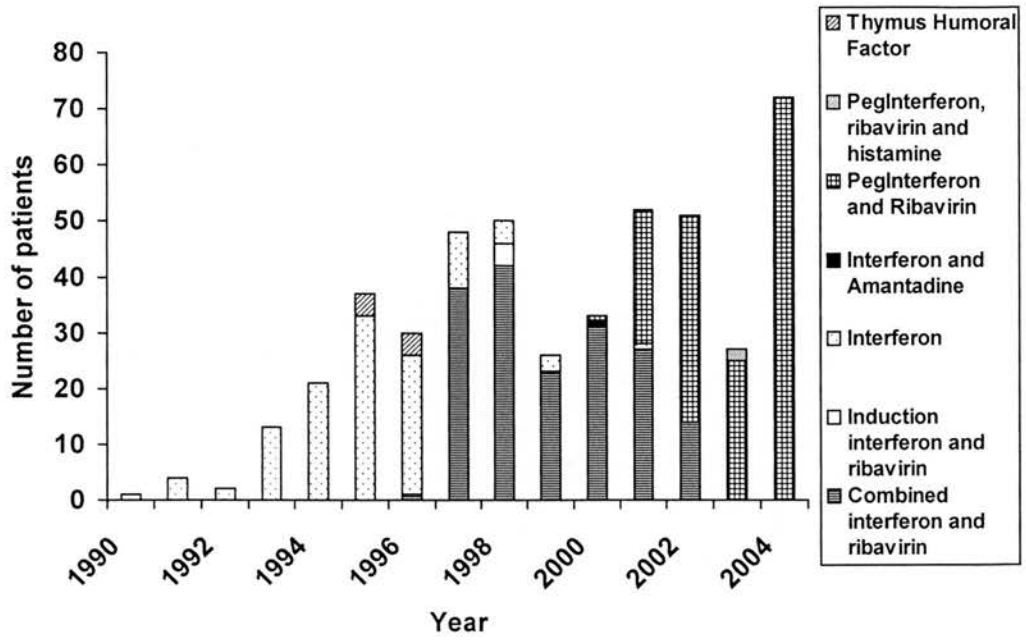
6.3 Results

6.3.1 Edinburgh cohort

6.3.1.1 Description of whole cohort

Four hundred and sixty-one treatment episodes have been initiated in the cohort during the time period. Figure 37 gives a breakdown of the number of patients initiated on each type of regime each year.

Figure 37 Graph of the number of patients started on different types of treatment each year.



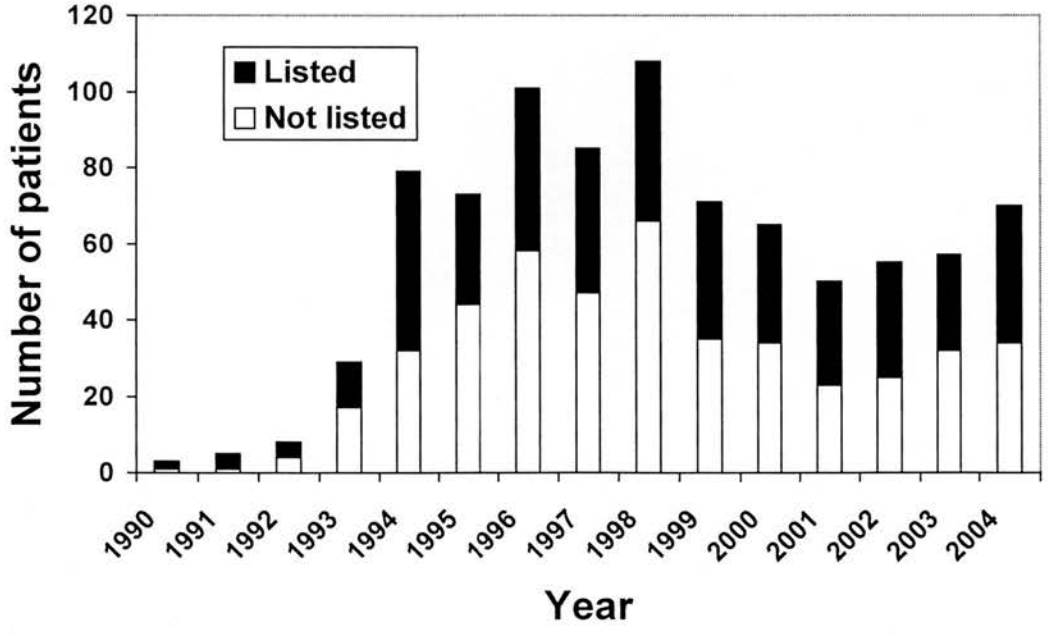
Three hundred and thirty patients have been initiated on treatment, and of these 236 have had only one treatment, 68 have had two treatments, 18 have had three treatments, five had four treatments and three had five treatments. Four hundred and eleven of the treatment episodes have sufficient follow-up and HCV RNA PCR data to allow a responder status to be determined. The overall response rates for each regime are given in Table 81.

Since 1992 on average 45.5% patients referred and seen for the first time each year have been thought to be suitable and listed for treatment (Figure 38).

Table 81 Overall response rates for each treatment regime.

Type of treatment	Response					
	Treatment incomplete	Non responder	Relapser	Sustained responder	Late relapser	Total
Interferon monotherapy	12	39	51	9	6	117
Combined standard interferon and ribavirin	30	50	26	63	1	170
Combination pegylated Interferon and ribavirin	29	24	15	41		109
Thymus Humoral Factor		8				8
Induction interferon and ribavirin				4		4
Interferon and Amantadine				1		1
Pegylated Interferon, ribavirin and histamine	1	1				2

Figure 38 Graph of the number of patients that eventually listed for treatment of according to the year that they first attended.



6.3.1.2 Standard Interferon monotherapy

6.3.1.2.1 Characteristics of the population

One hundred and seventeen patients were treated with standard IFN monotherapy. It has not been possible to differentiate those treated with IFN alpha-2a from those treated with IFN alpha-2b. Therefore a single analysis for IFN monotherapy has been performed. The base-line characteristics of these treated patients are described in Table 82. The proportions of the genotypes were similar to that previously described for the UK. 95.6% of patients were Caucasian and 3.2% had an Asian Subcontinent racial background. There were some patients that have had the same treatment regime more than once and some patients have had first standard IFN monotherapy, then combination standard IFN and ribavirin and finally combination PEG-IFN and ribavirin if the treatments before have failed. Therefore they are included in each of previous treatment rows.

6.3.1.2.2 Outcome of treatment

The primary end-point of a SVR was achieved in 15 patients (12.8%) of the 117 patients. However 6 of these subsequently had a relapse (Late relapsers). No response to treatment or relapse either during or in the 6 months following treatment occurred in 90 patients. Twelve patients received less than three months of treatment prior to discontinuation either due to major adverse event or patient choice and therefore it was not possible to determine their responder status. A breakdown of the reasons for discontinuing treatment in these patients and the sustained responders is given in Table 83.

Table 82 Baseline characteristics of patients treated with standard interferon monotherapy, Combination standard interferon and ribavirin and Combination pegylated interferon and ribavirin.

Characteristic	Standard interferon monotherapy	Combination standard interferon and ribavirin	Combination pegylated interferon and ribavirin
Number	117	170	109
Mean Age +/- SEM (yrs)	39.0 +/- 0.9	41.9 +/- 0.7	44.1 +/- 0.8
Mean duration of infection +/- SEM (yrs)	16.0 +/- 0.8	20.0 +/- 0.9	23.7 +/- 0.9
Mean Weight +/- SEM (Kgs)	74.0 +/- 2.0	72.8 +/-1.2	77.5 +/-1.7
Male:Female ratio	91:26	110:60	78:31
Previous treatments			
Std IFN monotherapy	14	40	27
Std IFN and ribavirin	N/A	6	32
PEG-IFN and ribavirin	N/A	N/A	4
Any IFN based treatment	14 (12.0%)	43 (25.3%)	49 (45.0%)
Cirrhotic	33 (28.2%) All Childs A	41 (24.1%) All Childs A	34 (31.2%) All Childs A
Previous alcohol abuse	33 (28.2%)	37 (21.8%)	25 (22.9%)
Methadone	11	11	6
HBsAg positive	3	1	5
HIV positive	3	4	5
Previous minor psychiatric history	9	6	9
Genotyped	92	130	106
1	37 (40.2%)	59 (45.4%)	55 (51.9%)
2	16 (17.4%)	9 (6.9%)	7 (6.6%)
3	37 (40.2%)	57 (43.8%)	41 (38.7%)
4	2 (2.2%)	3 (2.3%)	3 (2.8%)
6		2 (1.5%)	
Risk category			
IDU	55 (47.0%)	86 (50.6%)	49 (45.0%)
Blood/Tissue transfer	25 (21.4%)	28 (16.5%)	16 (14.7%)
Haemophiliac	10 (8.5%)	8 (4.7%)	11 (10.1%)
Tattoo / Body piercing	6 (5.1%)	8 (4.7%)	6 (5.5%)
Perinatal		2 (1.2%)	1 (0.9%)
Homo-/Bisexual contact	1 (0.8%)	2 (1.2%)	
Heterosexual contact	5 (4.3%)	9 (5.3%)	7 (6.4%)
Accidental needle	2 (1.6%)	9 (5.3%)	5 (4.6%)
Other			1 (0.9%)
Unknown	13 (11.1%)	18 (10.6%)	13 (11.9%)

Abbreviations: SEM: Standard error of mean, Std IFN: Standard interferon, PEG-IFN: pegylated interferon.

Table 83 The reason for stopping treatment with standard interferon monotherapy, when this occurred and the responder status of the patient.

Responder type	Reason for stopping treatment and length of treatment (months)								Totals
	PCR data	Completed planned treatment or PCR data			Major adverse event		Patient choice		
	<5.3	5.4 to 6.9	6.9 to 11.4	>11.4	<5.3	>5.3	<5.3	>5.3	
Treatment incomplete					4		8		12
Non-responder	18	12	7	1					38
Non-responder (dose reduced due major adverse event)			1						1
Relapser	17	19	7	5			1		49
Relapser (dose reduced due to major adverse event)	1		1						2
Sustained Responder		6		1			2		9
Late Relapser							4	2	6

For all patients with genotype 1 infections, 10.8% achieved a SVR compared with 9.4% with genotype 2 or 3 infections, while in those that had received at least 5.3 months of treatment this was 6.2% and 12.1% respectively (Table 84).

In IFN naïve patients with genotype 1 infection, the rate was 8.3% compared with 8.9% in those with genotype 2 or 3 infections (Table 85). Too few patients underwent IFN monotherapy retreatment to analyse response rates.

Table 84 The number of patients in each responder type and the infecting genotype when treated with standard interferon monotherapy.

Subgroup	Responder Type	Genotype					Totals
		1	2	3	4	Unknown	
Whole cohort (n=117)	Treatment incomplete	5	1	1		5	12
	Non-responder	18	3	13	1	4	39
	Relapser	10	9	21	1	10	51
	Sustained Responder and Late relapser	4	3	2		6	15
Patients that received at least 5.3 months of treatment (n=62)	Non-responder	10	3	6		2	21
	Relapser	5	6	14		7	32
	Sustained Responder and Late relapser	1	3	1		4	9

Table 85 The number of patients in each responder type according to whether they had previous interferon treatment and the infecting genotype, when treated with interferon monotherapy.

Subgroup	Responder Type	Genotype					Totals
		1	2	3	4	Unknown	
Interferon naïve (n=103)	Treatment incomplete	5	1	1		5	12
	Non-responder	18	3	12	1	3	37
	Relapser	10	6	18	1	7	42
	Sustained Responder and Late relapser	3	2	2		5	12
Previous interferon treatment (n=14)	Non-responder			1		1	2
	Relapser		3	3		3	9
	Sustained Responder and Late relapser	1	1			1	3

6.3.1.2.3 Predictors of sustained response

Binary logistical regression was performed with the covariates detailed above. This identified a younger age at treatment ($p<0.0001$), to be the only independent predictor of SVR in both the IFN naïve and previous IFN groups.

6.3.1.2.4 Adverse events

Fifty (42.7%) of the 117 patients had no adverse events recorded during and after treatment. Four patients (3.4%) had their treatment discontinued prematurely due a serious adverse event. This was due to depression in two patients and due to thrombocytopenia in two patients.

A further 16 patients (13.7%) discontinued their treatment prematurely without the occurrence of a serious adverse event. This was therefore defined as by patient choice but obviously the adverse effects of the medication may have been a contributory factor. A younger age pre-treatment and hypothyroidism during treatment independently predicted this outcome.

Five patients (4.3%) had the dose of their medication reduced due to a serious adverse event. The reason for dose reduction in one patient was anaemia, low mood in another and the rest were due to flu-like symptoms.

The prevalence of the common adverse effects of treatment that occurred in patients is given in Table 86, while Table 87 gives rarer ones.

Table 86 The common side effects in patients treated with standard interferon monotherapy, Combination standard interferon and ribavirin and Combination pegylated interferon and ribavirin.

Symptom	Standard interferon		Combination standard interferon and ribavirin		Combination pegylated interferon and ribavirin	
	Patients (n=117)	%	Patients (n=170)	%	Patients (n=109)	%
Influenza like						
Headache	6	5.1	8	4.7	9	8.3
Fatigue	30	25.6	26	15.3	21	19.3
Malaise	21	17.9	39	22.9	21	19.3
Myalgia	2	1.7	5	2.9	1	0.9
Arthralgia	1	0.9	9	5.3	5	4.6
Musculo-skeletal pain			1	0.6	1	0.9
Fever			1	0.6		
Psychiatric						
Anxiety			1	0.6	1	0.9
Impaired concentration						
Low mood	15	12.8	24	14.1	25	22.9
Emotional lability	5	4.3	13	7.6	6	5.5
Insomnia	2	1.7	8	4.7	7	6.4
Irritability					2	1.8
Dermatological						
Alopecia	1	0.9	6	3.5	1	0.9
Pruritis	1	0.9	8	4.7	5	4.6
Rash	4	3.4	21	12.4	15	13.8
Dry skin	1	0.9	7	4.1	7	6.4
Inflammation at injection site	1	0.9	4	2.4	2	1.8
Exacerbation of psoriasis			3	1.8	1	0.9
Gastrointestinal						
Anorexia	5	4.3	16	9.4	12	11.0
Dyspepsia						
Vomiting			3	1.8	1	0.9
Nausea	3	2.6	13	7.6	12	11.0
Diarrhoea			2	1.2	6	5.5
Abdominal pain					2	1.8
Weight loss			9	5.3	6	5.5
Respiratory						
Cough			1	0.6	1	0.9
Dyspnoea			5	3	1	0.9
Pharyngitis					1	0.9
Sinusitis						
Endocrine						
Hypothyroid	1	0.9	2	1.2	1	0.9
Hyperthyroid			3	1.8		
Ocular						
Blurred vision			1	0.6	6	5.5

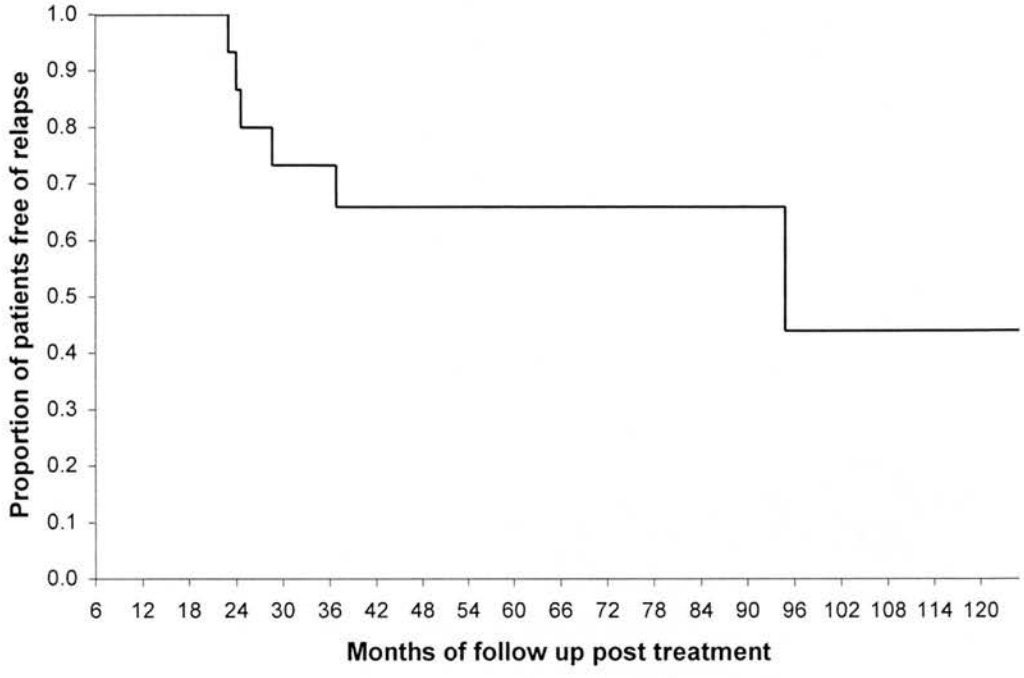
Table 87 The other side effects that were recorded in patients treated with standard interferon monotherapy, Combination standard interferon and ribavirin and Combination pegylated interferon and ribavirin.

System	Side effect	Standard interferon (n=117)	Combination standard interferon and ribavirin (n=170)	Combination pegylated interferon and ribavirin (n=109)
Hepatic	Right upper quadrant pain		1	
	Bilirubin elevation		1	
	Liver decompensation			1
	Hepatitis			
Oral	Mouth ulcers		1	
	Altered taste	2	1	1
	Dry mouth			2
	Candidiasis (oral)		2	
	Herpes simplex (oral)			1
GI	Exacerabation of colitis			1
Ocular	Dry eyes			1
CVS	Dizziness	1	2	1
CNS	Parasthesia in legs			1
Psychiatric	Acute confusional state		2	
	Alcohol intake increased	2	1	
	Suicidal ideation		2	1
	Loss of libido		1	
	Nightmares		2	
Miscellaneous	Nose bleeds	1	1	
	Exfoliative dermatitis			1
	Urinary Incontinence			1
	Increased blood glucose		2	
	Sweating	2	1	
	Night sweats		1	1
	Amenorrhoea			1
	Chest infection			1
	Increased VLDL			1

6.3.1.2.5 Late relapse

Fifteen patients treated with IFN monotherapy achieved a SVR at six months post treatment. Figure 39 shows that 4 of these patients relapsed and became HCV RNA PCR positive between 17 and 24 months of further follow-up.

Figure 39 A Kaplan Meier graph demonstrating the proportion of patients, treated with standard interferon monotherapy, that remain as sustained responders during the follow-up period after 6 months post treatment.



Cumulative relapsing patients	0	4	5		5	6	6
At risk patients	15	11	9		5	2	0

6.3.1.3 Combination standard interferon and ribavirin

6.3.1.3.1 Characteristics of the population

170 were treated with this treatment. All received IFN alpha-2b. The base-line characteristics of these treated patients are described in Table 82. The proportions of the genotypes were similar to that previously described for the UK. 91.7% of patients were Caucasian and 3.3% had an Asian Subcontinent racial background.

6.3.1.3.2 Outcome of treatment

The primary end-point of a sustained HCV clearance was achieved in 64 patients (37.6%) including one patient that subsequently had a late relapse. No response to treatment or relapse either during or in the 6 months following treatment occurred in 76 patients. Thirty patients received less than three months of treatment prior to discontinuation either due to major adverse event or patient choice and therefore it was not possible to determine their responder status. A breakdown of the reasons for discontinuing treatment in these patients and the sustained responders is given in Table 88.

For all patients with genotype 1 infections, only 11.9% achieved a SVR compared with 50.0% with genotype 2 or 3 infections, while in those that had received at least 5.3 months of treatment this was 17.9% and 71.4% respectively (Table 89).

Table 88 The responder type and the reason for stopping and length of treatment with combination standard interferon and ribavirin.

Responder type	Reason for stopping treatment and length of treatment (months)								Totals
	PCR data	Completed treatment or PCR data			Major adverse event		Patient choice		
	<5.3	5.4-6.9	6.9-11.4	>11.4	<5.3	>5.3	<5.3	>5.3	
Treatment incomplete					6		24		30
Non-responder at last HCV RNA PCR on treatment	23	17	4	1					45
Non-responder (dose reduced due major adverse event)	1	2	2						5
Relapser		18	3	1					22
Relapser (dose reduced due to major adverse event)		1	3						4
Sustained Responders and Late relapsers		39	11	4	2	1	3	4	64

Table 89 The number of patients in each responder type and the infecting genotype when treated with combination standard interferon and ribavirin.

Subgroup	Responder Type	Genotype						Totals
		1	2	3	4	6	Unknown	
Whole cohort (n=170)	Treatment incomplete	7	1	14	1		7	30
	Non-responder	32		10		2	6	50
	Relapser	13	2	6	2		3	26
	Sustained Responder and Late relapsers	7	6	27			24	64
Patients that received >5.3 months of treatment (n=112)	Treatment incomplete	1						1
	Non-responder	18		4		1	3	26
	Relapser	13	2	6	2		3	26
	Sustained Responder and Late relapsers	7	5	25			22	59

In IFN naïve patients the SVR rate was 11.6% and 52.2% for genotype 1 and genotype 2 or 3 infections respectively, compared with 12.5% and 45.0% in those that had previous IFN treatment (Table 90).

Table 90 The number of patients in each responder type according to whether they had previous interferon treatment and the infecting genotype, when treated with combination standard interferon and ribavirin.

Subgroup	Responder Type	Genotype						Totals
		1	2	3	4	6	Unknown	
Interferon naïve (n=127)	Treatment incomplete	5	1	11	1		5	23
	Non-responder	21		4		1	6	32
	Relapser	12	2	4	2		3	23
	Sustained Responder and Late relapsers	5	4	20			20	49
Previous interferon treatment (n=43)	Treatment incomplete	2		3			2	7
	Non-responder	11		6		1		18
	Relapser	1		2				3
	Sustained Responder and Late relapsers	2	2	7			4	15

6.3.1.3.3 Predictors of sustained response

Binary logistical regression with the covariates detailed above was performed and identified for IFN naïve patients a lower pre-treatment weight ($p<0.0001$) and a genotype 2 or 3 infection ($p=0.01$) to be independent predictors of SVR.

When the same analysis was stratified by genotype, for genotype 1 infections a lower pre-treatment weight ($p=0.003$) was found to be an independent predictor of SVR, but for the genotype 2 or 3 infections no independent predictors could be identified.

6.3.1.3.4 Adverse events

Sixty-seven patients (39.4%) of the 170 patients had no adverse events recorded during and after treatment. Nine patients (10.9%) had their treatment discontinued prematurely due a serious adverse event. The commonest cause was depression, which occurred in 5 patients. Two patients developed anaemia severe enough to discontinue all treatment. Binary logistical regression was performed and identified a lower weight as the only pre-treatment independent predictor of premature treatment termination due to a serious adverse event. Anxiety, inflamed injection site and low mood were the symptoms on treatment that independently predicted this outcome.

A further 23 patients (13.5%) discontinued their treatment prematurely without the occurrence of a serious adverse event. This was therefore defined as by patient choice but obviously adverse effects of the medication may have been a contributory factor. In two there was ongoing low mood, in two there were thyroid abnormalities and another one there was weight loss. A younger age pre-treatment and cough, malaise and weight loss during treatment independently predicted this outcome.

There were 22 episodes of dose reduction due to side-effects in 15 patients (8.8%). Three of these subsequently had their treatment discontinued prematurely. Anaemia was the reason for dose reduction in 8 of these episodes, neutropenia in 3, depression in 2, nausea in 2 and dyspnoea in 2.

The prevalence of common adverse effects of treatment that occurred in patients are given in Table 86. A younger pre-treatment age (and not a previous psychiatric history) was predictive of low mood whilst on treatment. A younger pre-treatment age was independently predictive of the occurrence of thyroid disorders whilst on treatment. Rarer adverse events are given in Table 87.

6.3.1.3.5 Late relapse

Sixty-four patients achieved a SVR at six months post treatment. At 2 years after treatment 42 patients out of 43 remained sustained responders (97.7%). The one late relapse in the whole cohort was documented to have occurred at 11.5 months after treatment with a positive HCV RNA PCR. At 3 years post treatment 19 patients remained sustained responders with no further relapses. The longest sustained responder has been followed up for 91 months post treatment without relapse.

6.3.1.4 Combination pegylated interferon and ribavirin

6.3.1.4.1 Characteristics of the population

One hundred and nine patients were treated with this treatment. The base-line characteristics of these treated patients are described in Table 82. 96.3% of patients were Caucasian and 2.8% had an Asian Subcontinent racial background.

6.3.1.4.2 Outcome of treatment

The primary end-point of a sustained HCV clearance was achieved in 41 patients (37.6%) of the 109 patients. No response to treatment or relapse either during or in the 6 months following treatment occurred in 39 patients. Twenty-nine patients discontinued their treatment prematurely either due to major adverse event or patient choice, at such a time that it has not been possible to determine their responder status. A breakdown of the reasons for discontinuing treatment in these patients and the sustained responders is given in Table 91.

For all patients with genotype 1 infections, only 20.0% achieved a SVR compared with 56.2% with genotype 2 or 3 infections, while in those that had received at least 5.3 months of treatment this was 41.6% and 67.7% respectively (Table 92).

Table 91 The number of patients in each responder type and the reason for discontinuing treatment with combination pegylated interferon and ribavirin.

Responder type	Reason for stopping treatment and length of treatment (months)								Total
	PCR data	Completed treatment or PCR data			Major adverse event		Patient choice		
	<5.3	5.4-6.9	6.9-11.4	>11.4	<5.3	>5.3	<5.3	>5.3	
Treatment incomplete					6		18	5	29
Non-responder at last HCV RNA PCR on treatment	16	4							20
Non-responder (dose reduced due major adverse event)	2	2							4
Relapser		2	4	4					10
Relapser (dose reduced due to major adverse event)		3	1	1					5
Sustained Responder	4	21	10	3	1		2		41

Table 92 The number of patients in each responder type and the infecting genotype when treated with combination Pegylated interferon and ribavirin.

Subgroup	Responder Type	Genotype						Totals
		1	2	3	4	6	Unknown	
Whole cohort (n=109)	Treatment incomplete	18	4	6			1	29
	Non-responder	17		6	1			24
	Relapser	9		5	1			15
	Sustained Responder	11	3	24	1		2	41
Patients that received > 5.3 months of treatment (n=101)	Treatment incomplete	4					1	5
	Non-responder	1		5				6
	Relapser	9		5	1			15
	Sustained Responder	10	2	19	1		2	34

In IFN naïve patients the SVR rate was 29.0% and 59.3% for genotype 1 and genotype 2 or 3 infections respectively, compared with 8.3% and 45.8% in those that had previous IFN treatment (Table 93).

Table 93 The number of patients in each responder type according to whether they had previous interferon treatment and the infecting genotype, when treated with combination pegylated interferon and ribavirin.

Subgroup	Responder Type	Genotype					Totals
		1	2	3	4	Unknown	
Interferon naïve (n=60)	Treatment incomplete	10	3	4			17
	Non-responder	5		3			8
	Relapser	7		1			8
	Sustained Responder	9	2	14		2	27
Previous interferon treatment (n=49)	Treatment incomplete	8	1	2		1	12
	Non-responder	12		3	1		16
	Relapser	2		4	1		7
	Sustained Responder	2	1	10	1		14

6.3.1.4.3 Predictors of sustained response

Binary logistical regression with the covariates detailed above was performed separately on those that had had previous IFN treatment and those that had not. This identified for IFN naïve patients a genotype 2 or 3 infection (p=0.047) to be the only independent predictor of SVR. For those that had received previous IFN based treatment a genotype 2 or 3 infection (p=0.011) and a younger age pre-treatment (p=0.033) were found to be independent predictors of SVR.

When the same analysis was stratified by genotype, no independent predictors could be identified either for genotype 1 infections or for the genotype 2 or 3 infections.

6.3.1.4.4 Adverse events

Twenty-six patients (23.8%) of the 109 patients had no adverse events recorded during and after treatment. Seven patients (6.4%) had their treatment discontinued prematurely due a serious adverse event. One developed depression, one patient developed anaemia severe enough to discontinue all treatment and one discontinued due to weight loss. Binary logistical regression was performed and identified a lower weight as the only pre-treatment independent predictor of premature treatment termination due to a serious adverse event. Vomiting and weight loss were the symptoms on treatment that independently predicted this outcome.

A further 19 patients (17.4%) discontinued their treatment prematurely without the occurrence of a serious adverse event. This was therefore defined as by patient choice but obviously adverse effects of the medication may have been a contributory factor. In three there was ongoing low mood, in two there was ongoing anaemia, in two there were severe flu-like symptoms and in another one there were thyroid abnormalities. A lower weight pre-treatment and dyspnoea during treatment independently predicted this outcome.

There were 56 episodes of dose reduction of their medication due to side effects in 41 patients (37.6%). Five of these subsequently had their treatment discontinued prematurely. Neutropenia was the reason for dose reduction in 23 of these episodes, thrombocytopenia in 16, anaemia in 8 and weight loss in 2.

The prevalence of common adverse effects of treatment that occurred in patients is given in Table 86. Only a younger pre-treatment age and a history of alcohol abuse (>50 units/week) were predictive of treatment being discontinued due to depression. A previous psychiatric history was not predictive. Rarer events are given in Table 87.

6.3.1.4.5 Late relapse

Forty-one patients achieved a SVR at six months post treatment. No patients have relapsed so far after a median of 9.3 months follow-up post treatment. Only three patients have been followed up for two years post treatment and all remain sustained responders.

6.3.2 Combination interferon and ribavirin treatment in hospital practice in the United Kingdom

6.3.2.1 Characteristics of the Patients

1220 patients received combination IFN alpha-2b and ribavirin in the 69 centres around the UK on a compassionate basis from Schering-Plough between 1997 and 2000. The patients from the Royal Infirmary of Edinburgh were excluded and have been analysed separately above. Data was received on 238 patients that had had treatment from six other centres. Two hundred and nineteen of these had follow-up allowing responder status to be determined. The base-line characteristics of these treated patients are described in Table 94. The proportions of the genotypes was similar to that previously described for the UK. 92.2% of patients were Caucasian and 2.3% had an Asian Subcontinent racial background.

Table 94 Base-Line characteristics of patients with follow-up post treatment that allows response to be determined.

Characteristic		
Mean Age +/-SEM (years)	40.6 +/- 0.6	
Mean duration of infection+/-SEM (years)	15.9 +/- 0.7	
Mean Weight +/-SEM (Kilograms)	76.5 +/-1.1	
Male:Female ratio	155:64	
Previous interferon based treatment	69	
Cirrhotic	19	(8.7%) All Childs A
Previous alcohol abuse	63	(28.8%)
HBsAg positive	5	
HIV positive	1	
Previous minor psychiatric history	20	
Genotyped	109	
1	52	(31.1%)
2	9	(5.4%)
3	43	(25.7%)
4	2	(1.2%)
5	2	(1.2%)
Other	1	(0.6%)
Probable route of infection		
Intravenous Drug Abuser	94	(42.9%)
Transfusion / Blood Products	59	(26.9%)
Sporadic / Unknown	45	(20.5%)
Tattoo	9	(4.1%)
Occupational	6	(2.7%)
High Risk Sexual Practices	5	(2.3%)

Abbreviations: SEM: Standard error of mean

6.3.2.2 Outcome

The primary end-point of a sustained HCV clearance was achieved in 108 patients (49.3%) of the 219 patients with follow-up. Three of these subsequently relapsed (Late relapsers). No response to treatment or relapse either during or in the 6 months following treatment occurred in 103 patients. Eight patients discontinued their treatment prior to three months due to major adverse event or patient choice, and therefore it was not possible to determine their responder status. A breakdown of the reasons for discontinuing treatment in these patients and the sustained responders is given in Table 95.

Table 95 The number of patients in each responder type and the reason for discontinuing treatment in all patients in whom responder status could be assessed.

Responder type	Reason for stopping treatment and length of treatment (months)								Total
	PCR data	Completed treatment or PCR data			Major adverse event		Patient choice		
	<5.3	5.4-6.9	6.9-11.4	>11.4	<5.3	>5.3	<5.3	>5.3	
Treatment incomplete					6		2		8
Non-responder at last HCV RNA PCR on treatment	2	15	9	5	2	3			36
Non-responder (dose reduced due major adverse event)	1	5							6
Relapser	18	7	3	9	4	2	6	2	51
Relapser (dose reduced due to major adverse event)		1	3	4				2	10
Sustained Responder and Late relapser		19	1	49	2	5	4	28	108

For all patients with genotype 1 infections, 19.2% achieved a SVR compared with 59.6% with genotype 2 or 3 infections, while in those that had received at least 5.3 months of treatment this was 22.5% and 67.4% respectively (Table 96).

Table 96 The number of patients in each responder type and the infecting genotype.

Subgroup	Responder Type	Genotype							Totals
		1	2	3	4	5	Other	Unknown	
Whole cohort (n=219)	Treatment incomplete	3		3				2	8
	Non-responder	18	3	5			1	15	42
	Relapser	21		10	2	1		27	61
	Sustained Responder and Late relapser	10	6	25		1		66	108
Patients that received at least 5.3 months of treatment (n=170)	Non-responder	17	3	5			1	11	37
	Relapser	14		6	1			12	33
	Sustained Responder and Late relapser	9	6	23		1		61	100

In IFN naïve patients the rate was 15.6% and 58.1% for genotype 1 and genotype 2 or 3 infections respectively, compared with 25.0% and 61.9% in those that had previous IFN treatment (Table 97).

Table 97 The number of patients in each responder type according to whether they had previous interferon treatment and the infecting genotype, when treated with combination standard interferon and ribavirin.

Subgroup	Responder Type	Genotype							Totals
		1	2	3	4	5	Other	Unknown	
Interferon naïve (n=150)	Treatment incomplete	1		2				2	5
	Non-responder	14	1	4			1	7	27
	Relapser	12		6	2			22	42
	Sustained Responder and Late relapser	5	4	14		1		52	76
Previous interferon treatment (n=69)	Treatment incomplete	2		1					3
	Non-responder	4	2	1				8	15
	Relapser	9		4		1		5	19
	Sustained Responder and Late relapser	5	2	11				14	32

6.3.2.3 Predictors of sustained response

Binary logistical regression was performed separately on those that had had previous IFN treatment and those that had not. This identified for IFN naïve patients a genotype 2 or 3 infection (<0.0001), a lower pre-treatment weight (p=0.005) and a longer treatment course (p=0.037) to be independent predictors of SVR. For those that had received previous IFN based treatments a genotype 2 or 3 infection (p=0.017) was found to be the only independent predictor of SVR.

When the same analysis was stratified by genotype, for IFN naïve genotype 1 infections a younger age at treatment was an independent predictor of SVR, while those with previous IFN based treatment it was a lower pre-treatment weight and a

longer treatment course. For the genotype 2 or 3 infections no independent predictors of SVR could be identified for IFN naïve or previous IFN based treatment patients.

6.3.2.4 Adverse events

Seventeen patients (7.1%) of the 238 patients had no adverse events recorded during and after treatment. Twenty-four patients (10.1%) had their treatment discontinued prematurely due a serious adverse event. The commonest cause was depression, which occurred in three patients. Two patients developed anaemia severe enough to discontinue all treatment and one discontinued due to neutropenia and one due to thrombocytopenia. In 16 patients the reason was not classified. Binary logistical regression was performed and identified a lower pre-treatment weight ($p<0.0001$), previous minor psychiatric history ($p<0.0001$) and no previous IFN based treatment ($p=0.009$) as pre-treatment independent predictors of premature treatment termination due to a serious adverse event. Sinusitis, vomiting, emotional labiality and the absence of fever were the symptoms on treatment that independently predicted this outcome.

A further 45 patients (18.9%) discontinued their treatment prematurely without the occurrence of a serious adverse event. This was therefore defined as by patient choice but obviously adverse effects of the medication may have been a contributory factor. A younger age at treatment and previous IFN based treatment independently predicted this outcome pre-treatment and sinusitis, the absence of anxiety and dyspnoea whilst on treatment.

Thirty-four patients (14.3%) had the dose of their medication reduced due to a serious adverse event. Four of these subsequently had their treatment discontinued

prematurely. Anaemia was the reason for dose reduction in 16 patients (6.7%), neutropenia in 4, depression in 2 and thrombocytopenia in 1.

The prevalence of the adverse effects of treatment that occurred in over 10% of patients is given in Table 98. A previous history of thyroid disorders, a lower pre-treatment weight and no previous IFN treatment were independently predictive of the occurrence of thyroid disorders whilst on treatment.

Table 98 The common side effects whose presence or absence was recorded for all patients that completed treatment (n=238).

Symptom	Number of patients	Percentage
Influenza like symptoms		
Headache	143	41.5
Fatigue	180	62.3
Malaise	71	19.7
Myalgia	51	17.2
Arthralgia	47	15.6
Musculo-skeletal pain	78	24.0
Fever	93	25.4
Psychiatric symptoms		
Anxiety	63	16.9
Impaired concentration	44	11.7
Low mood	65	21.3
Emotional lability	42	13.1
Insomnia	59	16.7
Irritability	60	16.4
Dermatological symptoms		
Alopecia	47	13.9
Pruritis	38	11.7
Rash	52	21.8
Dry skin	69	28.9
Inflammation at injection site	13	5.4
Gastrointestinal symptoms		
Anorexia	48	20.1
Dyspepsia	21	8.8
Vomiting	17	7.1
Nausea	74	31.0
Diarrhoea	23	9.6
Abdominal pain	21	8.8
Weight loss	68	28.5
Respiratory symptoms		
Cough	26	10.9
Dyspnoea	31	13.0
Pharyngitis	6	2.5
Sinusitis	4	1.7
Endocrine		
Thyroid	9	3.8

Other side effects of treatment that occurred less commonly are recorded in Table 99.

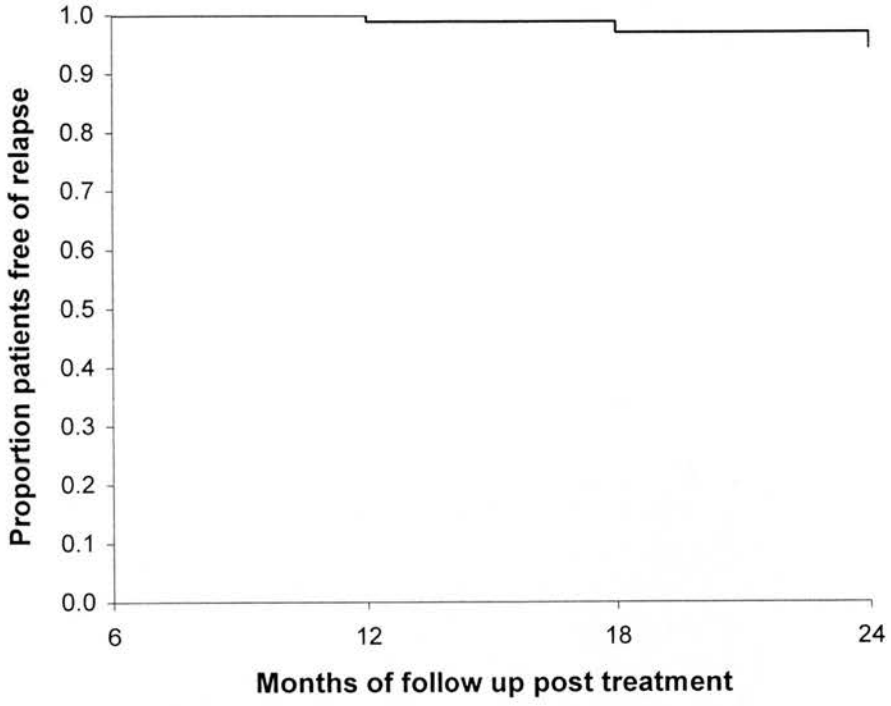
Table 99 The other side effects that were recorded (n=239)

System	Side effect	Number of patients
Hepatic	Right upper quadrant pain	3
	Bilirubin elevation	1
	Elevated ALT and Alk Phos	1
	Hepatitis	1
Oral	Mouth ulcers	6
	Dry mouth	5
	Sore tongue	3
	Gingivitis	1
	Tooth abscess	1
	Candidiasis (oral)	1
GI	Dysphagia	1
	Constipation	1
Ocular	Sore eyes	3
	Dry eyes	4
	Blurred vision	2
CVS	Dizziness	10
	Palpitations	4
	Atrial flutter	1
	Hypertension	2
	Cold hands	1
CNS	Parasthesia in arms	1
	Tinnitus	1
	Tremor	1
Psychiatric	Suicide attempt	1
	Hallucinations	1
Miscellaneous	Nose bleeds	3
	Psoriasis exacerbation	1
	Urinary tract infection	3
	Urinary frequency	1
	Sweating	1
	Hot flushes	1
	Night sweats	1
	Amenorrhoea	1
	Chest infection	1
	Gout	1
	Hay fever	1

6.3.2.5 Late relapse

One hundred and eight patients from the whole cohort achieved a SVR at six months post treatment. Figure 40 shows that 3 of 51 patients (5.9%) followed up for a further 18 months relapsed and became HCV RNA PCR positive.

Figure 40 A Kaplan Meier graph demonstrating the proportion of patients, treated with Combination standard interferon and ribavirin that remain as sustained responders during the follow-up period after 6 months post treatment.



At risk patients	108	89	66	50
Cumulative relapsing patients	0	1	2	3

6.4 Discussion

The studies described in this chapter are not randomised controlled trials and therefore should be interpreted with caution. Despite this, the study may demonstrate the SVR rates that are likely to be achieved in the less controlled environment of clinical practise.

6.4.1 Standard Interferon monotherapy

The primary end-point of SVR was achieved in 12.8% of patients which is similar to that reported in the Cochrane review (SVR for 6 months 7% and for 12 months 14%) (Myers et al. 2002). In the genotype 1 infected patients an overall SVR rate for the whole cohort of 10.8% is less than 17% reported in the trials (Davis & Lau 1997) where the intention to treat was 12 months. Analysing those that had at least six months of treatment the SVR rate was 6.2% which is difficult to explain as longer treatment has been shown to improve outcome for genotype 1 (Davis & Lau 1997). It has not been possible to look at a cohort of those that had at least 12 months of treatment, as this would automatically exclude any non-responders at six months.

In the genotype 3 infected patients an overall SVR rate of 9.4% is less than 54.9% reported in the trials (Davis & Lau 1997) where the intention to treat was at least 6 months. Analysing those that had at least 5.3 months of treatment the SVR rate was little better at 12.1%.

The only predictor found for SVR was a younger age at treatment, which has not been found in other studies.

Overall discontinuation of therapy due to a severe adverse event occurred in 3.4%. This compares to 4% seen in the trials with depression, as in this study, being the

commonest cause (Poynard et al. 1996). In addition to this a further 13.7% discontinued therapy prematurely due to patient choice, without a severe adverse event. This group, which was not clearly documented in the trials, makes the overall discontinuation rate much higher. Higher motivation levels in the trial patients might explain this, combined with stricter application of when a patient could discontinue treatment. Dose reduction occurred in 4.3% of patients which is less than the 9% seen the trials. As with the trials, the flu-like side effects predominated with fatigue occurring in 25.6% of individuals being the commonest. The incidence of these other side effects was significantly less than reported in the trails (Poynard et al. 1996) as reporting is likely not to have been so complete.

The long-term outcome of patients who are sustained responders to IFN monotherapy has been documented. The proportion who remain HCV RNA PCR negative has ranged from 96% at a mean follow-up of 4 years to 92% (Marcellin et al. 1997) at a mean of 5.4 years (Reichard et al. 1999).

This study includes follow-up of 15 sustained responders, and at 2 years post treatment only 73.3% remained negative.

6.4.2 Combination standard interferon and ribavirin

Previous randomised controlled trials have established that a combination of three times a week IFN and twice daily ribavirin is an effective treatment for chronic HCV infection (McHutchison et al. 1998b; Poynard et al. 1998).

6.4.2.1 Edinburgh cohort

The primary end-point of a sustained HCV clearance was achieved in 64 patients (37.6%). In the genotype 1 infected patients an overall SVR for the whole cohort of 11.9% is less than 28-31% reported in the trials where the intention to treat was 12

months. This may reflect that some genotype 1 patients were discontinued prior to six months of treatment for being PCR positive and a further patients were discontinued at six months when PCR negative and subsequently relapsed. A proportion of these may have gone on to be sustained responders if treatment had been continued. Analysing those that had at least six months of treatment the SVR rate was 17.9% which is similar to the 16-18% seen the six month treatment group in the trials, although the groups are not directly comparable. It has not been possible to look at a cohort of those that had at least 12 months of treatment, as this would automatically exclude any non-responders at six months.

In the genotype 3 infected patients an overall SVR rate of 50.0% is less than 64-69% reported in the trials where the intention to treat was at least 6 months. However analysing those that had at least 5.3 months of treatment the SVR rate was 71.4%.

Surprisingly the SVR rate in those that had had previous IFN monotherapy was not significantly different from those that had not. This probably reflects how few patients achieved a SVR with this form of treatment and therefore the composition, including genotype ratios, of the two groups was similar.

For IFN naïve patients, binary logistical regression identified a genotype 2 or 3 infection, a lower pre-treatment weight and a longer treatment course to be independent predictors of SVR. The previous trials have established this effect of genotype but also a low degree of fibrosis at baseline and female sex, which was not seen in this study. The length of treatment would obviously not have been looked at as the trial cohorts were divided into six and 12-month groups at the outset. When the same analysis was performed but stratified for genotype, for IFN naïve genotype 1 infections a younger age at treatment was an independent predictor of SVR. The

European randomised controlled trial also identified young age as an independent predictor of response (Poynard et al. 1998). The effect of baseline viral load was not assessed in this study but has been shown to be predictive of SVR if $2x10^6$ copies per millilitre or less (McHutchison et al. 1998b; Poynard et al. 1998).

Overall discontinuation of therapy due to a severe adverse event occurred in 10.9%. This compares to 8% for 24 weeks of treatment and 19-21% for 48 weeks of treatment seen in the trials with depression, as in this study, being the commonest cause (McHutchison et al. 1998b; Poynard et al. 1998). A lower pre-treatment weight was identified as an independent predictor of discontinuation for a serious adverse event. In addition to this a further 13.5% discontinued therapy prematurely due to patient choice, without a severe adverse event. Again this group, which was not clearly documented in the trials, makes the overall discontinuation rate much higher. Only 22.6% of these patients achieved a SVR. Dose reduction occurred in 8.8% of patients which is comparable to the 7%-10% seen the trials. Anaemia triggered this in 4.6% of patients compared with 7-9% seen in the trials. As with the trials, the flu-like side effects predominated with malaise occurring in 22.9% of individuals being the commonest. It is likely that there was incomplete recording of these minor side effects as the frequencies are so much lower.

It has been assumed that the persistence of the SVR following combination treatment will be equivalent to that in IFN monotherapy. The only data so far reported, followed up 25 sustained responders of which 84% remained HCV RNA PCR negative at a mean of 9.4 months (Barnes et al. 1999). This study includes follow-up of 64 sustained responders, of whom 43 had a HCV RNA PCR at 2 years post treatment, and 97.7% remained negative.

6.4.2.2 UK cohort

The primary end-point of a sustained HCV clearance was achieved in 108 patients (49.3%) of the 219 patients with follow-up. This is much higher than the Edinburgh cohort, as are the SVRs in the other subsets. In the genotype 1 infected patients an overall SVR for the whole cohort of 19.2% is less than 28-31% reported in the trials where the intention to treat was 12 months. Again this may reflect that some genotype 1 patients were discontinued prior to six months of treatment for being PCR positive and a further patients were discontinued at six months when PCR negative and subsequently relapsed. A proportion of these may have gone on to be sustained responders if treatment had been continued. Analysing those that had at least six months of treatment the SVR rate was 22.5% which is similar to the 16-18% seen the six month treatment group in the trials, although the groups are not directly comparable.

In the genotype 3 infected patients an overall SVR rate of 59.6% is less than 64-69% reported in the trials where the intention to treat was at least 6 months. However analysing those that had at least 5.3 months of treatment the SVR rate was 67.4%.

As with the Edinburgh cohort the SVR rate in those that had had previous IFN monotherapy was not significantly different from those that had not.

For IFN naïve patients, binary logistical regression identified a genotype 2 or 3 infection, a lower pre-treatment weight and a longer treatment course to be independent predictors of SVR. When the same analysis was performed but stratified for genotype, for IFN naïve genotype 1 infections a younger age at treatment was an independent predictor of SVR. For those with a previous IFN based treatment, it was a lower pre-treatment weight and a longer treatment course. This would be in

keeping with the trials that have shown that 12 months treatment achieves greater SVR rates than six. The effect of pre-treatment weight in IFN monotherapy was analysed in the two international trials. It revealed a lower weight increased SVR rates independently of genotype (McHutchison et al. 2001a). Similar associations were found in the Edinburgh cohort.

Overall discontinuation of therapy due to a severe adverse event occurred in 10.1%. This compares to 8% for 24 weeks of treatment and 19-21% for 48 weeks of treatment seen in the trials with depression, as in this study, being the commonest cause (McHutchison et al. 1998b; Poynard et al. 1998). In addition to this a further 18.9% discontinued therapy prematurely due to patient choice, without a severe adverse event. However despite this early discontinuation 72.7% of these patients achieved a SVR, suggesting it occurred late in their planned treatment course. Dose reduction occurred in 14.3% of patients which is comparable to the 7%-10% seen the trials. Anaemia triggered this in 6.7% of patients compared with 7-9% seen in the trials. As with the trials, the flu-like side effects predominated with fatigue occurring in 62.3% of individuals being the commonest. The incidence of these other side effects was comparable except there was less myalgia, fever, nausea, insomnia, pharyngitis and alopecia reported in this study (McHutchison et al. 1998b).

This study includes follow-up of 108 sustained responders, of whom 51 had a HCV RNA PCR at 2 years post treatment, and 94.1% remained negative.

There are a number of reasons to interpret the results with caution. Data was submitted on only 366 patients out of the total possible cohort of 1220 patients, and therefore selection bias may have also been introduced, although for each participating centre, all potential patients were identified and complete data

submitted. The reason for this low proportion was that many more centres expressed an interest in participating at the outset, but did not submit data when it became clear how much work was involved.

6.4.3 Combination pegylated interferon and ribavirin

The primary end-point of a sustained HCV clearance was achieved in 41 patients (37.6%) of the 109 patients with follow-up. In the genotype 1 infected patients an overall SVR for the whole cohort of 20% is less than 50% reported in the trials (Manns et al. 2001), (Fried et al. 2002) where the intention to treat was 12 months. Analysing those that had at least six months of treatment the SVR rate was 41.6%.

In the genotype 3 infected patients an overall SVR rate of 56.2% is less than 80% reported in the trials where the intention to treat was at least 6 months. However analysing those that had at least 5.3 months of treatment the SVR rate was 67.7%.

In IFN naïve patients the SVR rate was 29.0% and 59.3% for genotype 1 and genotype 2 or 3 infections respectively, compared with 8.3% and 45.8% in those that had previous IFN treatment. The large numbers that were receiving retreatment explains, in part, the lower overall SVRs.

Binary logistical regression identified for IFN naïve patients a genotype 2 or 3 infection to be the only independent predictor of SVR. For those that had received previous IFN based treatment a genotype 2 or 3 infection ($p=0.011$) and a younger age pre-treatment ($p=0.033$) were found to be independent predictors of SVR. When the same analysis was stratified by genotype, no independent predictors could be identified either for genotype 1 infections or for the genotype 2 or 3 infections. No effect of pre-treatment weight could be identified. In the PEG-IFN alpha-2b plus ribavirin trial an association with pre-treatment weight in genotype 1 infected

patients was observed and attributed to a dose-dependent response to ribavirin (Manns et al. 2001). However for genotype 3 patients this factor was not predictive of SVR. In a PEG-IFN alpha-2a plus ribavirin trial SVR rates appeared independent of the ribavirin dose in genotype 3 infected patients (Hadziyannis et al. 2002).

Overall discontinuation of therapy due to a severe adverse event occurred in 6.4%. This compares to 9.7% for 48 weeks of treatment seen in the trials (Manns et al. 2001), (Fried et al. 2002). Binary logistical regression identified a lower weight as the only pre-treatment independent predictor of premature treatment termination due to a serious adverse event. In addition to this a further 17.4% discontinued therapy prematurely due to patient choice, without a severe adverse event compared to 12.3% in the trials. A lower weight pre-treatment and dyspnoea during treatment independently predicted this outcome. Only 8% of these patients achieved a SVR. Dose reduction occurred in 37.6% of patients which is much higher than the 8.2% seen the trials and much higher that that seen with combination standard IFN and ribavirin. Neutropenia triggered this in 21.1% of patients compared with 3.5% seen in the trials. This is also much commoner than with combination standard IFN and ribavirin. As with the trials, the flu-like side effects predominated with fatigue occurring in 19.3% of individuals. However they were no commoner than with combination standard IFN and ribavirin. The commonest side effect was low mood at 22.9%, which was much commoner than with combination standard IFN and ribavirin. There was not a higher prevalence of injection site inflammation that was seen in the trials.

This study includes follow-up of 41 sustained responders. No patients have relapsed so far after a median of 9.3 months follow-up post treatment. Only three patients

have been followed up for two years post treatment and all remain sustained responders.

6.4.3.1 Conclusion

This study has demonstrated that the improvements in efficacy in treatment for chronic HCV infection seen in the randomised clinical trials are likely to be converted into improvements when used in routine hospital practise as the guidelines suggest.

Overall IFN monotherapy rates were similar to published meta-analysis although the SVR rates in non-genotype 1 patients appeared considerably lower. The adverse events appeared similar, although as with all treatment regimes a higher number of patients terminated treatment early compared with the trials. The long-term outcome was also similar.

The results from the combination standard IFN and ribavirin treatment analysis were lower in the Edinburgh cohort compared with the UK cohort. The UK cohort had lower SVR rates compared with the trials for genotype 1 patients, but similar for non-genotype 1 patients. Although there were higher discontinuation rates, when this was not for a serious adverse event, the SVR rate remained high. These differences may relate to treatment regimes not being established when the treatment was initially used, as is demonstrated with the improved SVR rates when at least 6 months of treatment has been given. Adverse events were lowest in the Edinburgh cohort but this may relate to underreporting. Dose reduction rates were similar to trials. Both cohorts demonstrate that SVR is very durable.

Overall the SVR rates for PEG-IFN were lower than the trials but improved significantly and were comparable when only IFN naive patients were analysed.

Discontinuations were similar to trials but higher than combination standard IFN and ribavirin. Dose reductions were much higher than those in the trials primarily for neutropenia and anaemia. This is important as these patients can be treated with GM-CSF and erythropoetin, which markedly increases the cost of treatment. The incidence of low mood appeared higher in the PEG-IFN and ribavirin group compared with combination standard IFN and ribavirin. There was no evidence to suggest the durability of the SVR is different with this treatment regime.

Chapter 7 Discussion

At the end of each chapter there is a detailed discussion of the analysis and placed in the context of previous published research in the relevant area.

7.1 Natural History of HCV infection

The patients that are referred to the Royal Infirmary of Edinburgh have been described in detail in this thesis. There is a significant proportion that has been found to be HCV RNA PCR negative at initial assessment and presumably have cleared the infection at the acute stage of the disease. No analysis has been performed on these, as referral of these patients is even more inconsistent than the HCV RNA PCR positive cohort. Therefore they may be even less representative of the HCV RNA PCR negative in the community. HCV RNA PCR positive cohort is made up of a significant proportion of middle-aged men who acquired the infection less than 20 years ago, principally through IDU, who have a significant history of alcohol abuse. This population, in its make-up is very different from the prospective community-based cohorts, such as the Irish anti-D cohort (Kenny-Walsh 1999). Therefore, unsurprisingly the outcome is very different and it is unlikely to be entirely down to referral bias in the hospital-based cohorts. A significant proportion are already presenting with cirrhosis and its complications, demonstrating the considerable burden on healthcare resources these patients are creating.

Analysis of the covariates that may influence progression in the HCV RNA PCR positive cohort, demonstrates how often they are significantly correlated. Importantly the covariates also significantly influence the time to presentation and therefore may lead to either an under- or over-estimation of their effect. In chapter 4 and 5 the progression to cirrhosis and the complications of cirrhosis respectively were analysed using Cox regression. Although Cox regression analysis is a multivariate method, its

use in this setting, where there is such high correlation of covariates, to identify independent predictors of progression is not foolproof. It merely produces the best model with the covariates available to recreate the observed results. In all models produced the R square scores were very low compared with the maximum possible suggesting the models are not good and there maybe other covariates untested that will improve the predictive value of the model.

The importance of left truncation of the risk set at the time of presentation has been emphasised and shown to influence the outcome of analysis. This is required as those referred are only a sample of the HCV RNA PCR positive population in the Edinburgh 'EH' postcode region. For this sample to be a valid representation of the whole population, referral has to be random. Clearly this is not the case as only a proportion are being referred and this referral process is significantly affected by the vast majority of those that have been found to have advanced disease in the cohort, presenting at or just before the time of this diagnosis of advanced disease. As mentioned above the covariates also affect the time of presentation. Therefore hospital-based cohorts, such as this, are very susceptible to this referral bias and it will have influenced the results. The left truncation method does require much larger cohorts, due to fewer patients being in the risk set at any given census time-point, compared with analysis without left-truncation.

Despite the acknowledged limitations of these studies, in particular the difficulty of recording accurately the date of infection and the previous alcohol history, they do provide further evidence of the key roles of the age of the patient at infection and the level of alcohol consumption in the progression to cirrhosis. It shows that previously established risk factors for progression, such as the sex of a patient, are not the same

in all cohorts of patients. This raises some concerns about applying the results of previous cohort studies to other populations, when trying to make accurate predictions of health impact of HCV infection for a local cohort.

The studies looking at the onset of the complications of cirrhosis do provide evidence the age of the patient does influence progression to these end-points. The influence of other covariates is less clear due to confounding. A beneficial effect of previous IFN-based treatment, especially in those that have a SVR on HCC occurrence is suggested, however selection of those that were treated was not random and therefore susceptible to bias. It attempts for the first time to estimate the duration of infection to the onset of clinically important complications for a hospital-based cohort. It confirms that at five years after the diagnosis of uncomplicated cirrhosis the risk of HCC is about 9-12%, the risk of a major complication of cirrhosis is about 11% and the risk of liver related death is about 8-10%. This progression is with conventional primary prophylaxis of varices, treatment of bleeding varices and treatment of about halve the cohort with IFN-based regimes. Clearly this is likely to have modified the outcome. Larger cohorts with longer follow up are required to clarify these issues further.

7.2 Treatment of HCV infection

This study has demonstrated a steady improvement in efficacy in treatment for chronic HCV infection with the introduction of each new treatment regime as suggested by randomised clinical trials. About 45% of all patients referred and assessed each year were deemed suitable for treatment and listed.

Overall IFN monotherapy rates were similar to published meta-analysis although the SVR rates in non-genotype 1 patients appeared considerably lower. The adverse

events appeared similar, although as with all treatment regimes a higher number of patients terminated treatment early compared with the trials. The long-term outcome was also similar.

The results from the combination standard IFN and ribavirin treatment analysis were lower in the Edinburgh cohort compared to the UK cohort. The UK cohort had lower SVR rates compared with the trials for genotype 1 patients, but similar for non-genotype 1 patients. These differences may relate to treatment regimes not being established, in terms of assessment of response and duration of treatment for each genotype, when the treatment was initially used. This was demonstrated with the improved SVR rates when at least 6 months of treatment has been given. Adverse events were lowest in the Edinburgh cohort but this may relate to underreporting. Dose reduction rates were similar to the trials. Both cohorts demonstrate that SVR is very durable.

Overall the SVR rates for combination PEG-IFN and ribavirin were lower than the trials due to the high number of patients being retreated. The SVRs for IFN naive patients were comparable. Discontinuations were similar to trials but higher than combination standard IFN and ribavirin. Dose reductions were much higher than those in the trials. They were most commonly for neutropenia and anaemia and therefore may necessitate the use of GM-CSF and erythropoietin. This markedly increases the cost of treatment and probably its cost-effectiveness. The incidence of low mood appeared higher in the PEG-IFN and ribavirin group compared with combination standard IFN and ribavirin. There was no evidence to suggest the durability of the SVR is different with this treatment regime.

7.3 Utilisation of the database application

A major positive outcome of this thesis has been the creation of a database application that can be used in any liver unit to collect epidemiological and audit data. The database is now successfully used in all the liver units in Scotland as part of an ongoirg project to collect data on all patients with HCV, that have attended hospital and been assessed. This will culminate in the creation of a National Clinical HCV database which will provide information for health planners and researchers to assess and combat the health burden poised by the infection in Scotland.

7.4 Conclusion

Chronic HCV is a significant health problem in Edinburgh with large numbers being referred for assessment, treatment and management of the complications of cirrhosis. Treatments are effective, although do have significant adverse effects that affect compliance. The numbers still requiring treatment in the cohort still remain high overall. The natural history of the infection and how it is influenced by therapy is becoming clearer, in particular the influence of alcohol and the age of the patient. However for further progress to be made, it will require the analysis of larger cohorts from a variety of settings.

7.5 Suggested future work

7.5.1 Natural History

- Further analysis of the factors that influence presentation to hospital.
- Further follow up over time of the cohorts identified in this thesis to identify further progression to the end-points.

- Performing the same survival analysis on patients on the National Clinical HCV database that has been set up. The larger numbers will allow left-truncation of the survival analysis to be more accurate.
- Staging of the liver biopsies using a scoring scheme to further analyse progression of fibrosis prior to cirrhosis.
- Assessing the influence of steatosis on progression to liver cirrhosis by documenting its presence on biopsies and how this correlates with the patients BMI and insulin resistence.
- Analysis of the HCV RNA PCR negative cohort.
- Further validation of Hyaluronic acid as a surrogate marker of cirrhosis and identifying the causes of false positive and negative results.

7.5.2 Epidemiology

- Collection of basic epidemiological data on all patients referred to hospital in Scotland using the National Clinical HCV database.
- Analysing how the cohort referred to hospital differs from those that have not in Lothian, by anonymised linkage with the antibody positive database held by the Health Protection Scotland in Glasgow.

7.5.3 Treatment

- Ongoing audit of the number of patients suitable for treatment, the reasons of unsuitability, treatment results, treatment adverse events and long-term outcome.
- Analysis of the reasons for discontinuation treatment and whether this can be predicted prior to initiation of treatment.

- Long-term outcome of patients that developed permanent complications of treatment such as thyroid disorders.
- Long-term effect of treatment on progression to complications of cirrhosis and HCC.
- Trials to assess efficacy of shorter courses of treatment in genotype 2 or 3 patients in hospital practise.
- Auditing GM-CSF and erythropoietin use in reducing dose reductions or discontinuations.

Appendix 1 The Hepatitis C management database

1.1 Aim

To create a Microsoft Access database application, to enable clinicians to manage, audit practise and collect epidemiological and natural history data on hospital cohorts of patients with HCV infection.

1.2 Description of database

1.2.1 Sign on

On opening the database the first screen that is displayed is the 'Hepatitis C Database Sign on' form. The user has to enter their name and password. This functions to limited entry to the database and it also identifies who is using the database, so that when a record is changed it is possible to identify who made that change and when. The user is then taken to the main switchboard.

1.2.2 Main switchboard

This is portal of entry into the database. It allows access to either a form that has all the patent possible patient fields or just the fields of the minimum dataset of the Scottish HCV database project. Reports and charts can be accessed based on the whole population or subgroups within the population, including those concerning treatment.

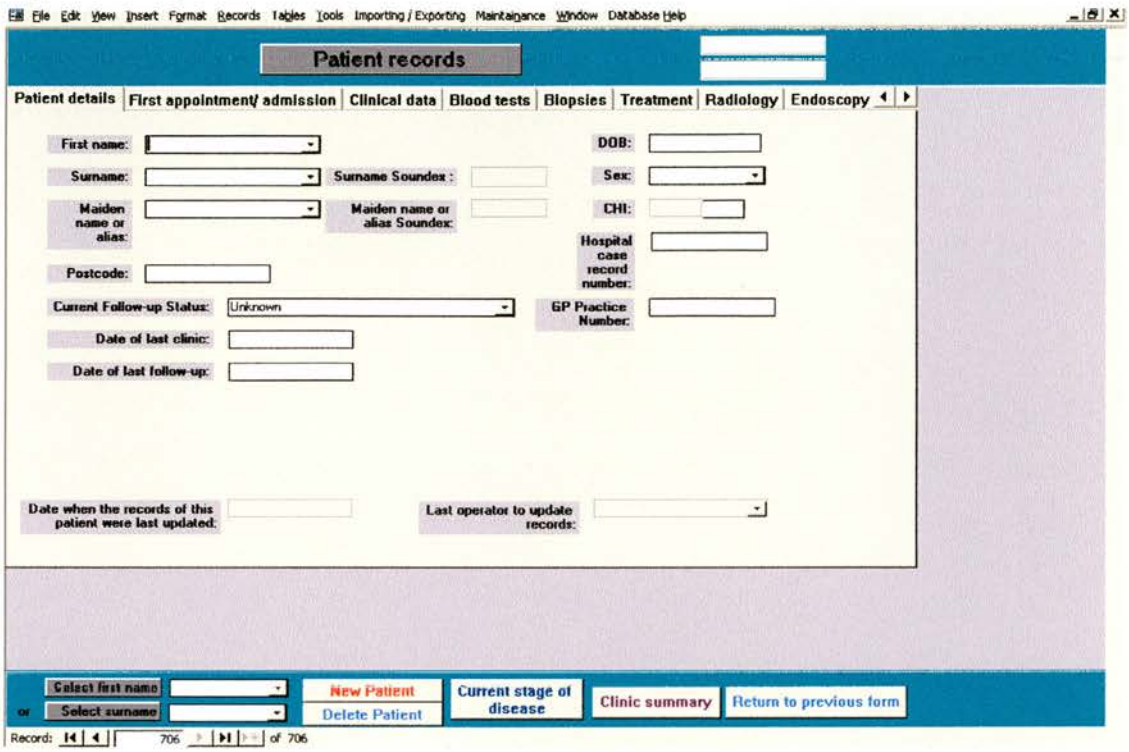
1.2.3 Patient Records

1.2.3.1 All patient records form

The 'All patient records' form (Figure 41) shows all the available fields / boxes for entering the patient's details. The form however, can also display information entered

on all the patients already entered into the database and allows editing of this information.

Figure 41 All patient records form.



Clicking the labelled tabs at the top of the form accesses the main subforms (**First appointment/admission**, **Clinical data**, **Blood tests**, **Biopsies**, **Treatment**, **Radiology**, **Endoscopy**, **Other Diagnoses** and **Outcome**).

1.2.3.2 Organising data entry

When a patient is seen for the first time in clinic then the main **Patient details**, **First appointment/admission**, **Outcome** and previous **Blood tests**, **Biopsies**, **Endoscopies** or **Radiology** procedures can be entered.

A new **Clinical Data** record should only be entered at presentation, at liver biopsy and if there is a change in the clinical findings. Therefore it is not necessary for each clinic visit.

Blood test data such as Bilirubin, Albumin and Prothrombin ratio should be entered at the time of first diagnosis of cirrhosis or HCC and thereafter at each clinic visit so that an accurate Childs grade can be calculated for the **Current Stage of Disease**. Subsequent procedures or interventions such as treatment episodes and outcomes can be entered as they occur.

1.2.3.3 Main areas

1.2.3.3.1 Patient details

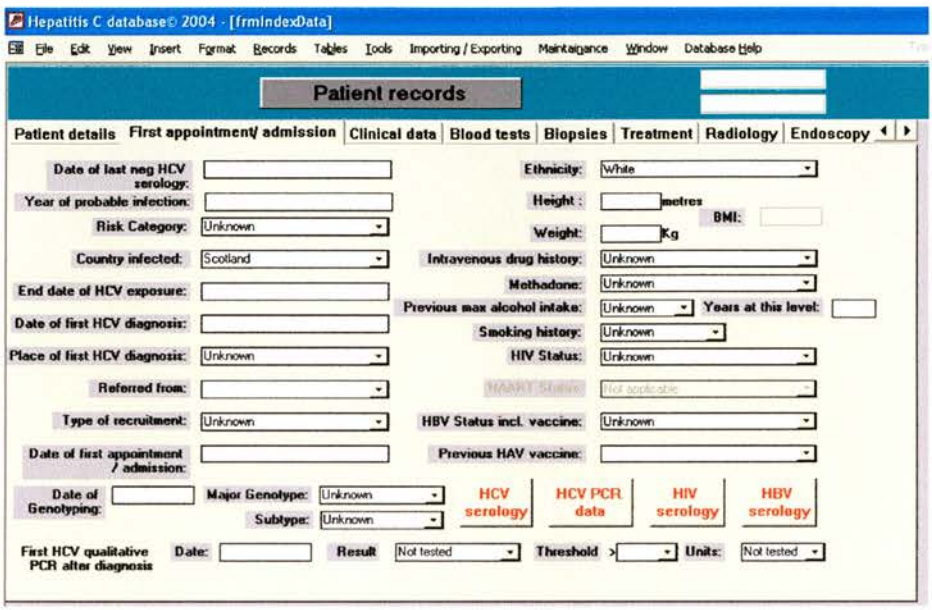
The **Patient details** page (Figure 41) includes boxes to record details of the patient that do not change.

- **Surname soundex** and **Maiden name or Alias soundex**: These fields are automatically calculated using an algorithm pre-programmed into the application (Mortimer & Salathiel 1995).
- **Current Follow up status:** This should be updated every time information is changed on a patient. This can be entered manually or it can be filled in automatically when entering information into other fields.
- **Date of last follow-up**: This is the date of the last contact with a patient i.e. endoscopies, radiology, death, transplantation etc. This can be entered manually or it can be filled in automatically when entering information into other fields.
- **Date when the records of this patient were last updated**: Automatically updated when data is changed on a patient.
- **Last operator to update records**: Automatically updated when data is changed on a patient.

1.2.3.3.2 First appointment

The **First appointment** page (Figure 42) includes boxes to record details of the patient that will usually be available at their first appointment.

Figure 42 First appointment page.



- **BMI**: The body mass index is automatically calculated.

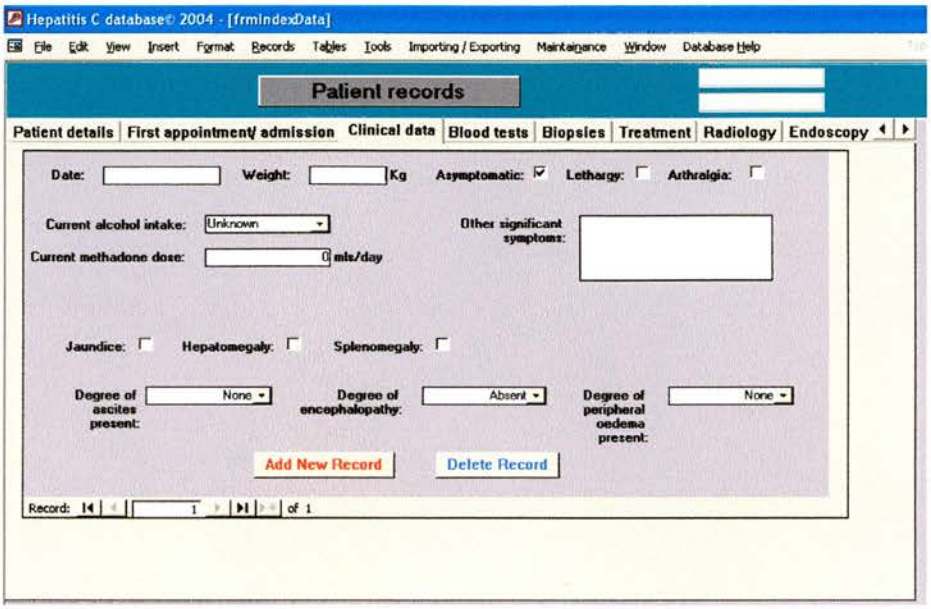
1.2.3.3.3 Clinical data

The **Clinical data** page (Figure 43) includes information on the common symptoms in patients with HCV. It also records the presence or absence of the signs of HCV and the signs of liver decompensation.

The **Degree of ascites** or the **Degree of encephalopathy** can be record in the drop-down lists. If presence of ascites or encephalopathy is recorded for the first time, the operator will be asked if they want to update either **Date of first episode of ascites** or **Date of first episode of encephalopathy** on the 'Outcome' tab. If this is the first time a complication of cirrhosis has been present and the patient is not known previously to be cirrhotic the operator will be asked if they want to fill in the **Date of**

first diagnosis of cirrhosis with this date and the **Mode of first diagnosis of cirrhosis** as 'Clinical' on the 'Outcome' tab.

Figure 43 Clinical data page.



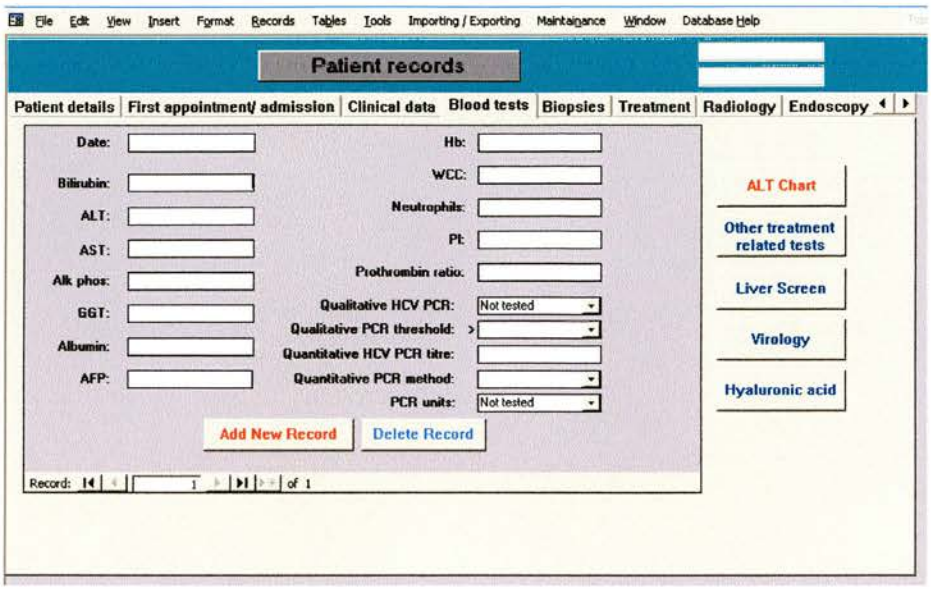
1.2.3.3.4 Blood tests

The **Blood tests** page (Figure 44) includes boxes for the most commonly requested blood tests on a patient with Hepatitis C.

- If the **Bilirubin** >35, **Albumin** <35 **Prothrombin ratio (PTR)** >1.2 and **Platelets** <100 then this might suggest that the patient is cirrhotic. This will prompt the option of automatic filling in of the **Date of first diagnosis of cirrhosis** and the **Mode of first diagnosis of cirrhosis** on the 'Outcome' tab.
- **AFP**: Alphafetoprotein. If a value of >20 is entered this may suggest the presence of an HCC. This will prompt the option of automatic filling in of the **Date of first diagnosis of HCC** and the **Mode of first diagnosis of HCC** as 'AFP' on the 'Outcome' tab. If the HCC was later confirmed then this should be done.

- **ALT chart** this will plot the patients ALT over time.

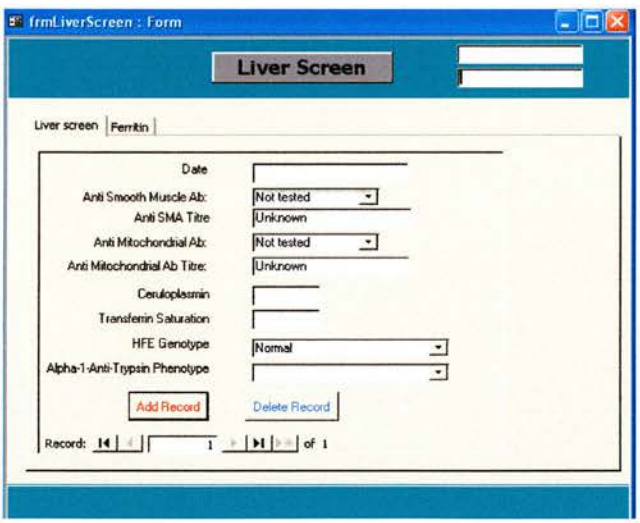
Figure 44 Blood tests page.



1.2.3.3.4.1 Liver screen

The **Liver screen** (Figure 45) includes boxes to record all the common liver screen blood tests except for virology serology.

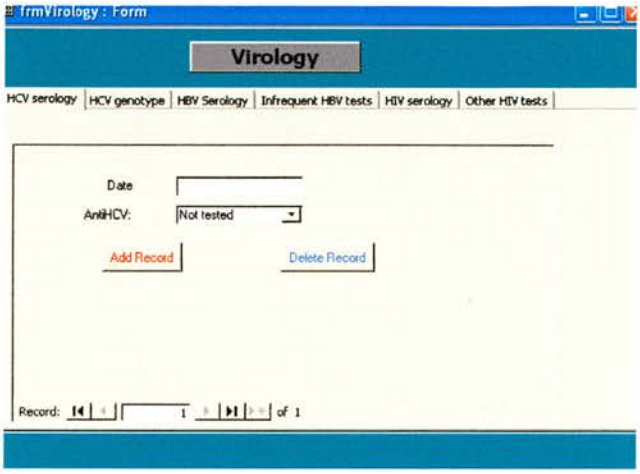
Figure 45 Liver screen form.



1.2.3.3.4.2 Virology

The **Virology** form (Figure 46) includes boxes to record all the common virology blood tests. The form is divided up into six subforms that can be accessed by clicking the appropriate tab headings:

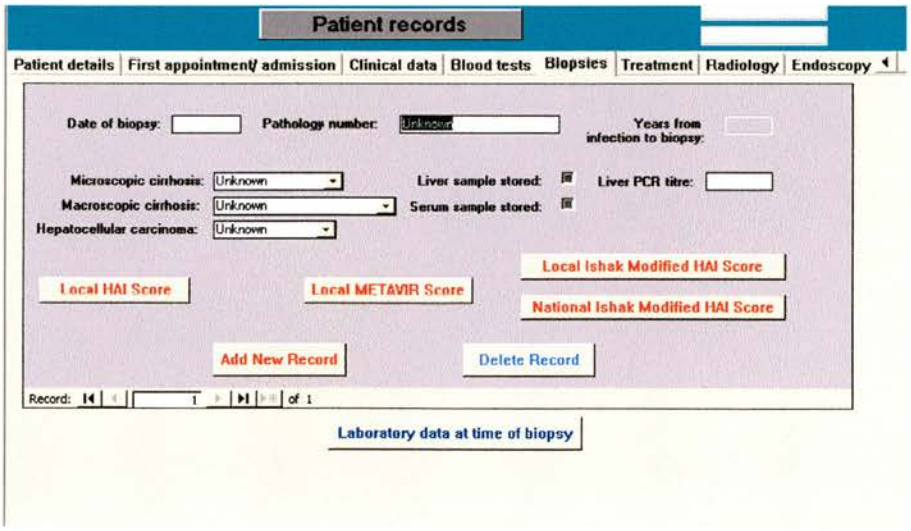
Figure 46 Virology form.



1.2.3.3.5 Biopsy data

The Biopsy data page (Figure 47) includes boxes for entering information on biopsies performed on patients.

Figure 47 Biospy data page.

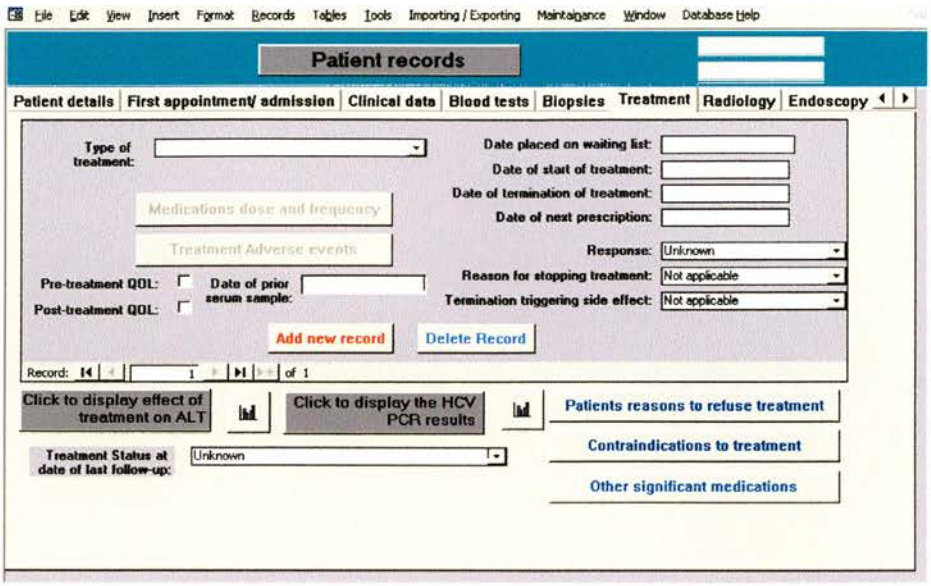


- **Microscopic cirrhosis**: This field will be updated if you classify the biopsy as cirrhotic using the scoring system forms.
- The **Macroscopic cirrhosis:** At laparoscopic procedures or at the time of surgery or transplantation it may be possible to determine if a liver is cirrhotic without biopsy. If the patient is recorded as not cirrhotic in the **Microscopic cirrhosis** field then the patient will not be classified as cirrhotic whatever is entered in this field.
- If the patient is recorded as cirrhotic using either of these fields it will prompt the option of automatic filling in of the **Date of first diagnosis of cirrhosis** and the **Mode of first diagnosis of cirrhosis**.
- **Hepatocellular carcinoma**: If HCC was seen on biopsy or macroscopically it can be recorded here and it will prompt the option of automatic filling in of the **Date of first diagnosis of HCC** and the **Mode of first diagnosis of HCC**.
- There is a choice of scoring systems available. One or more of these can be chosen depending the scoring system used by clicking on the appropriate button.

1.2.3.3.6 Treatment

The Treatment page (Figure 48) includes the **Treatment data** subform on which to record treatment episodes.

Figure 48 Treatment page.



- **Type of treatment** is descriptive name given to the treatment regime i.e. "Combined interferon and ribavirin".
- **Medications dose and frequency** button can be clicked to bring up the **Treatment regime** form. This allows each of the drugs that make up the treatment regime to be entered with its frequency and dose.
- **Treatment Adverse effects** button can be clicked to record treatment side effects. The action taken and the outcome can also be recorded.

Below the **Treatment data** subform there are a number of data boxes and buttons to collect and display additional treatment related data:

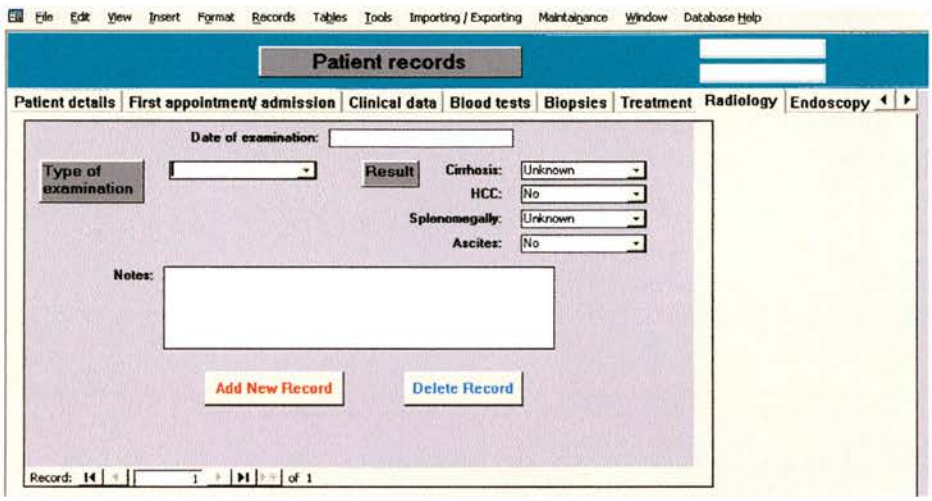
- There are two buttons to help determine the response to treatment. One plots the ALT from blood samples taken from one month prior to starting the treatment regime that is currently displayed in the **Treatment data** subform, and all subsequent measurements. The other button displays all the HCV PCR results in chronological order and plots the log10 quantitative PCR results.

- **Treatment status** is a field that can be used to classify all patients on the basis of whether they want or have been offered treatment and the results of this and whether they want further treatment. The options are selected from the fixed drop down list. If the patient is dead or transplanted then this can be recorded.

1.2.3.3.7 Radiology

The Radiology page (Figure 49) includes boxes to record radiology procedures.

Figure 49 Radiology page.



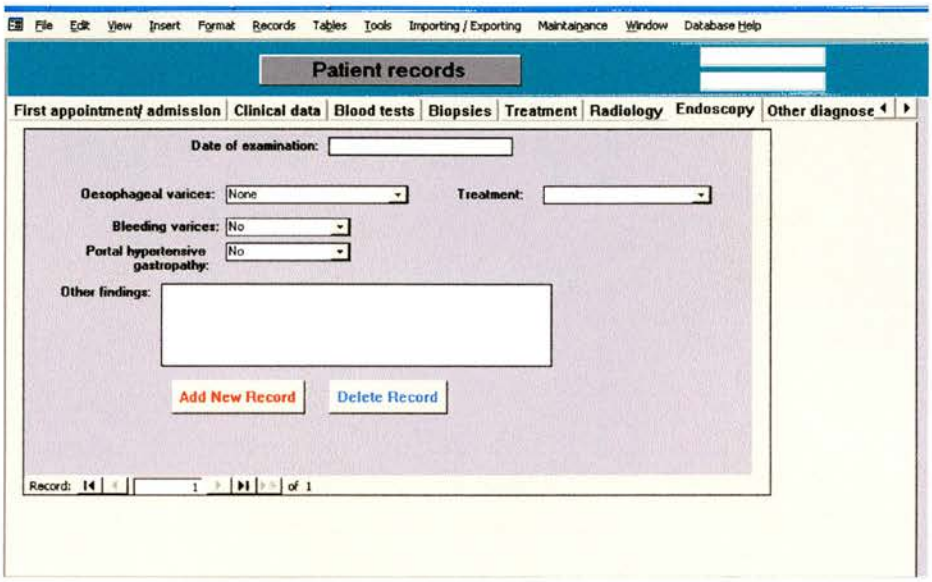
- **Cirrhosis:** A choice of options is available in the drop-down lists. If present it will prompt the option of automatic filling in of the **Date of first diagnosis of cirrhosis** and the **Mode of first diagnosis of cirrhosis.**
- **Hepatocellular carcinoma:** A choice of options is available in the drop-down lists. If present it will prompt the option of automatic filling in of the **Date of first diagnosis of HCC** and the **Mode of first diagnosis of HCC**.
- **Ascites:** If present and this is the first time that ascites has been diagnosed it will prompt the option of automatic filling in of the **Date of first ascites** and

the **Date of first diagnosis of cirrhosis** and the **Mode of first diagnosis of cirrhosis** if appropriate.

1.2.3.3.8 Endoscopy

The Endoscopy page (Figure 50) includes boxes to record endoscopic procedures.

Figure 50 Endoscopy page.

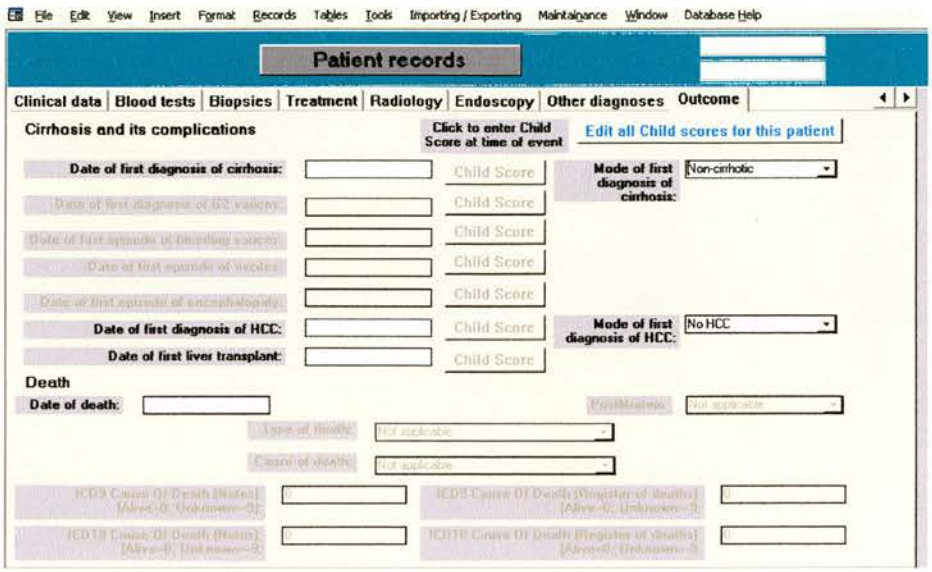


- **Oesophageal varices** drop-down list is to enter the presence or absence of oesophageal varices and their grade if present. If this the first time that Grade II varices have been diagnosed, it will prompt the option of automatic filling in of the **Date of first diagnosis of G2 varices**, **Date of first diagnosis of cirrhosis** and the **Mode of first diagnosis of cirrhosis** if this is appropriate.
- **Bleeding varices**: This can be recorded using the drop down list. If this the first time that bleeding varices have been present, it will prompt the option of automatic filling in of the **Date of first bleeding varices**, **Date of first diagnosis of cirrhosis** and the **Mode of first diagnosis of cirrhosis** if this is appropriate.

1.2.3.3.9 Outcome

The **Outcome** page (Figure 51) includes boxes to record the outcome of a patient.

Figure 51 Outcome page.



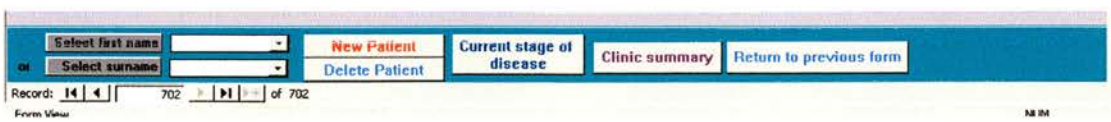
The dates of the first diagnosis of cirrhosis and its complications should be recorded on this form. These are often automatically filled in, as a result of entries elsewhere in the database. Next to each box is a button that opens a form that allows the entry of the components of the Childs-Pugh score. This will also be automatically filled in from the **Clinical data** and **Blood test**s records of the same date.

The **Type of death** box records the death category i.e. Liver-related death, while the **Cause of death** box records the exact cause of death.

1.2.3.4 Current stage of disease

Each patient can be staged by their biopsy fibrosis results and / or their clinical state. This can be displayed clicking the **Current stage of disease** button (Figure 52) at the foot of the **All patient records** form.

Figure 52 Current Stage of disease button



As the stage of fibrosis depends on the scoring system used for the liver biopsy the first screen asks the operator to select one that has been used.

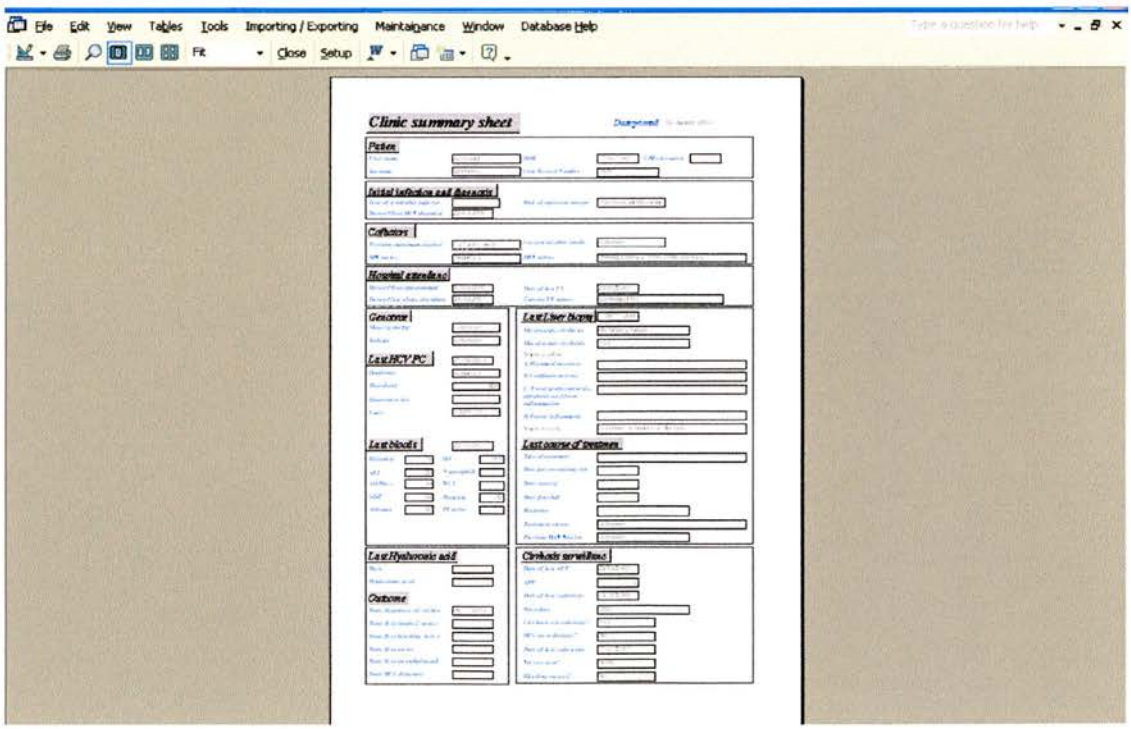
The hierarchical categorisation list is: Dead, Alive transplant, Cirrhosis with complications, Cirrhosis, Liver biopsy stage of fibrosis (depending on scoring system used), No evidence of cirrhosis but no biopsy, No clinical details and no liver biopsy.

For those patients that are cirrhotic two buttons will be displayed. One opens a screen that displays the **Current Childs-Pugh score**. The other displays when the last surveillance procedure for varices or HCC was carried out and whether any are due

1.2.3.5 Clinic summary sheet

This report (Figure 53) has been designed for use as a clinical summary that can be attached to a clinic letter following an outpatient appointment. Data cannot be entered on this sheet, it simply displays the information on the patient entered previously.

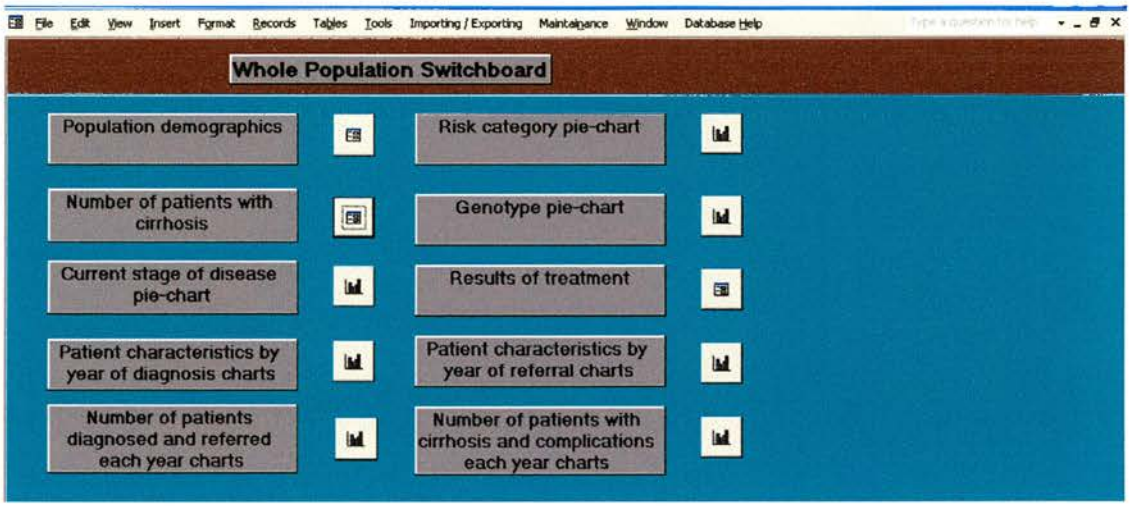
Figure 53 Clinic summary sheet.



1.3 Reports and charts on patient groups

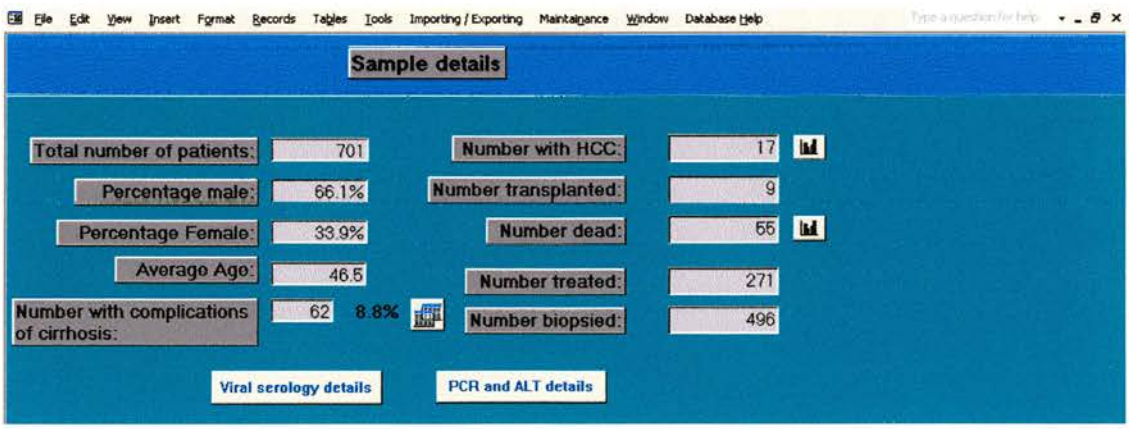
The database can provide pre-programmed reports on the data that has been entered. These can be on the whole population or on different subgroups (Followed up patients, biopsied patients, biopsied patients with a probable date of infection and patients with a probable date of infection irrespective of whether they have had a biopsy). These reports are accessed using switchboard forms (Figure 54).

Figure 54 Whole population switchboard.



Command buttons can then open the desired reports and charts (Figure 55).

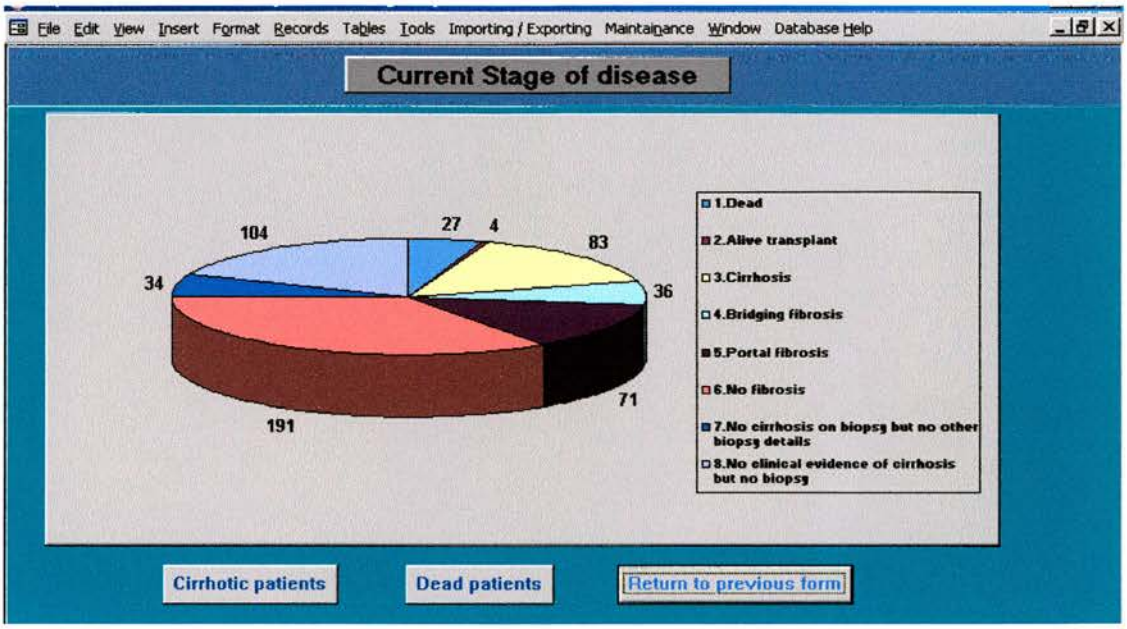
Figure 55 Population demographics.



Examples of other reports and charts on patient groups in the database include:

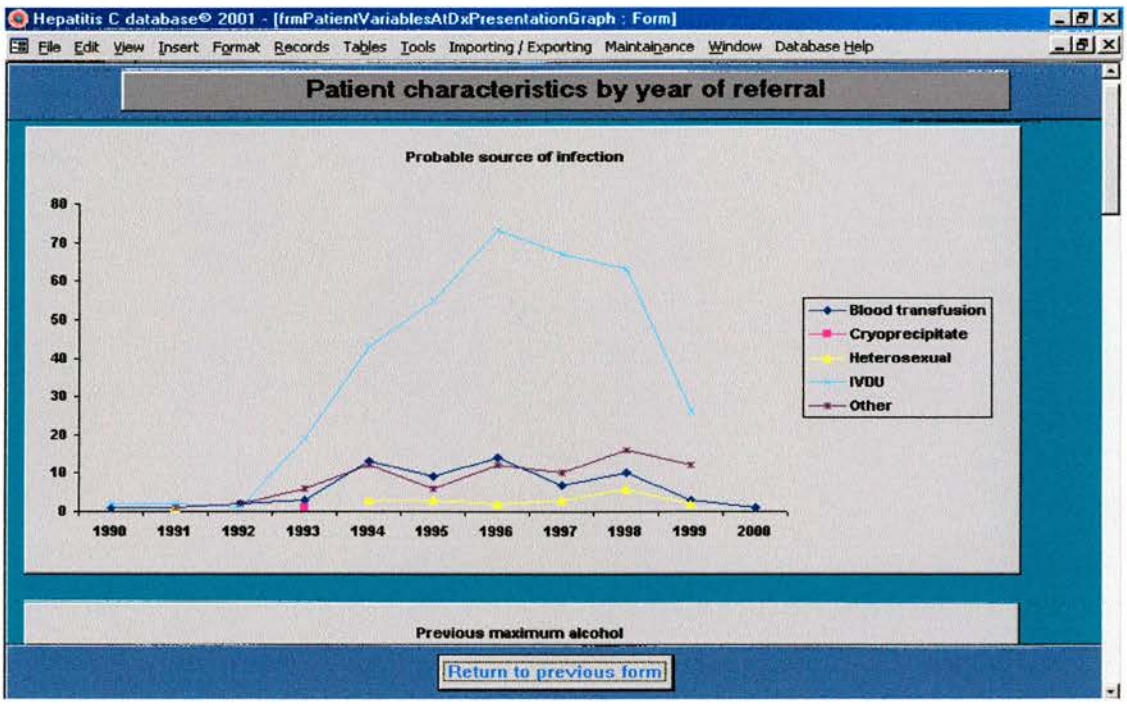
- Pie chart of the current stage of disease (Figure 56).

Figure 56 Pie-chart of current stage of disease.



- Pie charts of the Childs grade at diagnosis of cirrhosis, first complication of cirrhosis and HCC.
- The number of patients diagnosed with cirrhosis for each year.
- The number of patients presenting with their first complication of cirrhosis for each year.
- The number of patients presenting with HCC for the first time for each year.
- Patient characteristics at the time of diagnosis of HCV or at the time of referral. The aim is to identify changes in the characteristics of the population being diagnosed or referred. There are line graphs (Figure 57) for each key characteristic (probable source of infection, previous maximum alcohol intake, HIV serology and age at infection). The years are on the x-axis and the number of patients on the y-axis.

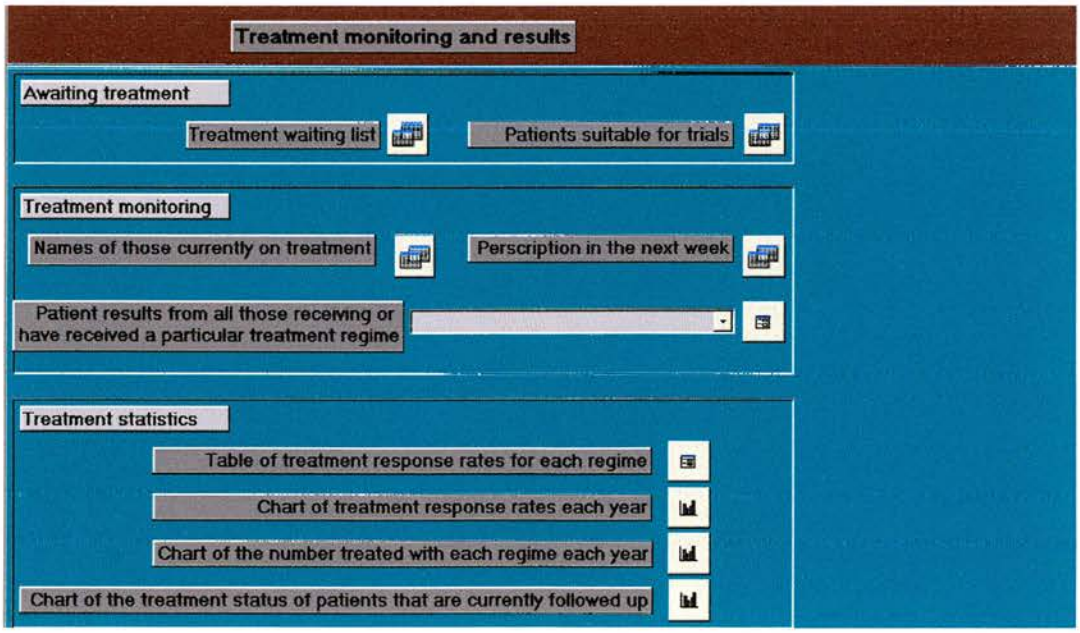
Figure 57 Patient characteristics by year of referral.



1.4 Identifying and tracking patients undergoing treatment

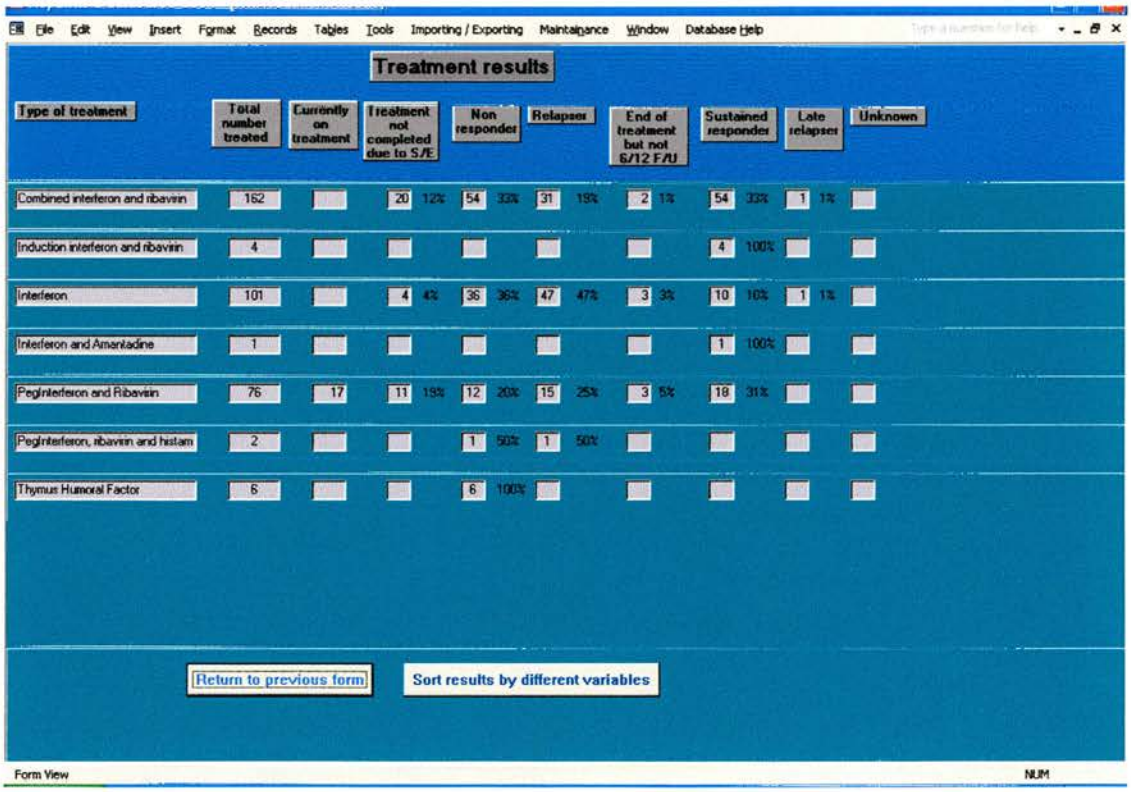
The treatment switchboard (Figure 58) can be accessed by clicking a command button on the main switchboard.

Figure 58 Treatment switchboard.



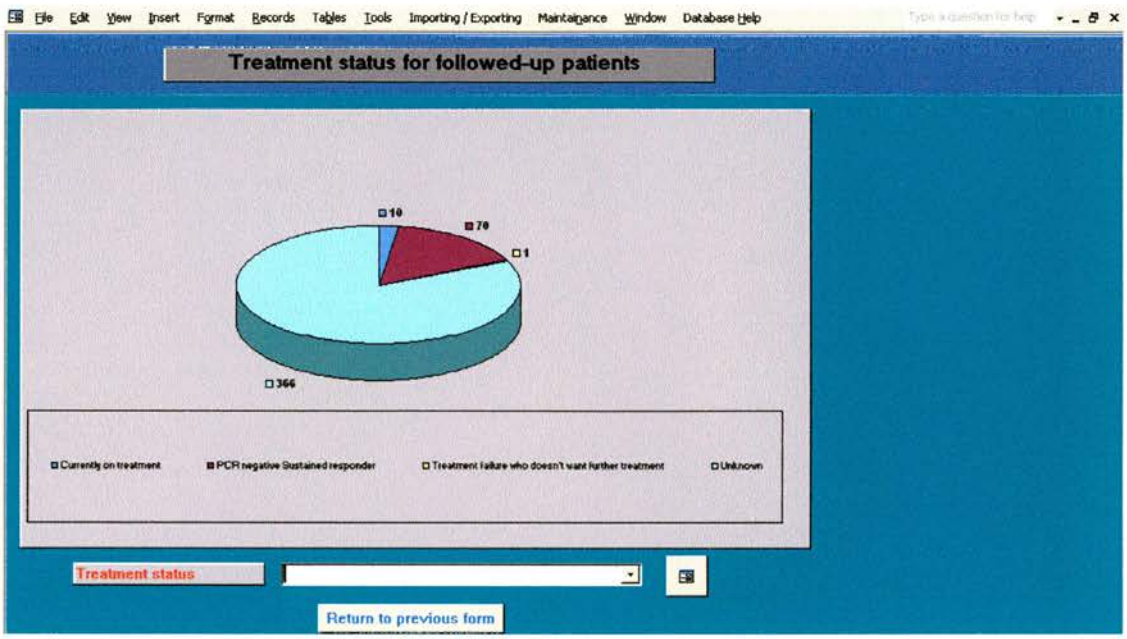
- **Treatment waiting list** is those patients that have not started treatment. The list is ordered according to whether the patient is prioritised and the date they were placed on the list.
- **Patients suitable for trials** are all the patients that are on the treatment waiting list who have had a biopsy in the last year, no previous treatment and whose last ALT was > 40.
- **Table of treatment response rates for each regime** (Figure 59). The **Sort results by different variables** command button displays the results of a particular regime sorted by a specific variable such as the presence or absence of cirrhosis.

Figure 59 Table of Treatment response rates.



- **Chart of the treatment status of patients that are currently followed up**: This displays a pie chart of the Treatment status of patients that are undergoing continuing follow-up (Figure 60).

Figure 60 Treatment status pie chart.



These are the possible categories:

PCR negative, no previous treatment, Mild/Early disease therefore not offered, Medical contraindication therefore not offered, Psychiatric contraindication therefore not offered, Liver decompensation therefore not offered, Ongoing IDU therefore not offered, Chaotic lifestyle therefore not offered, Lack of funding for treatment, Treatment offered but refused by patient, Awaiting start of treatment, Currently on treatment, PCR negative Sustained responder, Treatment failure who wants further treatment, Treatment failure who doesn't want further treatment, Unknown.

At the foot of the form there is a drop down list of categories. The details of the patients in a particular category can be displayed using this command button.

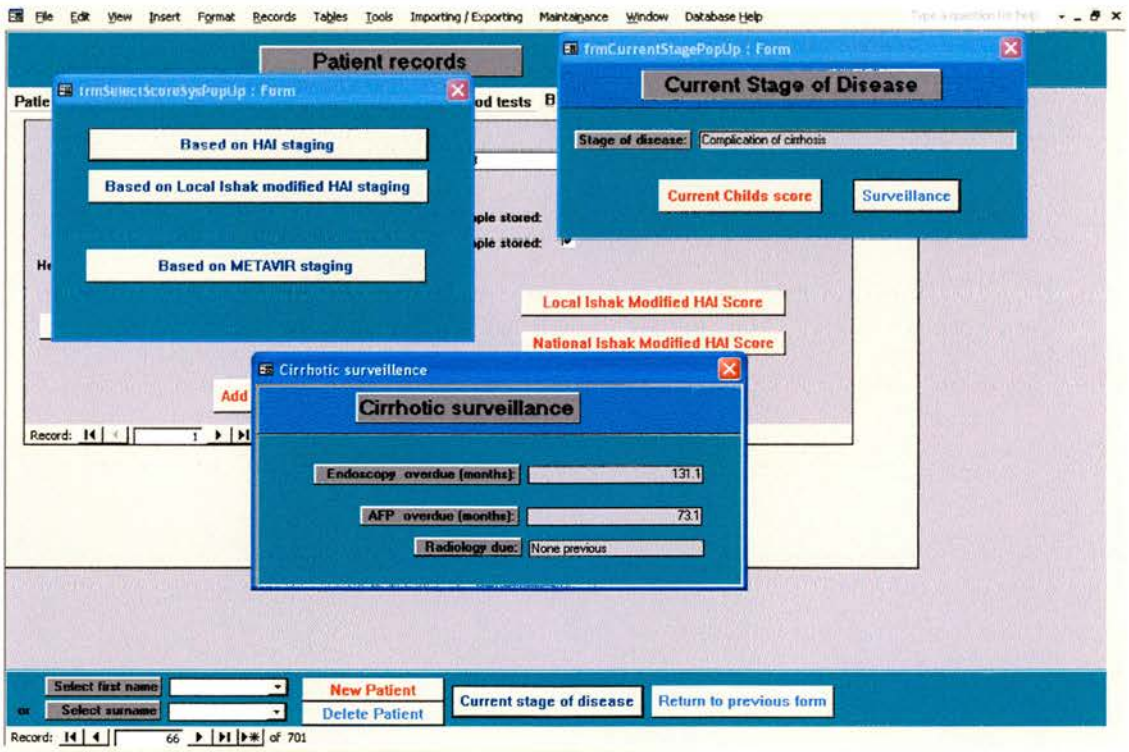
1.5 Surveillance for the complications of cirrhosis

All patients that have continued follow-up that are identified as cirrhotic should undergo surveillance for the treatable complications of cirrhosis. The surveillance protocols used in the Royal infirmary and therefore the database are:

- Endoscopy to diagnose varices every 2 years in those with no previous varices or 6 monthly in those with previous varices.
- USS or CT scans every 6 months.
- Alpha-fetoprotein every 6 months.

For an individual patient, information on recent surveillance procedures can be accessed by clicking on the **Current stage of disease** button at the bottom of **All patient records** form. If the stage of disease is cirrhosis or a complication of cirrhosis then a **Surveillance** button will be visible that can be clicked (Figure 61).

Figure 61 Surveillance forms for an individual patient.



A list of patients that have surveillance procedures are overdue, is available in the followed-up patient's **Reports and charts** section of the database.

1.6 Audit trail

There are two methods used to identify when data has been changed in the database and by whom.

- Whenever a patient record is changed the name of the operator and date that the whole record was last change is updated in the field boxes on the **Patient details** page of the **All patient records** form (Figure 41).
- Whenever data in a field in a record is added or changed, an entry is added to the 'AuditTrail' field in the table the field originates from. This entry details the name of the operator who made the change, the date it occurred and the old value. Therefore if a field is changed in error, it is possible to correct it retrospectively and inform the person who made the error of their mistake.

1.7 Help available to users of the database

Hovering the mouse pointer over a box or button can access an explanation of what should be entered into a field box.

An HTML help file has also been created for the database. It gives full explanations of all the forms and data entry boxes in the database. It utilises hyperlinks to allow easy navigation from topic to topic.

To display the help topic for a particular form, the form in question needs be opened. **Help** on the menu bar at the top of the screen can then be clicked. The drop-down list includes the item **Hepatitis C database help**. If this is clicked, the topic will be displayed.

A user manual for the database has also been created. It is 75 pages long and fully indexed. Screen shots of the database forms are used to illustrate the manual.

1.8 Consent from patients for their data to be held on the database

The final report of the Confidentiality and Security Advisory Group for Scotland (CSAGS) has provided useful guidance on the issues of consent. The two areas of particular relevance were concerning data collection relating to direct NHS Scotland Patient Care (applicable to the local databases, which provide an electronic patient record) and data collected for Multiple uses including disease registries, epidemiology and national databanks (applicable to a National HCV database). They concluded that "implied consent" for data to be collected in these settings would be acceptable, provided that "patients are clearly informed about such uses of their data, that data controllers use only the data needed for the task and that they have strict and monitored code of confidentiality. The right to opt-out must be integral to this although patients must be aware of the implications of this and any operational impediments in agreeing to such requests." They recommend that the information provided can be in two forms: (a) generic information leaflet on how NHS Scotland uses patient identifiable information. (b) A patient information leaflet detailing the specifics of the project concerned.

Therefore all patients that are under ongoing follow up at the Royal Infirmary are supplied with a version of the generic leaflet and a patient information sheet about the aims of the project, what information will be collected and how it will be used.

This explicitly states how to opt out. A time limit of four weeks for this opt out is also set, so that it is possible to determine who has or has not opted out.

This approach is taken because in practical and logistical terms it would be impossible to undertake this sort of epidemiological study, if written consent was required from all those in the target population.

The strategy to address the consent issues of with those subjects that are no longer attending follow-up (defined as those not attending the hospital for over one year and not attending their last scheduled appointment, or those discharged from hospital review) or those that have died, is different. The main concern about this group is that by trying to contact these persons to supply information about the project, by its very nature discloses that the person has Hepatitis C infection. Therefore, if the letter were to be opened by anyone other than the addressee, patient confidentiality would be broken. Therefore the information on the subjects defined above, has been collected as previously described and held on the local database. However, no patient identifiable information except the Master Index (see below) is associated with their data and therefore it is "acceptable anonymised". This process therefore does not compromise the patient's right to confidentiality, and complies with the CSAGS's recommendations on consent when data is "acceptable anonymised".

For those patients that opt out a master index reference for them is created. As this Master Index is "acceptably anonymised" and contains no clinical information, we feel it does not require patient consent. The data contained within the Master Index enables a description of those that participated and those that did not to be made in very basic terms. This gives information on whether our cohort is biased or unrepresentative in this respect.

1.9 Anonymisation

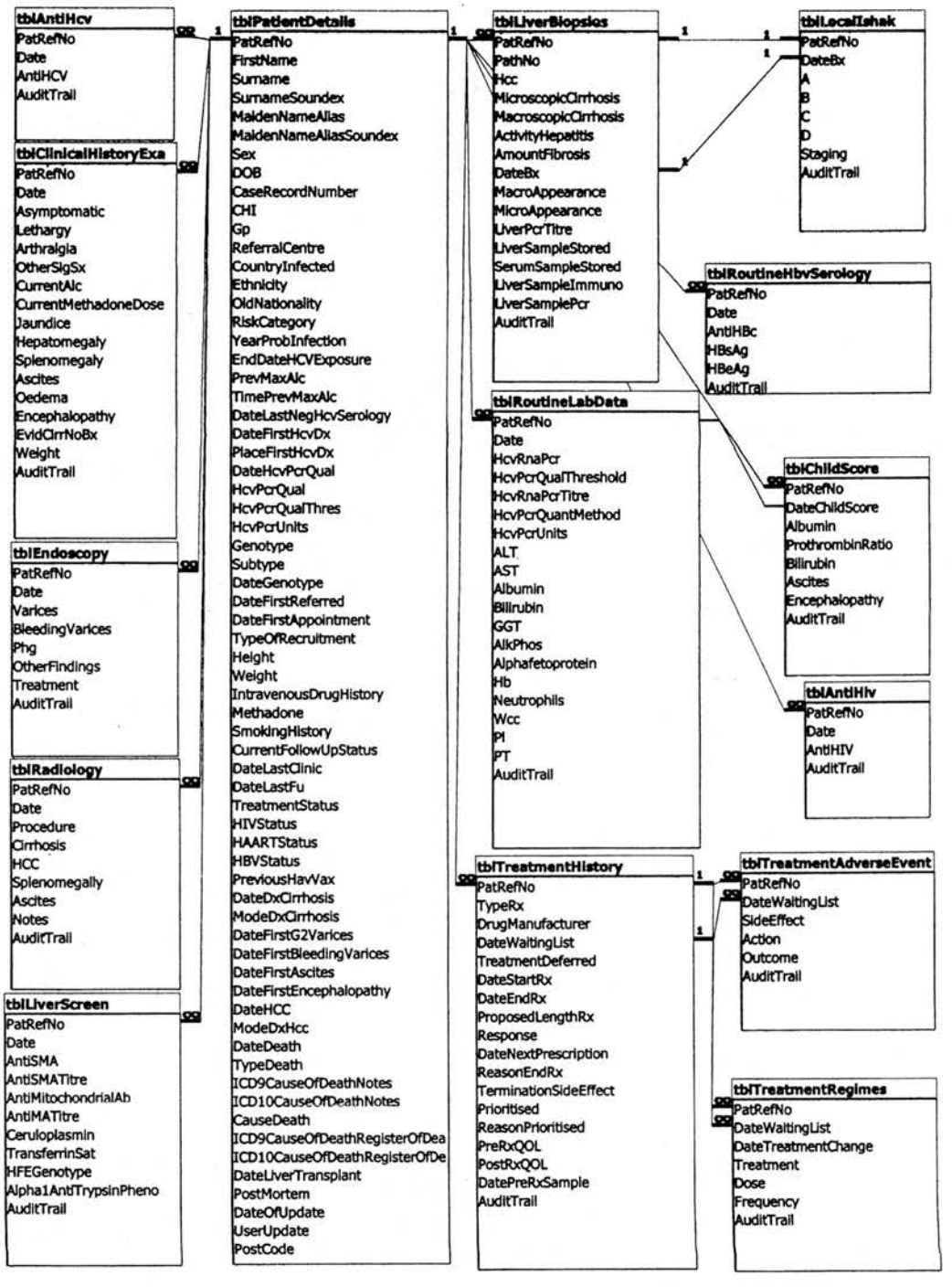
Anonymisation is undertaken by creating a Master Index reference of each patient. The constitutive parts are first name initial, 4 digit Soundex code (Mortimer & Salathiel 1995) of surname, Sex of patient, Date of birth, Postcode sector. For example a Sandra Bird, date of birth 18/05/1962, living in postal district EH03 would be coded as: [S] [B][6][3][0] [f] [1][8] [0][5] [1][9][6][2] [E][H][0][3]. A 4 digit Soundex code of maiden name or other alias surname will also recorded separately as appropriate for each patient. Health Protection Scotland, formerly known as the Scottish Centre for Infection and Environmental Health has used this method in similar projects in the past. It will allow database linkage to their HCV antibody positive database.

This process of anonymisation has been automated in the database by adding a command button on the footer of the **All patient records** form that removes the patient identifiable data and leaves the constituents of the master index. It is also possible to search the database using a surname and identify all the patient records with the soundex of that surname. This allows patients that have been lost to follow up and therefore anonymised, to be identified if they are re-referred.

1.10 The database structure

The database is based around numerous tables related by the patient reference number (PatRefNo) assigned to the patient. Each table has various fields depending on the type of information that is being stored in the table. Figure 62 lists all these tables and fields.

Figure 62 The tables and fields of the database.



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