

**NATURAL HISTORY AND MANAGEMENT OF
HEPATITIS C IN EAST LONDON**

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(M.D.)**

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ABSTRACT

Chronic infection with the hepatitis C virus infection (HCV) affects over 170 million individuals worldwide. In this thesis the natural history and management of hepatitis C in North- East London was investigated.

The prevalence of cirrhosis in patients with chronic hepatitis C rises with increasing duration of infection. In Asian patients infected at birth, infection over 60 years causes cirrhosis in 71% of infected individuals. Since the rate of fibrosis progression in Asian patients is the same as that seen in Caucasian patients, it is likely that similar rates of cirrhosis will be seen in all patients who are infected with HCV for over 60 years. Factors found to be associated with fibrosis progression were:- age and alcohol excess. Insulin resistance was associated with fibrosis progression. However, elevated serum ferritin or hepatic iron were not.

Knowledge of hepatitis C in the East of London was examined and found to be poor despite the Department of Health information campaign. Educational meetings and postal surveys improved the level of knowledge of HCV. However as our group only assessed knowledge immediately after completion of the

sessions, such a testing regime does not address long-term knowledge retention.

We examined current and novel management strategies for patients with chronic HCV. Current therapy involves pegylated interferon and ribavirin. We found that insulin resistance was a poor predictor of sustained virological response. Chinese herbal treatments for hepatitis C are widely used but poorly studied. Our group designed a randomised controlled double blind study to assess whether Chinese herbal treatment is effective and results from this study show that recruitment and retention in trials of alternative therapies are problematic and that the herbal remedy had little effect on viraemia and quality of life, although liver function tests did improve a little.

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DECLARATION

This work was performed during my tenure of research fellowship in the Hepatobiliary Group Department of Adult and Paediatric Gastroenterology, Queen Marys School of Medicine and Dentistry between October 2002 and October 2004. I conducted all the studies described, and collated the data together with the typing of the text.

Statistical Analysis for the natural history of hepatitis C was performed by Professor CA Sabin (Professor of Primary Care and Population Sciences, Royal Free and University College Medical School, London)

The General Practice questionnaire on hepatitis C was devised by myself and Professor Foster. This was assessed for content and relevance by ten senior practitioners chosen from the primary care trusts chaired by Dr Comfort Ossonoya (Senior Lecturer, Department of General Practice and Primary Care). Dr Ossonoya was also involved in setting up the educational meetings for GPs.

The Herbal study was originally designed by Professor Foster and was initiated three years ago. Owing to the bankruptcy of the original company the trial was suspended and recommenced in 2003 when Phynova assumed financial responsibility. I was

responsible for the re-initiation of the study. I enrolled and supervised the care of all patients at The Royal London. I collected data from the other centre, St Marys Hospital, London and analysed the results and prepared the final report. The trial was formally monitored by Dr E. Blair, Phynova.

This thesis reviews the natural history of hepatitis C in the North-East of London and assesses the level of knowledge of GPs in the East of London after the Department of Health campaign on hepatitis C. We assess the effect of insulin resistance on sustained virological response and alternative herbal treatments for hepatitis C. I believe that this work represents a new contribution to medical knowledge. Ethical approval was granted for all the work by the East London and the City Research Ethics Committee. The work in this thesis has not already been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed

Signed

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GLOSSARY OF ABBREVIATIONS

Ab	Antibody
a.m	Before noon
ADME	Absorption, distribution, metabolism, and excretion.
Aes	Adverse event (s)
ALT	Alanine aminotransferase, (ALAT, SGPT)
AMA	Anti-mitochondrial antibodies
ANA	Anti-nuclear antibodies
ANC	Absolute neutrophil count
ASMA	Anti-smooth muscle antibodies
AST	Aspartate aminotransferase, (ASAT, SGOT)
AUC	Area under the plasma concentration-time curve
Bid	Twice daily
BLD	Below the limit of detection
BMI	Body mass index
BUN	Blood urea nitrogen
CFR	Code of Federal regulations
CHC	Chronic Hepatitis C
C _{max}	Maximum plasma concentration
CRF	Case Report Form(s)
CNS	Central nervous system
DDX	Doctors and Dentists Certificate of Exemption
DM	Diabetes Mellitus
DRAM	Data Reporting and Analysis Manual
ECG	Electrocardiogram
EEG	Electroencephalogram
ELISPOT	Enzyme-linked immunospot assays
ESF	Eligibility screening form
EVR	Early virological response

FACS	Fluorescence activated cell sorting
FSS	Fatigue severity scale
g	Gram
G1	Genotype 1
G 2/3	Genotype 2/3
G/dL	Grams per deciliter
γGT	Gamma-glutamyltranspeptidase
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HBc	Hepatitis B core
HBsAg	Hepatitis B surface antigen
HCG	Human chorionic gonadotrophin
HCV	Hepatitis C virus
HCV RNA	Hepatitis C virus ribonucleic acid
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
hr(s)	Hour(s)
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IFNα	Interferon α
IFNb	Interferon b
IgM	Immunoglobulin M antibody
IL-	Interleukins
IND	Investigational New Drug (application)
IRB	Institutional Review Board
ITT	Intent to treat
IU/mL	International units per milliliter
IUD	Intrauterine device
IVRS	Interactive Voice Response System
kg	Kilogram

m	Meter
MAD	Multiple ascending dose
mg	Milligram
mg/dL	Milligrams per deciliter
mL	Milliliter
mL/min	Milliliter per minute
mm ³	Cubic millimeter
(m)RNA	(messenger) Ribonucleic Acid
NICE	National Institute for Clinical Excellence
NPV	Negative predictive value
NYHA	New York Heart Association (classification of heart disease)
PBMC	Peripheral Blood Mononeucleocytes
PCR	Polymerase chain reaction
PD	Pharmacodynamic
PE	Pharmacoeconomic
PEG-IFN alfa-2a	Peginterferon alfa-2a, Pegasys®, Ro 25-8310
PK	Pharmacokinetic
po	per os/ by mouth
POC	Proof of concept
PPV	Positive Predictive Value
PRC	Peoples Republic of China
q	every
qd	daily
qw	weekly
RBV	Ribavirin, Copegus®, Ro 20-9963
SAD	Single ascending dose
SAE	Serious adverse event
sc	Subcutaneous
SMT	Study management team
SPAM	Study procedures and administrative manual

SVR	Sustained virological response
TCM	Traditional Chinese Herbal Medicine
t_½	Half life
T3	Triiodothyronine
T4	Thyroxin
Th1	T-helper subset 1
Th2	T-helper subset 2
TNF	Tumor necrosis factor
TSH	Thyroid-stimulating hormone
µg	Microgram
U/L	Units per liter
ULN	Upper limit of normal
UK	United Kingdom
WBC	White blood cells

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'If you want a good fire in your stove you must put into it good clean wood and good coal, free from rubbish; therefore, put into your stomach proper food in a proper way, that your stomach may take good care of it, making good blood which is vitality'.

'As about nine tenths of the diseases flesh is heir to are caused by ignorant or wilful abuse of the stomach and liver, every person should make it their business to study themselves, their habits and appetites; and if wrong, correct them as far as possible, for good health is better than riches'.

Virtue's Household Physician. A twentieth Century Medica. Virtue and Co Ltd. 1920.

BACKGROUND AND AIMS

The aims of my thesis were as follows:-

1) Impact of HCV on immigrants from Bangladesh and Pakistan

Prior to starting research I worked as a hepatology registrar both on the wards and liver clinics at Barts and the London NHS Trust. Whilst there I noticed a large proportion of first and second generation patients from Pakistan and Bangladesh who were infected with HCV. When tracing their mode of acquisition and duration of infection I noted that the majority of these patients had acquired HCV infection in childhood whilst living in their country of origin. My clinical duties were to attend the liver histopathology meetings and check all histopathology reports prior to inserting them in the notes. I noted anecdotally that a high proportion of Bengali and Pakistani patients had developed cirrhosis or hepatocellular carcinoma in the fourth or fifth decades of life. The indigenous Caucasian patients did not seem to have this same degree of cirrhosis or rates of HCC. I hypothesised that patients born in Bangladesh or Pakistan acquire their infection at a younger age and are subsequently at a much higher risk of long-

term complications from this infection by the fourth or fifth decade of life. This thesis describes this work

2) GP knowledge of HCV

Many of our patients had received inaccurate information from their GPs about chronic hepatitis C. Also, around this time I had been asked to give a round the table discussion to local GPs about hepatitis C. At this meeting, it became evident that a lot of the GPs were misinformed about this condition which was very concerning as they are the first health care professionals to see patients with hepatitis C. I therefore set up a study to try and improve GPs knowledge about hepatitis C. Before evaluating ways to improve this knowledge it was necessary to examine their baseline knowledge about this condition.

3) Improving the care of patients with symptomatic HCV

Whilst conducting my research we were approached by a commercial group who wanted to study a new herbal remedy for the treatment of hepatitis C. There were a lot of patients in our hospital who had strong views on using antiviral therapy that had

been tested on animals. Also there were a large proportion of patients who had terminated treatment due to side-effects from treatment or relapsed after completing treatment. I agreed to manage the study on behalf of the group.

CHAPTER 1: INTRODUCTION

1.1 General Overview

The Hepatitis C virus (HCV) was first identified in 1989 (Choo et al. 1989). This discovery illuminated many dark corners in the natural history of the infection formerly known as non-A, non-B hepatitis. Hepatitis C is a global health problem and the World Health Organisation (WHO) estimates over 170 million individuals worldwide to be infected with hepatitis C virus. Approximately 80-90% of these individuals will progress over a number of years to chronic liver disease (Gish et al. 1997). The prevalence of infection varies in different countries and regions within the same country.

In the under-developed world the incidence of new infections with HCV is still high mainly as a consequence of the use of unscreened blood transfusions and unsafe parenteral exposure. Some countries for example Egypt have extremely high prevalence rates of 20%, which is thought to be caused by the use of unsterilised equipment during schistosomiasis prevention programmes, which were introduced after the Second World War (Arthur et al. 1997).

The virus is transmitted by blood-to-blood contact and most infections in the Western world were acquired either through intravenous drug abuse or through transfusion of blood or blood products prior to the advent of anti-HCV screening in 1992. In the developed world, the incidence of new infections with HCV is decreasing and the prevalence varies from 0.5- 2% (Anon 1997).

We face a substantial rise in the prevalence of end-stage liver disease as a high proportion of individuals with HCV infection in the USA and the UK were infected 20-25 years ago by sharing of drug injection equipment (Armstrong et al. 2000). In the UK, between 200 000 and 400 000 individuals are believed to harbour the infection, most of whom are asymptomatic and have yet to be diagnosed. In the underdeveloped world, iatrogenic transmission from inadequately sterilised medical equipment or unscreened blood transfusion is the main route of transmission and still persists today (El-Ahmady et al. 1994). Epidemiological studies suggest a limited role for sexual transmission, however sexual transmission of hepatitis C is facilitated by co-infection with HIV (Bresters et al. 1993). The risk of vertical transmission seems to be low (< 6% of children becoming HCV positive) unless the mother is HIV positive or has a particularly high level viraemia

(Giovannini et al. 1990). The virus has not been shown to be transmitted by breast feeding.

By studying patients infected 20 to 30 years ago the natural history of chronic hepatitis C infection over 2-3 decades has been determined and the outcome is variable with around 30% of patients developing cirrhosis after 30 years (Poynard et al. 1997). Hence chronic HCV is a slowly progressive disease which leads to cirrhosis over several decades. Independent factors associated with an increased rate of fibrosis progression include age at infection greater than 40 years, daily consumption of 50g or more of alcohol and male sex (Poynard et al. 1997). Other factors are immunodeficiency such as HIV and co-infection with HBV.

Infection with the hepatitis C virus results in a variety of hepatic and extrahepatic diseases. In a minority of patients, infection results in an acute hepatitis with symptoms resembling other forms of acute hepatitis. The mean incubation period is seven weeks and symptoms last for 2-12 weeks. 60-80% of patients with acute hepatitis progress to chronic HCV. Chronic HCV patients may have symptoms of fatigue, muscle aches, anorexia, right upper quadrant pain, and nausea but more often than not

they have no symptoms. Symptoms and signs of chronic liver disease often occur later in the disease although some patients with chronic HCV cirrhosis remain asymptomatic. HCV infections have been associated with a number of immunological disorders including autoimmune hepatitis, Sjorgren's syndrome, lichen planus, thyroiditis, membranous glomerulonephritis, polyarteritis nodosa and essential mixed cryoglobulinaemia (Lunel. 1994; Marcellin et al. 1993). Hepatocellular carcinoma is commonly associated with chronic HCV infection, probably as a consequence of cirrhosis or as a result of chronic necroinflammation, but HCV does not have a direct carcinogenic effect (Bruix et al. 1989). Suspected individuals with HCV infection should be tested for HCV antibody by enzyme-linked immunosorbent assay (ELISA) (Courouce et al. 1994). All patients with positive antibody tests and those patients thought to be at risk of HCV infection despite negative or indeterminate serological tests should undergo polymerase chain reaction (PCR) testing of serum for viraemia. The results of routine liver tests correlate poorly with both necroinflammatory and fibrosis scores found on liver biopsy. Liver biopsy is therefore usually performed before initiation of antiviral treatment and remains the most accurate

measure of the extent of liver disease. It is also useful to exclude other diagnosis such as alcohol induced liver disease.

All patients with chronic HCV should be referred for treatment and effective treatments are now available for hepatitis C. The goal of treatment is to achieve a sustained virological response (PCR-negativity six months after the end of treatment) (Davis et al. 2002). In chronic hepatitis C, variables such as low pre-treatment HCV RNA levels, HCV genotype (2 or 3), female sex, younger age, less hepatic fibrosis on liver biopsy and lower body weight have an increased likelihood of a sustained response (National Institutes of Health Consensus Development Conference Statement : Hepatitis C). The beneficial effects of interferon alpha in hepatitis C were first reported in 1986 (National Institutes of Health Consensus Development Conference Statement : Hepatitis C). In 1997 a course of interferon alpha administered subcutaneously at a dose of 3MU thrice weekly for 48 weeks was the optimal therapy of this disease with sustained virological response of 12% to 16% (National Institutes of Health Consensus Development Conference Statement : Hepatitis C). The addition of ribavirin to alpha interferon resulted in improved response rates of 35% to 45% . Pegylated interferons have further improved response rates, such that 50-60% of patients can expect to have a

sustained response with combination treatment using peginterferon and ribavirin. This has therefore been proposed as the new standard of treatment for chronic hepatitis C.

1.2 Incidence and Prevalence of hepatitis C in the UK

The prevalence in the UK is not known, but estimated to be between 0.1 to 1 per cent, whilst in Scotland it is around 0.6%. It is very difficult to gain the true prevalence of HCV infection in the UK as there is marked variation in the epidemiological studies performed. This is because prevalence varies from area to area and according to risk behaviour present in the population studied. Information is available from laboratories in England and Wales on positive testing for HCV infection. Since 1990, surveillance of HCV has been carried out by the Public Health Laboratory Service Communicable Disease Surveillance Centre. Between 1992 and 1996 a total of 5232 reports of confirmed HCV infection were received from laboratories in England and Wales. The majority of reports were in the 25-34 year age group (1974 of 5232 (38%)) and the 35-44 year age group (1417 of 5232 (27%)) with more than twice as many reports in males than females (Ramsey et al.

1998). Risk factor information was available for 2976 (57%) laboratory reports and the commonest risk factor was injecting drug use (80%) followed by receipt of blood products and transfusions. Laboratory reports of HCV mainly reflect population based reporting of positive HCV testing and there will be a large number of asymptomatic HCV patients who have not been tested.

Surveillance of blood-borne virus infections carried out by the Public Health Laboratory Service Communicable Disease Surveillance Centre and the National Blood Authority detected HCV infection in 0.002 per cent of donations from new blood donors in England and Wales. This however will be an underestimation of the prevalence in the general population as injecting drug users are prevented from donating. Public Health Laboratory Service (PHLS) data show that prevalence of hepatitis C is much higher in specific groups:- 0.2- 0.4% in antenatal clinic attenders, 0.72% in organ donors; 1.07- 2.75 % in Genito-Urinary Medicine and 60-85% amongst injecting drug users.

Ethnic minorities have been poorly represented in these epidemiological studies on prevalence of hepatitis C in the UK and there have been very few studies performed in Asia. The prevalence of HCV infection is believed to be similar in different

parts of Asia, with an average seroprevalence of HCV antibody less than 2.5 % in healthy adults although prevalence is much higher in at risk groups (Parker et al. 1999; Pradat et al. 2000; Akhtar et al.2002). The prevalence of HCV is high in Asia due to lack of routine screening of donated blood, injecting drug use, traditional medical practices or medical treatment under sub-optimal conditions that involve blood contamination.

In summary, the UK has a relatively low prevalence of HCV infection; estimated by the Department of Health to be 200 000 to 400 000 patients (Hepatitis C Strategy for the UK Department of Health). As few as 10% of these individuals are aware of their infection (Hepatitis C Strategy for the UK Department of Health). The remainder of patients with chronic hepatitis C are asymptomatic and are not aware that they are suffering from a progressive liver disorder. The main exposure group in the UK is injecting drug users who have the highest prevalence rates. The decline in the incidence of cases of HCV in the UK has been due to the elimination of the transmission of HCV by blood and blood products through exclusion of infected donors using the second – generation antibody assay to HCV and by virus inactivation

procedures; although injecting drug use is likely to be the main method of continued transmission. It may be expected that the reduction in incident cases will eventually lead to a decrease in the prevalence of HCV infection. However, although the incidence of HCV infection may be decreasing, the prevalence of liver disease caused by HCV is on the rise. This is because there is a significant lag, often 20 years or longer, between the onset of infection and clinical manifestations of liver disease.

1.3 Transmission of Hepatitis C

Large studies on transmission of hepatitis C have come from the United States; whilst information concerning transmission in the UK is gradually emerging. Post-transfusion hepatitis was first recognised as an entity in the 1940s, its occurrence was dramatically reduced by the implementation of HBsAg blood donor screening in the late 1960s (Alter 1995). However despite this 10% of transfusion recipients continued to develop post-transfusion hepatitis. The discovery of the hepatitis C virus in 1989 showed that this virus was responsible for the cause of more than 90% of non-A, non-B (NANB) post-transfusion hepatitis (Alter 1995). By 1992 with the availability of routine blood

screening for HCV antibody in most countries it soon became apparent that hepatitis C is efficiently transmitted by blood or blood products. It can survive in dried blood for longer periods than most other viruses (even HIV) – survival for up to 3 months has been reported (Alter 1995). It is imperative that health professionals educate their patients about transmission of hepatitis C so that measures can be taken to prevent infection. Information about the dangers inherent in any kind of blood to blood contact needs to be made freely accessible to the general public and HCV infected patients need to be provided with information which will prevent them from transmitting the virus.

Transfusion of blood and blood products

The widespread availability of donor screening in 1992 in developed countries meant that transmission of HCV via blood or blood products is virtually non-existent (Alter H. 1995; Lee et al. 1991; Donahue et al. 1992) . In the developing world transmission through unscreened blood or blood products still remains a major route of HCV spread (Mansell et al. 1995). In most Western countries this group is on the decrease and now represents at least 15% of the total HCV population. It comprises older patients who were infected between the late 1940s to the

early 1990s (Alter H. 1995). Nearly all patients with hemophilia born prior to 1986 were infected with hepatitis C because of the presence of the virus in blood supplies and products. Since 1986 donated blood products such as clotting factors have been subject to various inactivation processes designed to kill viruses and bacteria. Nevertheless, patient groups advocate quite understandably recombinant (genetically engineered) blood products to reduce the risk of inadvertent transmission of unidentified pathogens (Alter 1995).

Intravenous drug users (IDU)

In developed countries this group represents a significant proportion of the more recently infected HCV population. Intravenous drug users (IVDU) have infection rates as high as 60-80% and the majority contract hepatitis C within the first year of injecting (Alter et al. 1995). HCV is a very resistant virus that can survive the cleaning techniques of equipment recommended to prevent the spread of HIV. Therefore, it can also be spread by sharing of spoons, ampoules, water, filters, tourniquets and other paraphernalia as well as contaminated hypodermic needles and syringes (Alter et al. 1994). Needle exchange centres have been

found to be cost-effective and result in a reduction of prevalence of HCV in this population.

Sexual transmission

HCV is not spread very effectively by the sexual route possibly due to low levels of virus in genital fluids and tissues or due to lack of appropriate target cells in the genital tract (Vandelli et al. 2004; Clarke et al. 2006). Male homosexuals and sexual partners of drug addicts with HCV infection have low rates of HCV transmission (Alary et al. 2005). However co-infected patients with HIV are more likely to be able to pass on HCV via sexual intercourse possibly due to higher viral loads in body fluids such as semen (Vandelli et al. 2004; Clarke et al. 2006; Alary et al. 2005).

Intrafamilial transmission

Household contact may account for cases of intrafamilial transmission. The virus can be harboured in tiny quantities of blood or body fluids and there are extensive opportunities for it to jump to a new host (Alter et al. 1995). Injection of saliva from HCV infected patients into chimpanzees has resulted in HCV

transmission (Taber et al. 1978). Possible risks of transmission include toothbrush or razor sharing, although the risk is very small (Alter MJ. 1994).

Occupational exposure from needlestick injuries

Health care workers are prone to needlestick injuries. Needlestick injuries from a hepatitis C patient have a 2 % likelihood of transmission of HCV to the health care worker (Alter M. 1995; Alter M. 1994). Department of Health guidelines advocate that serum should be obtained from the worker at baseline, 6 weeks, 12 weeks, and 24 weeks after exposure. Serum is tested for HCV RNA at 6 and 12 weeks and for anti-HCV at 12 weeks and 24 weeks (Ramsay M. 1999)

Maternal-foetal transmission

Maternal-foetal transmission is rare occurring in less than 5% of deliveries from HCV-infected mothers in the Western world (Yeung et al. 2001; Steininger et al. 2003). When the transmission occurs is unclear – it may occur in utero, intrapartum or both. The risk of HCV transmission increases during vaginal delivery with high maternal viraemia ($> 10^6$ copies/ml), infantile hypoxia and intrapartum exposure to virus

contaminated maternal blood (Yeung et al. 2001; Steininger et al. 2003; Ruff A. 1994). There is no treatment for HCV infection that is currently approved for use in pregnancy or in the neonate. In non-infected babies clearance time of antibodies is significantly longer if the mother is viraemic.

Mothers with both HIV and HCV have about 8-12 % probability of passing the virus on to their baby (England et al. 2006; Pembrey et al. 2005). Elective caesarian section in these co-infected mothers results in a reduced risk of vertical transmission of HCV (England et al. 2006; Pembrey et al. 2005). HCV pregnant women and the delivery team need to discuss means of minimising the possibility of transmission to the baby.

Breast feeding does not appear to significantly increase the risk of neonatal HCV infection as long as the nipples are not traumatised and there is no blood contact (Pembrey et al. 2005; Pappalardo B; 2003). Neonates from mothers who replicate HCV should have a PCR HCV tested at 12-15 months of age. The diagnosis of perinatal infection is made from the persistence of anti-HCV in the serum for more than 12 months from birth and the detection of HCV RNA in at least two post delivery samples.

Rare cases of transmission

HCV may also be transmitted intranasally during cocaine use probably through blood contact. Nosocomial transmission (patient to patient) has been documented by inadequately sterilised EEG pads, filters of anaesthetic masks and colonoscopy and dialysis machines (Alter M. 1995; Roth D. 1995). Universal precautions and scrupulous aseptic techniques are recommended to limit the spread of infection in this setting.

There is around 50 % chance of the recipients of an organ from a HCV positive donor developing hepatitis after transplantation (Terrault et al. 1995).

Transmission in developing countries

The mode and timing of acquisition of HCV infection in Bangladesh, India and Pakistan differs significantly from that in the UK. HCV is spread by the lack of routine screening of donated blood, injecting drug useage, traditional medicine practices or medical treatment under suboptimal hygienic conditions that involve blood contamination and tattooing (Parker et al. 1999; Aktar et al. 2002; Abdel-Aziz et al. 2000; Mollah et al. 2003). They acquire their infection at a younger age, and are subsequently at

much higher risk for long-term complications of this infection by the fourth or fifth decade of life (Nguyen et al. 2003).

Other practices which are implicated in HCV transmission in developing countries include the practice of circumcision and tattooing in un-sterile conditions and the presence of injection shops in some countries. The highest prevalence of HCV is found in Egypt which may be due to the use of parenteral anti-schistosomal therapy (Mansell et al. 1995). A significant proportion of 'sporadic' cases of HCV may have been infected by cosmetic contact, especially tattooing. In past years acupuncture and vaccination programmes were strongly implicated as vectors in the spread of HCV, due to the fact that traditional measures may not have been sufficient to kill HCV or as a result of 'shared needle' vaccination programmes. All vaccination programmes should be carried out by personnel who are well educated about the risks of HCV transmission and this programme should be extended to barbers, dentists, cosmetic surgery professionals, tattooists and acupuncturists.

In summary, HCV infection will continue to have a global impact on health in the future. Healthcare professionals and the public must be made aware of HCV, and treatment of drug misuse and dependency needs to be increased (Hepatitis C. Strategy for

England Published by the Department of Health). There must be raised public awareness of those at high risk of infection, especially injecting drug users and those in prison. Needle exchange programmes have been shown to be a useful strategy in lowering HCV prevalence amongst drug users in the USA (Alter M. 1994). The high rates of progression to chronic infection with end-stage liver disease and resultant liver complications means that more effective education and public health programmes are required to prevent transmission; as to date treatments with pegylated interferon and ribavirin are poorly tolerated and have a 50% rate of cure (Hepatitis C. Strategy for England Published by the Department of Health).

1.4 Natural History of Hepatitis C

Hepatitis C virus is a common cause of cirrhosis and hepatocellular carcinoma and is now the single leading indication for liver transplantation. However, the natural history of this infection is variable and difficult to assess as it often has a largely asymptomatic onset together with a protracted highly variable silent course of 20-40 years (Seeff et al. 2002). In this disease there are often multiple host, viral or environmental factors that

can affect the rate of fibrosis progression. By understanding the natural history and factors influencing progression of chronic hepatitis C to the development of cirrhosis and hepatocellular carcinoma we may be able to halt this progression.

The majority of our information on natural history comes from three types of studies:- retrospective, prospective and cohort studies (Seeff et al. A & B 2002) (see Table 1).

Table 1 - Natural History of Hepatitis C- retrospective, prospective and cohort studies and cross-sectional studies with mathematical modelling.

Method of study	Author (reference)	Country	No of patients	Interval from exposure (mean or range of means)	Cirrhosis (%)	HCC (%)	Liver death (%)
RETROSPECTIVE	Kiyosawa 3	Japan	231	10-29	35.1	23.4	-
	Tong 4	USA	131	14-28	51.0	10.6	15.3
	Yano	Japan	70	-	50.0	-	-
	Niedarau	Germany	838	9-22	16.8	2.0	3.7
	Gordon	USA	215	19	55.0	3.7	-
	Gordon	USA	196	20	21.0	1.0	-
	Takahashi	Japan	333	19	17.4	19.0	-
PROSPECTIVE	DiBisceglie	USA	65	9.7	12.3	0	3.7
	Koretz	USA	80	16.0	7.0	1.3	1.3
	Mattson	Sweden	61	13.0	8.0	-	1.6
	Tremolada	Italy	135	7.6	15.6	0.7	3.7
	Hopf	Germany	86	3-20	24	-	6
COHORT	Seeff	USA	103	20	15	1.9	2.7
	Seeff	USA	17	45-50	5.9	0	5.9
	Kenny-Walsh	Ireland	376	17	2.0	0	0
	Vogt	Germany	458	17	0.3	0	0
	Rodger	Australia	98	25	4	0	1
	Thomas	USA	919	9-16	1.0	0	2.1
	Wiese	Germany	1018	20	0.4	0	0
Cross-sectional Study with mathematical modelling using estimated duration of infection	Poynard (1997)	France	2236 single biopsies 70 paired biopsies	0.133 Metavir units per year 0.183 Metavir units per year	Mean time from HCV infection to cirrhosis of 30 years		

1.41 Retrospective Studies

Retrospective studies have produced the most severe outcomes with a high rate of progression to cirrhosis and hepatocellular carcinoma (Kiyosawa et al. (1996); Tong et al. 1995; Yano et al. 1996; Niedarau et al. 1998; Gordon et al. 1993; Takahashi et al. 1993). These studies assess patients with established chronic hepatitis and attempt to trace back in time to the moment of acute onset based on the presumed time of infection. These seven retrospective studies are a representative group of studies from Japan, USA and Europe with an interval from exposure ranging between 9-29 years. Cirrhosis was identified in 17 % to 55% (mean 35%), development of HCC in 1-23% and liver related mortality in 4-15%. Kiyosawa et al. (1996) was one of the first studies to show from frequent serial biopsies that there was sequential progression from acute hepatitis to chronic persistent hepatitis to chronic active hepatitis to cirrhosis and then to HCC.

The severe outcomes can be explained firstly by the fact that all these studies took place in academic centres which result in 'referral bias' because these tertiary referral centres were likely to attract patients with well-established and serious liver disease. They are not representative of all HCV- infected patients in the

general population (Seeff et al. 2002). Secondly, in Japan chronic HCV infection is common and strongly associated with HCC and it is difficult to extrapolate these results to other populations (Seeff et al. 2002).

1.42 Prospective Studies (Transfusion associated HCV infection)

Long- term prospective follow-up of patients with transfusion-associated HCV infection revealed that acute non-A, non-B hepatitis progressed to chronic hepatitis (Di Bisceglie et al. 1991; Koretz et al. 1993; Mattson et al. 1993; Tremolada et al. 1992; Hopf et al. 1990). These studies were extensions of previous six month trials of the incidence and causes of transfusion associated hepatitis. This is the preferred way of studying natural history and focuses on a whole population. They yielded differing results from the retrospective studies. Five prospective studies with a known onset of acute infection were conducted in US and Europe; with an interval from exposure ranging between 8-20 years. These studies revealed that progression from acute to chronic hepatitis C was common. Study results showed wide variations in the frequency of histologically defined cirrhosis (8-24 %), HCC (0.7-

1.3 %) and liver related death (2 -6 %). These studies were not initially designed for long follow-up (8-20 years) and are insufficient to determine most of the serious sequelae of chronic hepatitis C (Seeff et al. 2002). Biopsies were mainly performed on patients with more severe liver disease. The role of alcohol in progression was not taken into account (Seeff et al. 2002).

1.43 Retrospective-Pro prospective Studies

In these studies the date of initial exposure to HCV was known retrospectively and investigators traced these patients 15 years later; in this way all patients were prospectively reviewed even those who cleared infection (Seeff et al. 1998; Seeff et al. 1998; Kenny-Walsh E. 1999; Vogt et al. 1999; Rodger et al. 2000; Thomas et al. 2000; Wiese et al. 2000). Seven retrospective-prospective studies have been performed; six comprising young patients (Seeff et al. 1998; Kenny-Walsh 1999; Vogt et al. 1999; Rodger et al. 2000; Thomas et al. 2000; Wiese et al. 2000) and one study middle-aged patients (Seeff et al. 1998). The time interval from exposure ranged from 9-50 years. Cirrhosis developed in 0.3 to 15%, HCC in 0 -1.9% and liver related mortality from 0 -2.8%. These studies have the advantages of studying a heterogenous population with a variety of ages and

sexes from the outset of the disease. The lowest rates of progression appear to be in young people especially young women (Kenny-Walsh 1999; Vogt et al. 1999). However, both these groups are special cases in that they were infected with HCV due to a point outbreak by contaminated anti-D immunoglobulin.

Seeff et al. (2002) noted discordance in mean frequency progression to cirrhosis according to study design. The mean frequency progression was 42% for retrospective studies, 11% for prospective studies and 2.1% for retrospective- prospective cohort studies (Seeff et al (2002)).

1.44 Other Studies assessing natural history

Poynard et al. (1997) performed a single biopsy cross-sectional study of 2 235 patients in whom duration of infection was estimated from the date of acquisition of HCV. A series of risk factors were analysed to determine influence on progression of fibrosis. This study comprised 30% of people who were infected through blood transfusion and 25% through iv drug abuse. Poynard's group developed a scoring system called the Metavir scoring system for assessing the grade and stage of histopathological changes seen in chronic hepatitis C, that is used

mainly in France. The median rate of fibrosis progression using Metavir was 0.133 unit per year. This gave a mean time from HCV infection to development of cirrhosis of 30 years. Three factors increased the rate of fibrosis progression:- age greater than 40 years at the time of infection, followed by alcohol consumption more than 50g per day and then male sex. There were 70 patients who had paired biopsies in which fibrosis rates were 0.183 unit per year. These patients were in the control arms of interferon treatment trials and no information as to the time interval between these biopsies were available. Some individuals progressed rapidly to cirrhosis (102 patients in 9.5 years) (Table 2). In 68 patients minimal fibrosis was present after 20 years infection with an estimated time from infection to cirrhosis of greater than 50 years. This study relies heavily on a single liver biopsy and an estimated duration of infection.

Table 2. The length of time of progression to cirrhosis in HCV-infected patients due to risk factors (Poynard et al. 1997).

Age <40	alcohol <50	Male	36 yrs to cirrhosis
Age <40	alcohol <50	Female	42 yrs to cirrhosis
Age > 40	alcohol <50	Male	13 yrs to cirrhosis
Age > 40	alcohol <50	Female	20 yrs to cirrhosis
Age >40	alcohol > 50	Male and Female	15 yrs to cirrhosis

A subsequent cross-sectional study of 2313 patients in 2001 was reported by Poynard (Poynard et al. 2001). Patients were infected either through blood transfusion or iv drug abuse. Disease progression was modelled using the Hazard Function in terms of linearity. During the first 10 years of infection there was little progression (except patients infected after the age of 50), the next 15 years involved slow progression, an intermediate period occurred during the next 10 years, followed by a further five years where progression was greatest. At 60 years of age 50% of patients would develop severe disease. These estimates are in concordance with the progression intervals of retrospective studies and prospective studies.

However, these studies firstly rely heavily on a single liver biopsy and an estimated duration of infection and there is little prospectively collected data. Poynard does include 70 paired biopsies of patients making up the control arms of treatment studies, but the time interval between these biopsies is not available. Secondly, the variable outcomes among HCV-infected patients and studies from patients infected via blood transfusion argues against linear progression

The untreated control arm of a study on antiviral therapy helped to address this further. Patients with mild hepatitis C who were not offered treatment with interferon were followed regularly with repeat biopsy every 31 months (Ryder et al. 1999). In this control group of 151 patients, 27% progressed by one or more fibrosis points. This data suggests that even mild hepatitis C is a progressive disease with progression of fibrosis occurring at a rate of 0.146 Knodell fibrosis unit per year. Assuming the development of fibrosis is linear the authors estimate the overall time from infection to cirrhosis is 27.5 years.

1.45 Outcome in Children

Hepatitis C infection of children is rare as perinatal spread is uncommon. Vogt et al. (1999) has reported the largest outcome study in 458 children (14.6% were HCV antibody positive) undergoing cardiac surgery before the implementation of blood donor screening and followed up 12-27 years later. 45 % of children spontaneously cleared the virus during this period. A liver biopsy was performed in 17 of the 37 (55%) remaining viraemic patients and 1 (0.3%) child was found to have developed cirrhosis.

Losasciulli et al. (1997) followed up 114 children with childhood leukaemia of which 56 (49%) were found to be HCV RNA positive after chemotherapy. After a period of 17 years all the HCV RNA positive cohort were asymptomatic and sixteen of the 56 (29%) had spontaneously cleared the virus. No liver biopsy was performed. Garcia-Monzon et al. (1998) compared 24 HCV infected children and 22 HCV infected adults over a mean follow-up of 11 years and despite similar viral loads fibrosis was markedly less in children. Luban et al. (1999) reviewed 5,446 children that had received blood transfusion between 1982 and 1992. Of 1,753 children tested so far 36 (2 %) are HCV positive and 7 out of the 36 were biopsied a mean 13.6 years from exposure with only one child having early bridging fibrosis.

Data for children is still sparse and duration of follow-up insufficient to determine most of the serious sequelae of chronic hepatitis C. However, the studies so far suggest mild outcomes over the first two decades of infection with a high rate of spontaneous recovery (29-45%).

1.46 Fibrosis progression in patients with normal liver enzymes

10 to 40 % of HCV infected patients have persistently normal liver enzymes and this subgroup has a lower risk of progression to cirrhosis. Patients who have normal ALT levels recorded on several occasions, usually have mild degrees of hepatitis disease activity and either no or minimal fibrosis (Marcellin P. 1999, Martinot-Peignoux et al. 2001; Persico et al. 2000). Mathurin et al. (1998) reviewed 204 patients and found that the median progression rate of fibrosis was significantly lower in patients with normal liver enzymes (0.05 Metavir points per year) compared with 0.13 points per year in those with elevated ALT levels.

1.47 Outcome after the development of cirrhosis

The outcome after the development of cirrhosis has been reviewed in two studies. The EUROHEP study was a retrospective 5 year follow-up study of 384 patients with compensated cirrhosis due to HCV (Fattovich et al. 1997). The risk of decompensation after a mean of 5 years (9-124 months) was 18% and of HCC 7%. The 5 year survival was 91% in all patients, but 50% in those with decompensated cirrhosis. Serfarty

et al. (1998) followed 668 cirrhotic patients for a mean of 40 months (6-72 months) (31). The risk of decompensation was 14.5%, with a risk of HCC of 1.6%. The 2 year and 4 year survival rate was 96% and 84% respectively.

Benvegnu et al. (2004) revealed that there was significant morbidity and mortality during the first decade after diagnosis of compensated cirrhosis due to HCV and/or HBV. Hepatocellular carcinoma was the most frequent and life threatening complication in both HCV and HBV/HCV related cirrhosis (Benvegnu et al. 2004)..

1.48 Fibrosis progression in ethnic minorities

There are few studies on natural history of chronic hepatitis C in minority populations however information from cross-sectional studies suggest that fibrosis progression differs among ethnic groups.

A retrospective study of 355 chronic hepatitis C patients in America revealed that African-Americans had significantly less piecemeal necrosis and less chance of cirrhosis than non-African Americans. This was despite the fact that they had a longer mean

duration of infection and were older at the time of biopsy (Wiley et al. 2002). African-Americans have a higher rate of HCC. These findings have been confirmed by other studies (Reddy et al. 1999) and are thought to be due to differences in phenotypic human leukocyte antigen (HLA) gene expression and less immune recognition of HCV-infected hepatocytes (Wiley et al. 2002; Reddy et al. 1999).

Subgroup analysis of other minority groups such as Hispanics in America show higher histological activity and fibrosis score than Caucasians (Mallet et al. 2002), and a study of 256 patients showed that Hispanics have a three-fold higher fibrosis progression rate (n=66; 0.215 mean stages/year) compared with Caucasians (n=47; 0.084 mean stages/year; $p < 0.02$) which was thought to be related either to a longer estimated duration of disease or higher prevalence of metabolic syndrome amongst this group (Bonacini et al. 2001).

1.49 Factors associated with more rapid progression

Factors associated with more rapid disease progression include older age of infection, male sex and history of heavy alcohol use (Freeman et al. 2001; Poynard et al. 1997; Poynard et al. 2001).

Immune deficiency such as hypogammaglobulinaemia or HIV infection together with steatosis, obesity and diabetes are associated with more rapid progression of fibrosis. Virological factors such as viral load and genotype have not been associated with the rate of progression of fibrosis (Poynard et al. 2001).

Age at onset of infection is an important determinant of disease progression and severity. Poynard et al. (1997) found that the rate of progression was correlated directly with age of onset of infection, male sex and alcohol consumption. In the hazard function model, nearly all patients infected after the age of 40 developed cirrhosis within 16 years (Poynard et al. 1997; Poynard et al. 2001). Male sex and high consumption of alcohol (> 50 g per day) were associated with greater fibrosis progression (Freeman et al. 2001; Poynard et al. 1997; Poynard et al. 2001; Ryder et al. 1999). Darby et al. (1997) reported that suppressed immunity such as HIV can have a faster rate of progression with significantly higher mortality from liver disease in patients co-infected with HIV. This also is found in liver transplant recipients

where cirrhosis occurs in up to 30% of patients after 5 years (Berenguer et al. 2000). Similarly co-infection with HBV has a synergistic effect on fibrosis progression .

There is strong evidence of an association between chronic hepatitis C and diabetes mellitus (Allison et al. 1994). Insulin resistance plays an important role in the development of type 2 diabetes mellitus, preceding diabetes by 10 to 20 years. HCV infection can induce insulin resistance and insulin resistance may increase the rate of fibrosis progression in HCV patients (Hui et al. 2003).

Chronic hepatitis C is associated with hepatic steatosis to a greater extent than other liver conditions. There is growing evidence that hepatic steatosis is associated with more severe liver disease in patients with hepatitis C (Adinolfi et al. 2001).

The natural history of hepatitis C is difficult to determine due to an asymptomatic acute phase hepatitis followed in 60-80% of cases by a silent protracted chronic phase of 20-40 years. A mixture of retrospective, prospective and cohort studies reveal that the natural history of hepatitis C is highly variable, however during the first 20 years it appears that about 20% of patients have evidence of cirrhosis. After this period we can only make estimations about the long-term outcome, it could theoretically plateau, progress

linearly or increase exponentially. Each patient differs according to the duration of infection and presence of factors associated with more rapid disease progression and should be assessed accordingly.

1.5 Diagnosis and Treatment of Hepatitis C

Hepatitis C virus (HCV) is a major cause of chronic hepatitis that progresses in some patients to cirrhosis and hepatocellular carcinoma (HCC). The natural history of HCV-related liver disease is variable among individuals, but without effective treatment strategies, hepatitis C-related morbidity and mortality is expected to increase nearly 3-fold over the next few years. This is because most patients were infected in the 70s and 80s when many people experimented with intravenous drugs. Since infection leads to cirrhosis in 20-30 years it is probable that many of these patients will develop end stage liver disease in the next few years. Accordingly, current hepatitis C therapies are aimed at eradication of HCV infection to prevent progression to end-stage liver disease and its associated complications.

1.51 Diagnosis of HCV

The incidence of HCV is decreasing in Europe, as a result of available and accessible medical and diagnostic facilities for a large part of the population (WHO). All patients, who are antibody positive for hepatitis C virus, require their diagnosis confirmed by PCR HCV testing, and the severity of disease assessed before initiating treatment. Liver function tests are a poor screening tool for HCV- as 50% of infected patients have normal transaminases, despite having evidence of necroinflammatory liver disease with or without cirrhosis (Alberti et al. 1992).

The diagnosis of acute and chronic hepatitis C is based on antibody tests (anti-HCV). All patients who have detectable antibodies against the hepatitis C virus should have a sample of serum analysed for the presence of virus (qualitative assay) by a sensitive nucleic acid detection assay (a polymerase chain reaction (PCR) assay) to detect viral RNA (Pawlotsky J. 2002).

Patients may also be seen who are antibody negative, but have a detectable viral load on PCR. These patients may be heavily immunosuppressed or have contracted the infection within the previous few weeks (antibody response can take a few months to develop). Some patients have detectable antibodies, but are

persistently PCR negative; they have been exposed to HCV but have eliminated the virus spontaneously.

Following confirmation of the diagnosis it is important to counsel the patient about infectivity and the effects of infection on partners and family members. Prior to beginning therapy, other virological tools, including liver biopsy, HCV genotype determination and HCV quantification come into play to tailor treatment to the individual patient and determine its efficacy (Pawlotsky J. 2002). Six major genotypes and more than 50 subtypes of HCV have been described. The different HCV genotypes have marked geographic variation in their relative frequencies and response together with duration of treatment (Pawlotsky J. 2002; Saadeh et al. 2001).

In patients with genotypes 2 and 3, the frequency of sustained responses to short-term (6 months) therapy is so high that under these circumstances the need for liver biopsy is less compelling. For most patients with other genotypes the value of pre-treatment liver biopsy may outweigh its risks as it excludes other causes of liver injury and helps in deciding the timing of treatment (Saadeh et al. 2001).

Acute hepatitis C

Acute hepatitis C is rarely recognised and largely under-diagnosed (Gerlach et al. 2001). Up to 15% spontaneously clear the virus after an acute phase whilst the remaining 85% develop chronic hepatitis. HCV rarely causes acute liver failure. Factors such as age, genetic factors and ALT profile have been associated with an increased risk of chronicity (Gerlach et al. 2001; Gerlach et al. 2001).

Pegylated interferon therapy in acute hepatitis C can induce high sustained virological response rates, is well tolerated and reduces the risk of evolution of chronicity (Kamal et al. 2006; Wedemeyer et al. 2006). Initiation of pegylated interferon at week 8 or 12 weeks resulted in higher sustained virological rates than initiation at week 20 (Kamal et al. 2006; Wedemeyer et al. 2006).

Chronic hepatitis C

All patients with chronic HCV should be assessed for treatment. Variables that correlate with a higher likelihood of a sustained response to therapy include low pre-treatment HCV RNA levels, HCV genotype (2 or 3), female sex, younger age, less hepatic fibrosis on liver biopsy and lower body weight (Seeff L. 2002). Patients with HCV should be warned that alcohol abuse has been shown to increase the severity of hepatitis C with more rapid progression to cirrhosis and hepatocellular carcinoma (Harris et al. 2001; Peteres et al. 2002). Only recently have effective treatments become available for hepatitis C. The goal of treatment is to achieve sustained virological response (PCR negative) 6 months post-treatment which leads to histological improvement (Davis G. 2002). The beneficial effects of interferon alpha in hepatitis C were first reported in 1986 (National Institutes of Health Consensus Development Conference Panel Statement: Management of hepatitis C: 2002). In 1997 a course of interferon alpha administered subcutaneously at a dose of 3MU thrice weekly for 48 weeks was the optimal therapy of this disease with sustained virological response of 12% to 16% (National Institutes of Health Consensus Development Conference Panel Statement: Management of hepatitis C: 1997 and 2002). The addition of

ribavirin to alpha interferon improved response rates to 35% to 45% (McHutchison et al. 1999). Pegylated interferons have further improved response rates, such that 50-60% of patients can expect to have a sustained response with combination treatment using peginterferon and ribavirin (National Institutes of Health Consensus Development Conference Panel Statement: Management of hepatitis C: 2002).

1.52 Standard treatment for hepatitis C

Pegylation is the process by which an inert molecule of polyethylene glycol (PEG) is covalently attached to a protein giving it a higher molecular weight and resulting in an increase in serum half-life. Two forms of pegylated interferon have been developed: Pegylated interferon alfa 2a (interferon a2a bound to 40kDa molecule of PEG) and Pegylated interferon alpha 2b (interferon-a2b bound to 12kDA size PEG) (National Institutes of Health Consensus Development Conference Panel Statement: Management of hepatitis C: 2002; Alberti et al. 2003). The pegylated interferons are a once per week subcutaneous injection. Both Pegylated interferon alfa 2a (40 KD Peg/IFNa2a) and Pegylated interferon alpha 2b (12 KD Peg/IFNa2b) have different pharmacokinetics and pharmacodynamic properties; 40 KD Peg/IFNa2a having a longer half-life and smaller volume of distribution compared to 12 KD Peg/ IFNa2b ((National Institutes of Health Consensus Development Conference Panel Statement: Management of hepatitis C: 2002). 40 KD Peg/IFNa2a is excreted mainly by the liver and is administered at a standard dose of 180ug; whilst 12 KD Peg/IFNa2b is excreted mainly by the kidney and is dosed by body weight (Alberti et al. 2003).

With the combination of peginterferon and ribavirin patients with genotype 1 achieve sustained viral response rates of 40-45% after 48 weeks of treatment compared with rates approaching 80% with genotypes 2 or 3 after a 24 week course (Manns et al. 2001; Fried et al. 2002). Patients with genotype 2 and 3 may only need 24 weeks of treatment. Trials with the 40 KD Peg/IFNa2a show that patients with genotype 2 and 3 require a dose of ribavirin of 800mg daily to achieve optimal response rates, whereas 1000 to 1200 mg daily is needed for patients with genotype 1 (Manns et al. 2001; Fried et al. 2002). Analysis of quantitative PCR HCV at 12 week in genotype 1 treated patients is a predictive tool of future response (Pawlotsky J. 2002). Patients who are PCR HCV negative or who have >2 log HCV RNA drop have a high chance of achieving a sustained virological response at 48 weeks (Pawlotsky J. 2002).

The side-effect profile of the pegylated interferon is similar to that seen with unmodified interferons (National Institutes of Health Consensus Development Conference Panel Statement: Management of hepatitis C: 2002; Alberti et al. 2003).

Interferon based therapies are administered by subcutaneous injection and have a wide range of side-effects that significantly

impair an individuals' quality of life whilst they are receiving therapy. However, the majority of the unwanted side effects settle when therapy is discontinued. Pegylated interferon produce a similar side effect profile to unmodified interferon and include fatigue and flu-like symptoms (headache, myalgia, fever, rigors and joint pain) which usually occur a few hours after injection and may improve with oral paracetamol. Symptoms gradually improve by perseverance after 4-6 weeks of treatment. In addition patients may develop neuropsychiatric symptoms (irritability, depression, insomnia), nausea and thinning of the hair. The mild depression associated with interferon based therapies usually responds well to standard anti-depressant agents. Serious neuropsychiatric side effects to interferons can occur and include depression, paranoia, severe anxiety and psychosis (Alberti et al. 2003). In patients with a history of substance abuse, the psychiatric changes can lead to a disastrous relapse in alcohol or drug abuse. All patients receiving interferon should be closely monitored. Severe, suicidal depression does occasionally occur (and is more common in those with a previous history of serious psychiatric illness) and if this occurs treatment should be discontinued (National Institutes of Health Consensus Development Conference Panel Statement: Management of

hepatitis C: 2002; Alberti et al. 2003). In a majority of cases interferon causes mild neutropenia and thrombocytopenia and regular full blood counts and, if necessary dose adjustments, are required. Ribavirin comes in tablet form and may cause a mild haemolytic anaemia with a 2-3 gm fall in haemoglobin concentration and, again, regular monitoring and dose modifications are necessary. It is best avoided in patients with significant pulmonary or cardiovascular disease. Erythropoietin analogues can be used to treat the anaemia. Other side effects associated with ribavirin therapy include a dry cough, nausea and mouth ulceration. Very occasionally interferon based therapies induce autoimmune diseases, in particular thyroid disease.

Ribavirin is teratogenic and female patients should have pregnancy tests performed prior to treatment and must avoid pregnancy during therapy and for six months thereafter as ribavirin has a very long terminal half life. Since ribavirin may accumulate in sperm, men should not father children whilst receiving therapy and for six months after discontinuation. Pegylated interferon monotherapy should be given to those patients in which ribavirin is contraindicated.

Combination treatment with PEG-IFN (40 KD Peg/IFNa2a and 12 KD Peg IFNa2b) and ribavirin has a side-effect profile very similar

to unmodified interferon and ribavirin, although the frequency of depression may be reduced with the 40 KD Peg/IFNa2a (PegIntron(r) Product Information; PEGASYS(r) Product Information). Premature withdrawal from therapy due to adverse events in the two pivotal clinical studies with pegylated interferons were of the order of 10-14%, which is similar to that with unmodified interferon and ribavirin (11%) (Manns et al. 2001; Fried et al. 2002) suggesting that the increase in efficacy seen with the pegylated interferons is not associated with an increase in adverse events.

1.53 Which groups of patients should be treated?

All patients should be assessed for the benefits and risks of therapy. For many patients a liver biopsy aids the assessment by both identifying the aetiology of liver disease and the degree of liver damage. When selecting patients with chronic hepatitis C for treatment one needs to identify those patients who are likely to develop significant liver damage. At present at our center all liver biopsies are scored using the modified histological activity index (modified HAI (Ishak) scoring system), and those patients with a fibrosis score greater than 2 qualify for treatment. A proportion of patients with minimal fibrosis but marked necroinflammation (total

necroinflammatory score of greater than 3), have a higher potential for developing progressive disease and are also eligible for treatment. In chronic hepatitis C as described previously fibrosis progression is not linear. Therefore the cohort of patients with mild disease who do not qualify for treatment should have a repeat liver biopsy every 3-5 years to assess fibrosis progression. Those patients with fibrosis progression are offered treatment whilst those with no progression should continue being followed up. Our practice is very similar to national guidelines (National Institutes of Health Consensus Development Conference Panel Statement: Management of hepatitis C: 2002; Alberti et al. 2003).

Treatment is also considered for extrahepatic manifestations of HCV such as cryoglobulinaemia (National Institutes of Health Consensus Development Conference Panel Statement: Management of hepatitis C: 2002; Alberti et al. 2003).

Treatment of patients with cirrhosis have revealed response rates to be lower with frequent serious side-effects (Lee et al. 2006). In compensated cirrhosis combination therapy with interferon alpha (3MU thrice daily) and ribavirin (1000mg-1200mg/day) results in SVR of 34% to 44% (Lee et al. 2006). Treatment of decompensated cirrhosis or recurrent hepatitis C after

transplantation may not be beneficial and should be undertaken with caution ideally in clinical trials.

1.54 Previous non-responders or relapsers

The success of retreating a patient who has failed a first course of antiviral therapy is dependent on a number of factors:- a previous relapse, previous treatment with interferon monotherapy, HCV genotype (2 and 3), low HCV RNA and having a significant decrease in HCV RNA levels during the initial course of treatment (Davis G 2002). Retreatment of non-responders to standard interferon monotherapy using peginterferon and ribavirin has yielded SVR rates of 34% to 40% (Davis G. 2002). Retreatment of patients who relapsed after interferon monotherapy using peginterferon and ribavirin resulted in SVR of about 60% (Davis G. 2002).

1.55 Other Treatment Groups

Treatment of children and pregnant women with HCV

An estimated 100 000 children in the United States are chronically infected with HCV (Guido et al. 1998). Although the natural history of HCV infection acquired in childhood seems benign in the

majority of instances, infection can take an aggressive course in a proportion of cases leading to cirrhosis and end-stage liver disease during childhood. There should be increased awareness of prevention of perinatal transmission which is the major cause of new cases of HCV in children. There are very few studies on treatment in childhood; small studies on interferon monotherapy have reported sustained virological response rates of 35% to 40% (Guido et al.1998). Treatment for pregnant women with HCV is contraindicated.

Treatment of mild disease

Treatment with unmodified interferon and ribavirin was associated with relatively low response rates and unpleasant side effects. Therefore many patients and their clinicians felt at the time that treatment for this slowly progressive disease was often worse than the disease itself. As a result most treatment algorithms recommended that only patients with biopsy proven moderate/severe disease should receive treatment and this approach proved popular with both patients and their physicians. Lately, the increased response rates and reduced side effects seen with the pegylated interferons and ribavirin suggests that this approach may no longer be appropriate and increasingly clinicians

recommend that patients receive therapy without undergoing a liver biopsy. This approach is particularly appropriate for patients with genotypes 2 and 3 where 24 weeks of therapy is sufficient to eradicate the virus in nearly 80% of patients.

A particular subset of patients with mild disease are those with normal liver function tests. Patients with normal serum alanine transaminases on three successive measurements, each measurement several weeks apart are classified as patients with 'normal ALTs'. Up to 25% of patients with chronic HCV infection have normal serum transaminases and the majority of these patients have minimal disease on liver biopsy (Bacon et al. 2002). Early studies suggested that disease flares with an increase in serum ALT could occur when such patients were given interferon. Zeuzem et al. (2000) examined the benefits of treating such patients with pegylated interferon and ribavirin by conducting a randomised controlled clinical trial in this population using the 40KD pegylated interferon alfa 2a plus ribavirin. There was no evidence from this study that patients with 'normal ALTs' develop disease exacerbations when treated with the 40KD pegylated interferon and sustained virological response rates in these patients were similar to the response rates seen in other clinical

trials with this pegylated interferon. The SVR in patients treated for 24 and 48 weeks with 40KD pegylated interferon alfa 2a and ribavirin (at a dose of 800mg) were 30% and 52% respectively (Zeuzem et al. 2000). This study was in concordance with previous observations which suggests that patients with genotype 1 HCV infection need 48 weeks of therapy whilst patients with genotypes 2 and 3 require 24 weeks treatment.

Treatment of Patients With Hepatitis C and HIV Co-Infection

Until recently coinfecting patients with HIV and HCV often died from the opportunistic infections associated with HIV related immunosuppression. However with the introduction of effective antiretroviral therapies the life expectancy of patients with HIV has been markedly lengthened (Graham et al. 2001). As a result, in the Western world liver disease secondary to chronic HCV infection is now becoming a major cause of morbidity and mortality among HIV-infected patients. Co-infected patients who are infected with both HIV and HCV as a result have more rapidly progressive liver disease and clinical trials with standard treatment regimes suggest that they respond poorly to combination therapy with standard interferon and ribavirin (Graham et al. 2001).

The effectiveness of pegylated interferons in the management of patients with HIV/HCV co-infection has been studied in two randomised clinical trials. The RIBAVIC study compared PEG IFN a2b and ribavirin with standard combination treatment (unmodified interferon and ribavirin) resulting in a SVR of 15% in genotype 1 and 43% in genotype 2/3 patients (Perronne et al. 2004). PEGIFN was significantly more effective than IFN but 34% of patients discontinued pegylated interferon and ribavirin treatment due to side effects (compared to 36% in standard combination group) (Perronne et al. 2004). The APRICOT study comprised three arms :- standard combination, peginterferon a2a and placebo, and peginterferon a2a and ribavirin (800mg/day). SVR were 29% in genotype 1 and 62% in genotype 2/3 and 15% of patients discontinued treatment due to adverse events (Torriani et al. 2004).

Interactions between antiretroviral drugs and ribavirin can be harmful. Zidovudine should be used with caution when ribavirin is given, because both may produce anaemia (Dieterich D. 2002; Fried M. 2002). Didanosine should be avoided when taking ribavirin as there is a high risk of pancreatitis and lactic acidosis in all treated patients as well of liver decompensation in cirrhotic

patients (Salmon-Ceron et al. 2001; Kakuda et al. 2001). Patients should be advised of the possibility of experiencing severe weight loss, mimicking a rapid progression of lipoatrophy, due to the potentiation of mitochondrial damage in the subcutaneous fat tissue when taking ribavirin and some nucleoside analogues (Garcia-Benayas et al. 2002).

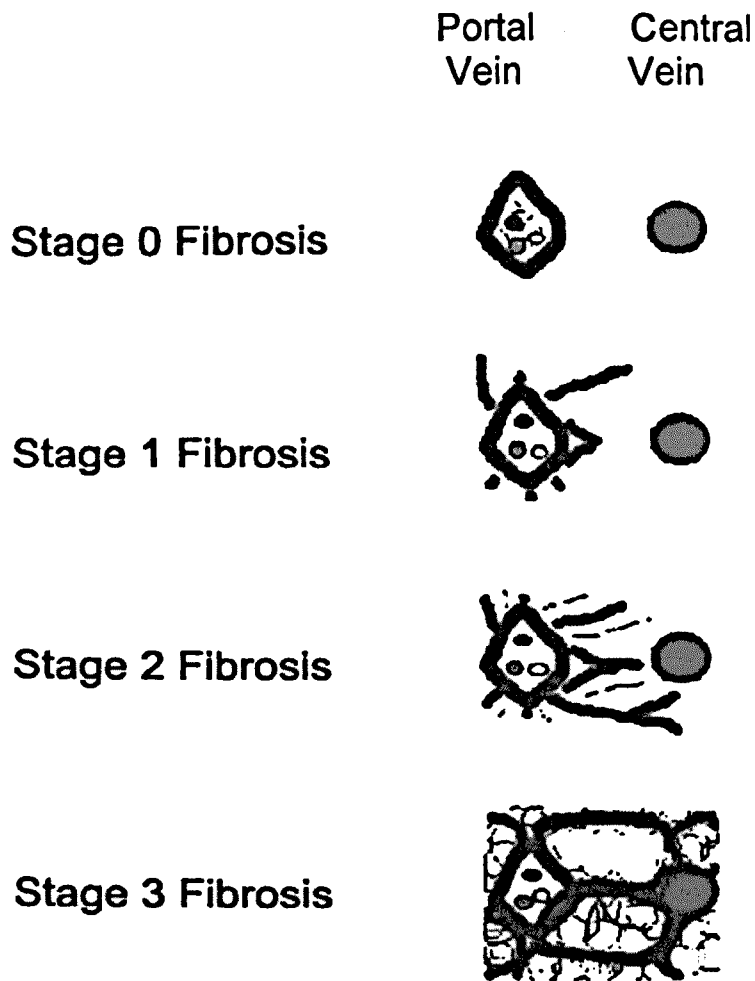
1.6 Fibrosis and Disease Progression

In many patients with hepatitis C infection, progression of disease leads to fibrosis which is progressive and largely irreversible. It is the progression of fibrosis that ultimately leads to architectural distortion of the liver and cirrhosis. For these reasons, the rate of progression of fibrosis is the defining feature of the natural history of chronic hepatitis C. In some individuals the rate of fibrosis progression is rapid with its associated long term complications such as liver failure, portal hypertension and hepatocellular carcinoma whilst in other patients progression is slow after many decades of infection. The assessment of the stage and rapidity of progression are essential in determining prognosis and the need of therapy in individual patients.

Fibrosis is characterized by the deposition of collagen and other extracellular matrix proteins and their organization in complex polymers, which are insoluble and induce loss of the liver architecture. In chronic hepatitis C active fibrosis begins around the portal areas (periportal or zone 1 fibrosis) and gradually extends out into the lobules towards the central veins (zone 3) with septa formation and then bridging fibrosis (Dhillon et al. 1995). The final stage of fibrosis constitutes cirrhosis with extensive fibrosis linking portal and central areas and the nodular regeneration of the liver parenchyma (Figure 1). The hepatic stellate cell has emerged as the key fibrogenic cell in the liver and are activated from a quiescent lipocyte phenotype to a fibroblastic phenotype. In chronic hepatitis C the major fibrogenic cytokine, transforming growth factor (TGF- β) is increased and produces connective tissue growth factor which correlate with the degree of liver fibrosis (Olaso et al. 1998). Conversion of stellate cells to a fibrogenic state occurs in the presence of TGF- β . This new myofibroblastic phenotype can proliferate, attract leucocytes and produce extracellular collagen and matrix proteins. During the fibrogenesis process, a basal membrane appears separating the hepatocytes from sinusoidal blood and disrupting the exchange of nutrients between blood and hepatocytes (capillarisation of the sinusoids). Liver fibrosis is

characterized by the transformation from normal extracellular matrix (basal membrane) into a reticular and dense matrix (fibrillar type) which is more resistant to enzymatic degradation.

Figure 1 The Metavir fibrosis staging system.



Liver biopsy remains the gold standard to assess the status of liver inflammation, potential progression of fibrosis, and the presence or absence of cirrhosis. Transcutaneous liver biopsy is the usual method, but transjugular biopsy may be useful for patients with clotting problems or ascites. A number of scoring systems have been defined. The Metavir scheme has been used by the French Metavir Cooperative study Group (Figure 1). The modified histological activity index or modified Ishak has been validated as a

suitable scoring system for chronic hepatitis C (Ishak et al. 1995; Westin et al. 1999). Fibrosis is identified in liver biopsies by connective tissue stains, such as trichrome, Azan or picrosirius red that identify extracellular matrix. Immunostaining of collagen can also be performed. Histologically chronic hepatitis C is difficult to distinguish from acute hepatitis and is often mild, but cirrhosis commonly develops. Other features include lymphoid follicles in portal tracts, damaged interlobular bile ducts, acinar activity including apoptotic bodies, large droplet steatosis, lymphocytes in sinusoids, granulomas and Mallory bodies (Dhillon et al. 1995; Scheuer et al. 1996). However liver biopsy is prone to sampling error, it cannot be used to quantify the amount of scarring and has the potential for medical complications. The development of non-invasive markers is an important future research goal, because they could be used to detect the extent of liver fibrosis, to define the natural history of disease, and to follow disease progression. There have been many studies on fibrosis in chronic hepatitis C ; most studies have been cross-sectional based on a single liver biopsy whilst a smaller number are longitudinal and based on patients who have two liver biopsies. There have been few prospective studies in chronic hepatitis C and those that have been performed have been limited in size.

Cross-sectional studies using mathematical modeling performed on large numbers of patients with a single liver biopsy suggest that the average (median) rate of progression of fibrosis in chronic hepatitis C is 0.13 Metavir fibrosis points per year (Poynard et al. 1997). The average patient would develop cirrhosis in 30 years. The rate of fibrosis progression was higher for men than women (0.15 vs 0.11 fibrosis units per year), older than younger patients (0.33 if infected over the age of 50 vs 0.09 if below the age of 20 years) and in heavy alcohol drinkers than in non-drinkers (0.17 vs 0.12). The estimated average time for development of cirrhosis ranges from as short as 13 years in men to as long as 42 years in women.

A more recent cross sectional study with mathematical modeling of 2 213 patients proposed a new model for fibrosis progression in chronic hepatitis C in which fibrosis progression occurs at different linear rates in 4 consecutive phases of infection with acceleration after 50 years of age (Poynard et al. 2001). The first phase of infection represents the initial 10 years of infection followed by the second phase (lasting 15 years) where fibrosis progression is slow. This then proceeds to an intermediate third phase (lasting 10 years) and lastly a rapid fourth phase. In this model the delay to the development of cirrhosis is approximately 40 years. In this

study only a small proportion of patients had an estimated duration of infection more than 20 years. The risk estimates suggested by Poynard et al. will probably be lower in unselected populations and progression is probably non-linear with advanced stages associated with accelerating, non-linear progression (Lagging et al. 2002).

There have been three recent longitudinal studies (1 prospective and 2 retrospective) of untreated patients with chronic hepatitis C who underwent 2 liver biopsies 3 to 8 years apart (Marcellin et al. 2001; Ghany et al. 2000; Alberti et al. 2001). These three studies showed similar average rates of progression of fibrosis that predicted the development of cirrhosis in the average patient after 30 to 40 years of infection.

It is host rather than viral factors that are associated with disease progression. There is no relationship between genotype or viral load and fibrosis progression. The major factors known to be associated with fibrosis progression are i) older age at infection (>50 years of age); ii) male gender; iii) excessive alcohol consumption (> 50g per day); iv) co-existent HBV infection or immunosuppression (Poynard et al. 2001.) Hepatic steatosis, obesity, diabetes mellitus and iron overload may contribute to more

rapid progression of fibrosis (Hourigan et al. 1999; Angelucci et al. 2002).

1.7 Relationship of Hepatitis C to Steatosis, Diabetes Mellitus and Insulin Resistance

1.71 Steatosis and Hepatitis C

Liver steatosis is common in patients with HCV, occurring in approximately 50% of biopsy specimens and is higher than in patients with chronic hepatitis B or autoimmune hepatitis (Scheuer et al. 1992; Bach et al. 1992).

Liver steatosis develops in chronic hepatitis C patients by two mechanisms.

Metabolic steatosis is independent of the HCV virus and is due to factors that cause non-alcoholic fatty liver disease (NAFLD). These are due to the metabolic syndrome such as diabetes, hypertension, obesity and dyslipidaemia (Adinolfi et al. 2001; Monto et al. 2002). This occurs in genotypes other than 3 and the degree of steatosis correlates with body mass index and visceral fat suggesting that insulin resistance may play a role.

The second mechanism of hepatocyte steatosis is **HCV or viral induced** and is due to a direct effect of the HCV itself (Rubbia-Brandt et al. 2000), occurring mainly in genotype 3 strains. The degree of steatosis correlates with the level of HCV viremia and intrahepatic concentration of HCV RNA. This type of steatosis is directly linked with HCV replication and disappears on viral eradication of the genotype 3 strain. Relapse of the infection results in recurrence of the steatosis.

Several cross-sectional studies confirm that body mass index (BMI) and infection with genotype 3 are two independent risk factors for steatosis in patients with chronic hepatitis C (Adinolfi et al. 2001; Monto et al. 2002).

The mechanisms of HCV – induced steatosis in patients infected with genotype 3 are not fully understood. In humans the degree of steatosis is directly related with the amount of core protein. In vitro and in vivo studies confirm that HCV core protein can induce steatosis in transfected cells and transgenic mice (Barba et al. 1997; Moriya et al. 1997). Studies on hepatoma cell lines have shown that HCV-induced steatosis is a result of the interaction between HCV via non structural protein NS5A or core protein, and triglyceride turnover (Shi et al. 2002). HCV is associated with

betalipoproteins (LDL and VLDL) in sera from infected patients and the LDL receptor is the receptor for the entry of HCV into hepatocytes (Thomssen et al. 1992; Monazahian et al. 1999). Serum levels of apoprotein B (a component of VLDL) is low and inversely related to the degree of steatosis and is corrected by HCV eradication. This suggests that steatosis in patients infected with genotype 3 could be the result of HCV- induced hypobetalipoproteinemia. These findings are consistent with the observation that the HCV core protein inhibits the activity of microsomal triglyceride transfer protein and the secretion of VLDL in transgenic mice developing steatosis (Perlemuter et al. 2002). Another mechanism involved in HCV induced steatosis could be mitochondrial dysfunction as is shown in transgenic mice expressing HCV proteins and developing steatosis (Okuda et al. 2002).

There is no clear explanation why only steatosis associated with genotype 3 in human is viral in origin. In vitro expression models do not show a distinction between genotype 3 transfected hepatoma-cell lines compared to HCV core type 1b, however these models may not be helpful as the level of HCV core protein is much higher in transfected cells than in the liver of infected patients (Abid et al. 2002).

The impact of steatosis on the natural history of chronic hepatitis C

Cross-sectional studies have proved the relationship between the degree of steatosis and the severity of fibrosis. In patients with a known date of acquisition of HCV, the progression rate of fibrosis is significantly higher in those with moderate to severe steatosis than those with no or mild steatosis (Adinolfi et al 2001; Serfarty et al. 2002). In patients with paired biopsies the degree of steatosis at initial biopsy is predictive of fibrosis progression (Westin et al. 2002; Serfarty et al 2001).

Correlation between metabolic steatosis and fibrosis has been shown in obese patients, where non-alcoholic fatty liver disease (NAFLD) is contributing as suggested by the presence of perisinusoidal and centrilobular fibrosis (Ong et al. 2001; Clouston et al. 2001). The association between viral steatosis and fibrogenesis is still controversial at present as studies show conflicting results; and this could be due to the younger age of patients infected with genotype 3.

The effect of antiviral treatment on steatosis

In viral steatosis patients infected with genotype 3 strains of HCV treated with interferon alone or combination treatment have shown

significant reductions in the degree of steatosis in sustained virological responders (Poynard et al. 2002). This effect is independent of weight loss or reduction in alcohol consumption. In contrast there is no significant change of steatosis in sustained responders with non-genotype 3 strains. Normalisation of cholesterol in non-genotype 3 patients can result in reversal of steatosis (Poynard et al. 2002).

Three large cohort studies have investigated the impact of steatosis on antiviral therapy (Poynard et al. 2002; Bjora et al. 2002; Akuta et al. 2002). In two studies steatosis was characterised into viral or metabolic steatosis (Poynard et al. 2002; Bjora et al. 2002), whilst in one study all patients were genotype 2 and hence metabolic (Akuta et al. 2002). The presence of moderate to severe steatosis (ie > 30%) at pre-treatment liver biopsy in metabolic steatosis was highly predictive of failure to achieve a sustained viral response (SVR). Steatosis in genotype 1 patients resulted in two-fold decrease of the response rate (combination therapy) and threefold in genotype 2 (interferon monotherapy). Patients with non-genotype 3 strains should be advised to correct their risk factors for steatosis before starting antiviral treatment as severe steatosis is a predictive factor of

treatment failure. Conversely, in genotype 3 response rates were similar between patients with and without steatosis.

1.72 Hepatitis C and Diabetes Mellitus

Chronic liver disease that leads to cirrhosis is a major cause of death in many areas of the world. Hepatocellular carcinoma (HCC) is one of the most common cancers in the world and develops in people with advanced chronic liver disease. Recently there is growing evidence that type 2 diabetes mellitus increases the risk of chronic liver disease and HCC.

There is strong evidence of an association between chronic hepatitis C and diabetes mellitus. Allison et al. (1994) reported that in patients with cirrhosis being assessed for liver transplantation those patients with an aetiology of HCV were independently associated with type 2 diabetes.

HCV infection has a significantly stronger association with diabetes than other liver diseases irrespective of whether cirrhosis was present. The Third National Health and Nutrition Examination Survey (N-HANES 111) showed that the rate of type 2 diabetes was three times more likely in HCV infected patients 40 years of age or older than patients without HCV infection. Conversely,

HCV positivity was higher among diabetic patients than the general population. Patients who undergo liver transplantation for HCV are more likely to become diabetic than for other liver conditions.

Hepatocellular carcinoma is also associated with diabetes mellitus. Early epidemiological studies showed no association between diabetes mellitus and hepatocellular carcinoma, however lately several studies show a significant association (El-Serag et al. 2001; El-Serag et al. 2004). Lawson et al. (1986) reported a four fold increased incidence of diabetes in patients with HCC compared to controls. A large cohort study in Denmark reported a standardised incidence ratio of HCC of 4.0 in diabetic men (95% CI: 3.5-4.6) and 2.1 (95% CI 1.6-2.7) in diabetic women as compared to the general population. Subsequent studies from United States and Europe have also confirmed this association (Wideroff et al. 1997).

1.73 Insulin Resistance In HCV

Insulin resistance plays an important role in the development of type 2 diabetes mellitus, preceding diabetes by 10 to 20 years. HCV infection can induce insulin resistance and insulin resistance

itself may increase the rate of fibrosis progression in HCV patients (Hui et al. 2003). The mechanisms by which HCV leads to insulin resistance and predisposes to Type 2 diabetes mellitus is not known. Insulin resistance is higher in non-genotype 3 patients. It is hypothesised that obesity especially central type body fat distribution and metabolic steatosis is associated with insulin resistance and secondary hyperinsulinaemia. Intracellular fat accumulation in mice models can result in defects in insulin signalling and insulin resistance (Kim et al. 2001).

Studies from transgenic mice that specifically express the HCV core protein at high levels in hepatocytes show that hepatic insulin resistance can be induced by HCV core protein alone and is due to increased levels of tumour necrosis factor alpha (TNF- α) (Shintani et al. 2004). Insulins' action is mediated by binding to a cell surface insulin receptor, a ligand activated tyrosine kinase. Once insulin binds there is phosphorylation of the tyrosine kinase leading to a cascade of phosphorylation of a family of insulin receptor substrates (IRS 1-4). TNF- α inhibits phosphorylation of IRS-1 which may result in insulin resistance and type 2 diabetes. Insulin resistance and defects of IRS-1 are present before the development of steatosis suggesting that insulin resistance occurs first. The mice did not become diabetic until they were challenged

with a high fat diet which is similar to humans where an environmental stressor leads to diabetes.

SUMMARY

In the first part of the introduction I have set the scene and reviewed the knowledge of hepatitis C. At that time, hepatitis C represented a major public health problem world-wide and was a common cause of cirrhosis and hepatocellular carcinoma as well as the most common reason for liver transplantation. Hepatitis C is carried in the blood which is the main vehicle of infection and I have reviewed the common routes of transmission in both developed and developing countries and discussed which high risk groups should be offered treatment. Studies on natural history of chronic hepatitis C have differed widely and rarely exceeded two decades. Certain factors are associated with rapid progression to severe liver disease such as age > 40, alcohol consumption, male gender and co-infection with HIV and hepatitis B and immunosuppressive treatment. Finally, I discussed the methods of diagnosing hepatitis C and treatment of this condition.

1.80 Knowledge of Hepatitis C amongst Primary Health Care Professionals

General practitioners (GPs) are often the first health care professionals to see infected individuals. Given the fact that HCV infection is often silent until the very late stages it is imperative that GPs are aware of the risk factors, diagnosis, management and complications of this common, treatable infection to facilitate early diagnosis and referral.

In France and Australia, primary care physicians have little knowledge about hepatitis C (Ouzan et al. 2003; Ouzan et al. 2003; Shehab et al. 2001). In France, primary care physicians knowledge of HCV screening practices and investigation of risk factors are poor as is the natural history of this disease. In Australia, there was a wide range of responses, especially from non-English speaking backgrounds, regarding modes of acquisition of HCV in patients, rates of progression to cirrhosis and hepatocellular carcinoma and optimal antiviral therapy. This suggests a need to improve provision of appropriate educational resources that will facilitate general practice management of a

common and important public health problem. An educational outreach programme to improve health professionals knowledge about hepatitis C was piloted in Minnesota (Fischer et al. 2000). It achieved significant improvements in knowledge and high satisfaction ratings amongst the 1150 delegates that took part. The authors conclude that for an educational initiative to be successful it needs to be valuable to healthcare staff, have good incentives, repeated exposures, commitment by key stakeholders and a well- organized implementation plan.

In the last few years hepatitis C has been extensively covered at national meetings, medical journals and popular press. The UK has a relatively low prevalence of HCV infection; it is estimated by the Department of Health to be between 200 000 to 400 000 patients. As few as 10% of these individuals are aware of their infection. The remainder of patients with chronic hepatitis C are asymptomatic and are not aware that they are suffering from a progressive liver disorder. Considering the high proportion of people who are still unaware of their infection the Department of Health wrote to all GPs to inform them about hepatitis C and provided them with educational material to improve their

knowledge of this virus. The 45 page booklet sent to all GPs was developed with experts and key stakeholders in line with the principles set out in the NHS Plan (Hepatitis C Strategy for the UK; Department of Health). Its main aims are to prevent new cases of hepatitis C infection, identify those who are chronically infected by increasing testing for hepatitis C and to offer specialist advice and appropriate treatment via co-ordinated pathways of patient care thus facilitating prevention, diagnosis and appropriate referral at an early stage

1.81 Screening

Prevention and control of HCV infection in the UK must focus not only on reducing transmission in groups at high risk of infection, but also on the early identification of persons with chronic infection. The early treatment of hepatitis C with pegylated interferon and ribavirin is more likely to be successful in eradicating the virus, reducing transmission, and halting progression of fibrosis. Screening programmes are in place for a variety of medical conditions and are successful if the condition is common, and the screening test inexpensive, noninvasive and

sensitive. Once identified the condition must be treatable and effective.

Hepatitis C screening offers several benefits:- treatment can be offered to cure the patients or slow disease progression. Lifestyle modifications can be made such as ceasing alcohol intake and vaccinations for hepatitis A and B. Family members can take precautions against transmission and public awareness is increased. The National Hepatitis Screening Survey investigated a screening programme for hepatitis C in 40 centres in the United States. 13 997 subjects took part and 9 270 completed the study (Kaur et al. 1996; Lapane et al. 1998). This involved a detailed questionnaire followed by liver function tests and two antibody tests for HCV (EIA-2 followed by confirmatory RIBA). 7% of patients were HCV positive, however this figure was higher than would have been expected from the prevalence of HCV in the United States. The reason for this is due to selection bias. 73% of patients had at least one risk factor, whilst Flamm et al. (1998) found that only 12% had no risk factor. The study concluded that it was much more cost-effective to pre-screen patients for hepatitis C risk factors and then only to test patients who had one or more risk factor.

The Department of Health (DoH) have advised testing of patients with the following risk factors:- past or current history of injecting drug use, history of receiving transfusion of blood or blood products, organ transplantation prior to the introduction of blood screening in 1991, needlesick injuries, tattooing or body piercing, sexual relations with a HCV infected partner, babies of a hepatitis C mother, renal dialysis patient, and people exposed to hepatitis C in other countries.

SUMMARY

In this section of the introduction, I have reviewed the present knowledge of general practitioners concerning chronic hepatitis C in the UK and discussed the current initiatives from the Department of Health to improve awareness of this disease (Hepatitis C Strategy for the UK. Department of Health). Primary care professionals knowledge in Europe and the United States and ways of improving this knowledge were also discussed (Ouzan et al. 2003; Ouzan et al. 2003; Shehab et al. 2001; Fischer et al. 2000; Dev et al. 2002; Zdanuk et al. 2001).

1.90 Complementary and Alternative Treatments of Chronic Hepatitis C

The only approved treatment for hepatitis C is pegylated interferon and ribavirin which results in a sustained virological response of 50-60% of patients (Baker D. 2003). This treatment is poorly tolerated and expensive (Liang et al. 2000). It is highly desirable to develop alternative treatments for those patients who are not suitable candidates for standard treatment or who do not respond to treatment. Due to the high incidence of viral hepatitis in China, traditional Chinese Medicine has had a long history of treating chronic hepatitis. It is a popular treatment amongst hepatitis C patients with 41% of patients in American liver disease outpatient clinics having used some form of Chinese herbal medicine within the past four weeks (Seeff et al. 2001).

Coon et al. (2004) recently performed a systematic review of complementary and alternative treatments for hepatitis C. They searched six electronic databases for randomised clinical trials: Medline (via Pubmed), Embase, CINAHL, Amed (Alternative and Allied Medicine Database, British Library Medical Information Centre), The Cochrane Library and the British Library Index of Conference Proceedings (from October 2002). Reference lists of

all papers were extensively searched for further publications. Each study was assessed using the Jadad scoring system for quality of the study (Jadad et al. 1996).

The search revealed a total of 142 relevant studies of which finally 27 studies (1709 patients in total) were included. The remainder were excluded due to no specific hepatitis diagnosis, mixture of hepatitis B and C patients, non-randomised trials or where clinical end-points were not measured. Only 11 out of 27 studies had well executed study design.

Supplements

1. Antioxidants

Seven randomised trials of antioxidants comprising 463 patients have been published. In one trial Vitamin E treatment was compared to placebo (Von Herbay et al. 1997). There were statistically significant reductions in alanine aminotransferase (ALT) in the vitamin E arm, however reductions did not occur exclusively in every patient and normalisation of liver enzymes were not seen. The remaining six studies combined antioxidants with interferon-alpha and no significant virological response between treatment arms were seen (Look et al. 1999, Neri et al.

2000, Andreana et al. 1997; Grant et al. 2000; Bernhard et al. 1998; Ideo et al. 1999).

No serious adverse events were reported in these trials at these doses.

2. Thymic extracts

Five randomised clinical trials of thymic extracts totalling 256 patients have been published. In two trials patients received the synthetic polypeptide thymosin alpha 1 (Andreone et al. 1996) or complete thymic formula alone (Raymond et al. 1998). The complete thymic formula comprises bovine glandular extracts of thymosin, thymopoeitin and thymic humoral factors together with herbs, vitamins, enzymes and minerals. No significant difference in virological or biochemical response was seen between thymic extract or placebo. Thymosin alpha1 in two patients produced localised irritation at the injection site (Andreone et al. 1996) and complete thymic extract produced thrombocytopenia in one patients also taking naproxen (Raymond et al. 1998).

In the three remaining studies the thymosin alpha 1 was combined with interferon and compared with either interferon or placebo alone (Sherman et al. 1998, Moscarella et al. 1998, Sherman et al. 1994). The combination arm was superior in terms

of significantly higher virological and biochemical responses at the end of treatment, together with increased numbers of patients with a sustained response at 6 or 12 months. Nausea and vomiting were more common in the combination arm.

3. Zinc

One randomised clinical study with zinc combined with interferon alpha was performed (Takagi et al. 2001). The combination group had significantly higher numbers of complete or incomplete responders (18/32 vs 8/36; $p < 0.006$). This may be explained by the increased number of low viral loads in patients in the combination arm as compared to interferon alpha arm alone. Side-effect profile was similar in both groups. Seven patients withdrew from the study; four from the interferon-alpha group alone (erythema multiforme, severe fatigue, headache, loss of consciousness) and three from the combination group (loss of sleep, serious headache and interstitial pneumonia).

4. Traditional Chinese medicine

The main function of the liver in Chinese herbal medicine is to control the flow of intrinsic vital energy called qi. By controlling the free flow of qi it produces harmony of four systems: emotions,

digestion, menstruation, and secretion of bile. The liver also stores blood and controls function of tendons. Liver dysfunction results in following states: stagnation and depressive syndromes, deficiency syndromes and excess syndromes.

Hepatitis is not mentioned in Chinese herbal medicine, however acute hepatitis resembles the damp heat syndrome. Patients who develop symptoms later in the chronic stage have a low-grade damp heat syndrome, with excess combined with deficiency. The herbs should drain the liver of excess heat and dampness, and support deficiency conditions while calming the emotions.

Seven randomised studies of traditional Chinese medicine were found; six of which were of poor quality (score one on Jadad scale). (Han et al. 1997; Pei et al. 1996; Batey et al. 1998; Chen et al. 1998; Jiang et al. 1999; Xiao et al. 1999; Yu et al. 1995). The best quality trial compared Chinese herbs with placebo and found a significant reduction in ALT levels during treatment, but no decreases in HCV RNA levels (Batey et al. 1998). However four patients on the active herbal preparation developed palpitations, diarrhoea and abdominal discomfort. No significant difference was seen between Chinese herbal preparations and interferon (Chen et al. 1998). In combinations of herbal treatment

and interferon-alpha there was a trend towards normalisation of ALT and clearance of HCV RNA in the combination group. The problem with some of these studies is their poor study design, small numbers and the unknown effects of the control treatment preparations. More work is needed to evaluate the safety of these herbal preparations.

5. Glycyrrhiza glabra (licorice)

Stronger Neo Minophagen C (SNMC) is a formulation consisting of 0.2% glycyrrhizin, 0.1% cysteine and 2 % glycine in physiological saline. Four randomised studies have been performed (Fujiyama et al. 1998; Suzuki et al. 1996, Van Rossum et al. 1999; Tsubota et al. 1999). Patients receiving SNMC combined with interferon alpha found no significant difference in biochemical or virological response. The European study comparing SNMC alone with placebo showed reductions in ALT only during the treatment period (Van Rossum et al.1999). This was also the case for the study comparing SNMC against SNMC and ursodeoxycholic acid, where there were statistically significant differences in liver enzyme levels between the treatment groups but these re-elevated after the study (Tsubota et al. 1999). There was no statistically significant difference in the

occurrence of side-effects in the SNMC arm, although Van Rossum et al (1999) reported cold/flu like symptoms in the 160mg SNMC group in his study.

6. Oxymatrine (constituents of Sophora japonica)

In one study oxymatrine was compared to general liver protective agents (Li et al. 1998) and at the end of treatment were found to have significant differences in the number of patients with normalised HCV RNA levels between groups. Information regarding the safety of oxymatrine was not available in this study.

7. Silymarin (milk thistle)

Silymarin is an extract of Milk Thistle (*silybum marianum*). 90% of the herb's components contains silibinin which has been used as a treatment for liver disease and jaundice (Flora et al. 1998). Silymarin in experimental studies acts as an antioxidant or free-radical scavenger preventing glutathione depletion and free radical destruction (Campos et al. 1989). One large scale trial showed benefits of silymarin in patients with cirrhosis of all causes especially in patients with Child's A cirrhosis and alcohol-induced liver disease (Ferenci et al. 1989). However, the design of this study was poor. Silymarin had no significant side effects.

There have been no randomised controlled studies of silymarin therapy alone on chronic hepatitis C. However, pilot studies suggest that silymarin can decrease ALT levels but has no effect on viral load.

Significant improvements in biochemical and/or virological response were found in studies using Vitamin E, thymic extract, zinc, traditional Chinese medicine, Glycyrrhiza glabra and oxymatrine. Studies of thymic extract, zinc and Bing Gan decoction in combination with interferon alpha and oxymatrine alone have shown greater clearance of hepatitis C virus than placebo. The methodological design was poor in a number of studies and extrapolation of the results is difficult for the following reasons:-

- i) The sustained virological response 6 months after stopping treatment was not measured.
- ii) Trials consisted of no more than 26 weeks of treatment, whilst conventional therapy (pegylated interferon and ribavirin) for genotype 1 patients continues for 48 weeks (Di Bisceglie et al. 2002).
- iii) Patients response to conventional therapy is dependent on several favourable host and viral characteristics and whether this is also the case for complementary

treatment has not been elucidated as yet. It is not clear if non-responders were recruited into these studies.

- iv) Over half of the studies did not have proper blinding procedures or reporting of randomisation and withdrawals.

Several of the studies have poor study design and more studies are needed to evaluate the role of complementary and alternative treatments together with their safety profiles for hepatitis C.

1.91 Quality of Life Assessments in Chronic HCV

In the last 10 years there has been an impetus to develop subjective measures of health to monitor medical outcomes (Geigle et al. 1990). These should incorporate measures of physical mobility, emotional wellbeing, social life and overall wellbeing. Any measure must be short, easy to complete, acceptable to patients and be fully validated for the illness process to be investigated. One of the more widely used questionnaires that meets these criteria has been the Nottingham health profile which comprises 38 items (see appendix). However this has been criticised due to its inability to detect low levels of disability.

A new questionnaire, the short form 36 (SF 36) is a shortened version of a battery of 149 health status questions that was developed from the Rand Corporation's health insurance experiment in the United States (Ware et al. 1980; Stewart et al. 1992). The SF36 is a short questionnaire (Table 3) with 36 items measuring eight multi-item variables: physical functioning (10 items), social functioning (2 items), role limitations due to physical problems (4 items), role limitations due to emotional problems (3 items), mental health (5 items), energy and vitality (4 items), pain (2 items), and general perception of health (5 items).

- 1) Physical functioning (PF) - extent to which health limits physical activities such as self-care, walking, climbing stairs.
- 2) Role Functioning- Physical (RP) – extent to which physical health interferes with work or other daily activities such as accomplishing less than wanted.
- 3) Role Functioning – Emotional (RE) – extent to which emotional problems interfere with work or other daily activities.
- 4) Bodily Pain (BP) – intensity of pain and effect of pain on normal work.
- 5) Vitality (VT) – feeling energetic and full of pep.

- 6) Mental Health (MH) – general mental health, including depression.
- 7) Social Functioning (SF) – extent to which physical health or emotional problems interfere with normal social activities.
- 8) General Health (GH) – personal evaluation of health.

The last item relates to changes in respondents' health over the last year. Modifications to six items were required to make it acceptable to British patients. Each variable item scores are coded, summed and transformed on to a scale from 0 (worst possible health state) to 100 (best possible health state). If an item is left blank then the developers suggest a method of gaining scores for missing values.

The SF 36 has been shown to be acceptable to patients, has good test-retest properties and a valid measure of the health status of a wide range of patients (Brazier et al. 1992; Garratt et al. 1993). However it does not contain a variable on sleep. There was also a lower response rate among those over 65 years and different questionnaires may be required (Brazier et al. 1992).

Many patients with chronic hepatitis C are asymptomatic; however non-specific symptoms such as fatigue, irritability, nausea, anorexia, headache, abdominal discomfort, right upper abdominal pain and even cognitive impairment have been

described (Conry et al. 1996; Poynard et al. 2002; Forton et al. 2002; Bayliss et al. 1999). A number of studies have now challenged this previous belief that hepatitis C is an asymptomatic disease. Patients with chronic hepatitis C have been found to have a reduced health related quality of life (Ware et al. 1994; Davis et al. 1994; Carithers et al. 1996; Bayliss et al. 1998). The causes of impaired health related quality of life in chronic hepatitis C is unknown, however psychiatric symptoms and emotional distress are more common amongst hepatitis C patients (Fontana et al. 2002). This study reviewed 220 untreated patients and detected emotional stress in 35% of the study population (compared to 10% in the general population) and the emotional stress correlated with a lowered health related quality of life. Both previous exposure to alcohol or intravenous drug abuse were not associated with emotional distress. Significant elevated scores for depression, anxiety, psychoticism, somatisation and obsessive-compulsive disorders were detected in 28-40% of patients, whilst psychiatric and medical co-morbidities arose in 71% of patients.

Patients without cirrhosis also show reductions in both mental and physical health-related quality of life (SF36) (Foster et al. 1998). Patients with previous intravenous drug use have psychological

disturbances that impair their quality of life (Kendall et al. 1995; Lipsitz et al. 1994). The study by Foster et al. (1998) excluded patients with drug abuse and Davis et al. (1994) made adjustments for co-morbidities which can be associated with impaired quality of life. Both studies reported that the reduced health related quality of life is due to infection with hepatitis C itself. Three studies have looked at the effect of treatment for hepatitis C on health related quality of life data (Davis et al. 1994; Hunt et al 1997; Bonkovsky et al. 1999). Davis et al. (1994) found that the measure of health related quality of life (Sickness Impact Profile - see appendix) was insensitive to distinguish between responders and non-responders and Hunt et al. (1997) used a small sample that had insufficient power to detect a difference. Bonkovsky et al. (1999) concluded that health related quality of life (SF 36) was significantly impaired in patients with chronic hepatitis C in the presence or absence of cirrhosis. The impairment is as large as other chronic illnesses such as diabetes mellitus and chronic arthritis. Treatment with interferon results in improvements in quality of life and the degree of improvement is directly related to the sustained virological and/or biochemical response to treatment. It appears that the SF 36 is better suited

for evaluating health related quality of life in patients with chronic hepatitis C and measuring the efficacy of treatment outcomes.

SUMMARY

In the last section of the introduction, I have reviewed the efficacy of complementary and alternative therapies of chronic hepatitis C together with quality of life assessments in chronic hepatitis C.

CHAPTER 2: METHODS

2.1 Patient Population and Databases

i) Patient Population

The patient population described in this thesis all originate from North East London and all were attending Barts and the Royal London Hospitals for clinical care. This patient population has been used in several of the studies that follow:-

- 1) Natural History of Chronic Hepatitis C Infection: Life-Long follow-up of an unselected population infected in early childhood.
- 2) Factors involved in progression of liver fibrosis in patients with Non-alcoholic steatohepatitis (NASH), hepatitis C and hepatitis B
- 3) The Role of Steatosis in Fibrosis Progression
- 4) Influence of Iron on Fibrosis Progression and Causation of Diabetes Mellitus in a Population with Chronic Hepatitis C
- 5) Clinical Significance of Insulin Resistance in Hepatitis C Virus Infection

The North East sector of London contains a diverse population. A high proportion of immigrants - over 30% are from 'ethnic minority

groups' which include first and second generation immigrants from Pakistan and Bangladesh (Figure 2). Health care services for immigrants in North East London are provided free under the provisions of The United Kingdom National Health Service. The mature immigrant communities in East London have excellent access to local health care services with a well developed system of Bengali speaking health care advocates and translators.

Until 2003 patients with HCV and other hepatological disorders in North East London were managed by local gastroenterologists with an interest in liver disease working in one of the three regional hospitals (Barts and The London, The Homerton and Newham District General Hospital). During the period 1992 -2003 none of the hospitals provided a tertiary referral service for patients with liver disease (although a transjugular liver biopsy service was provided by Barts and The Royal London in response to a request from the haemophilia service). Patients with advanced liver disease were referred to The Liver Unit at The Royal Free Hospital in the adjacent health care sector (London Central). Patients with hepatological disorders that did not require transfer to a tertiary referral center were managed locally. Patients from hospitals and practitioners in the West of the sector who use

	All people	White British	Irish	Other White	Mixed: White and Black Caribbean	Mixed: White and Black African	Mixed: White and Asian	Mixed: Other Mixed	Asian or Asian British: Indian	Asian or Asian British: Pakistani	Asian or Asian British: Bangladeshi	Asian or Asian British: Other Asian	Black or Black British: Caribbean	Black or Black British: African	Black or Black British: Other Black	Chinese or other ethnic group: Chinese	Chinese or other ethnic group: Other ethnic group	
% of Population		86.96	1.27	2.66	0.47	0.16	0.37	0.31	2.09	1.44	0.56	0.48	1.14	0.97	0.19	0.46	0.44	
Population number	40138631	42746869	624063	1307003	230962	78622	181814	152330	1027002	707699	276177.6	236866.4	660182.6	476646.7	93363.78	221125	216211	
HCV prevalence (estimated population)		170983	2496	5228	924	314	727	609	30810	21227.97	137588.7	11793.32	5601.82	4766.467	933.6378	2211.25	2162.1	274648.48
HCV prevalence (estimated % of population)		0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.03	0.03	0.05	0.05	0.01	0.01	0.01	0.01	0.01	
% of Hackney Population		44.12	3.02	12.26	1.52	0.79	0.78	1.11	3.76	1.07	2.94	0.82	10.29	11.98	2.39	1.17	2	
Population number (Hackney)	202824	89485	6125	24866	3082	1602	1582	2251	7626	2170	5963	1663	20970	24298	4847	2373	4056	
HCV prevalence			24	99	12	6	6	9	228	65	298	83	208	242	48	23	40	1397
% of Newham Population		33.78	1.32	4.31	1.22	0.68	0.68	0.8	12.14	6.46	8.9	3.12	7.35	13.11	1.12	0.96	2.14	
Population number (Newham)	243891	82386	3219	10511	2975	1658	1658	1951	29608	20633	21462	7609	17926	31074	2731	2341	5219	
HCV prevalence		329	12	42	11	6	6	7	898	618	1073	380	179	319	27	23	52	3980
% of Tower Hamlets Population		42.91	1.95	6.54	0.8	0.4	0.69	0.6	1.53	0.76	33.43	0.9	2.66	3.36	0.47	1.82	1.18	
Population number (Tower Hamlets)	196106	84149	3824	12825	1568	784	1353	1176	3000	1490	65558	1764	5216	6589	921	3569	2314	
HCV prevalence		336	15	51	6	3	5	4	90	44	3277	88	52	65	9	35	23	4109

2: Census Data

the virology service at Barts and The Royal London but refer patients with viral liver disease to a hospital outside the sector were excluded from the analysis.

For patients with chronic HCV infection the diagnosis was established by serological and virological testing at a single, central laboratory.

In accordance with national guidelines all patients who were viraemic (i.e. HCV RNA positive by PCR testing) had a liver biopsy performed, at their local hospital, and the tissue samples were sent to Barts and The London where they were examined by one of three consultant pathologists with an interest in gastroenterology/hepatology. All 3 pathologists operated under the same guidelines and scored the liver biopsy specimens according to a common system. These guidelines state that all patients who are HCV RNA positive should have a liver biopsy performed to assess the degree of fibrosis and/or suitability for treatment (Department of Health, NICE guidelines, Freeman et al. 2003).

Patients were recruited into these studies by searching through two independently maintained clinical databases. Appropriate patients were retrieved from each database (see relevant sections for the details of selection criteria for each of the studies).

ii) Central Virology Database

This database has been used in several studies described below between the period 1992 -2003. The central virology database is stationed at Barts and the Royal London Hospital NHS Trust and contains a computerized record of all viral hepatitis patients dating from 1992. In this database information comprising viral serology (hepatitis B and hepatitis C) together with estimations of viral load (qualitative and quantitative) using Polymerase Chain Reaction for both HBV DNA and HCV RNA and genotyping for hepatitis C patients are available. From the virology database patients who were HCV RNA polymerase chain reaction positive (by qualitative PCR testing with a sensitivity of 50 IU/ml) were identified. HCV genotype was determined through sequencing of polymerase chain reaction amplicons from the 5' Non-Coding Region (TRUGENE™ HCV 5'NC Genotyping Kit , Visible Genetics Inc., NJ, USA). This was performed by Dr Ines Ushiro-Lumb in the Department of Virology.

The duration of antiviral therapy for HCV is determined by the genotype of the infecting virus. Patients infected with HCV genotype 2 or 3 received 24 weeks of therapy; whilst patients infected with HCV genotype 1 received 48 weeks treatment. If the

qualitative PCR HCV RNA assay (Cobas Amplicor™ HCV Monitor [version 2.0], Roche Diagnostics) was negative six months after completion of full course of antiviral treatment then the patient was considered to have had a sustained virological response to treatment.

iii) Central Histopathology Database

The central histopathology database has been used in several studies described below between the period 1992 -2003. This database is at Barts and the Royal London Hospital NHS Trust. The pathology department at Barts and the Royal London provides a pathology service to all three local district general hospitals and therefore contains a complete record of all liver biopsies throughout the region.

There is a computerized record of all cytology and pathology reports that are stored at three sites:- St Bartholomews' Hospital, Royal London Hospital or London Chest Hospital. A reference number for the liver biopsy specimen can be found on the computer and this then has to be searched manually in the respective pathology library. Liver biopsies since 1983 were searched using this database.

Histopathology reports for patients with HCV who had undergone

a liver biopsy were retrieved from the local pathology database by manual searching under the terms 'viral hepatitis' or 'hepatitis C'. Biopsies were fixed, paraffin-embedded, and stained with haematoxylin, eosin, reticulin, PAS, Perls', Van Geison and Victoria Blue. Each biopsy was reviewed by one of the three histopathology consultants who specialise in gastrointestinal/liver pathology and then rescored by a second independent pathologist. Disagreements were resolved by discussion to reach a consensus score.

According to Desmet et al. (1994) scores for necroinflammatory changes and architectural alterations were graded separately. The liver biopsies were scored using the Modified Ishak scoring system (Ishak et al. 1995) with fibrosis stage scored on a scale from 0 to 6. Portal and lobular necroinflammatory grades were also recorded using this same scoring system (Ishak et al. 1995). Steatosis was assessed as the percentage of hepatocytes containing macrovesicular fat droplets and was graded as no steatosis, mild (<33% of hepatocytes affected), moderate (33%-66% of hepatocytes affected) or severe (> 66% of hepatocytes affected) steatosis. Two independent histopathologists graded hepatocyte and macrophage iron stores on a scale of 0 to 4 on Perls' Prussian blue liver sections (Searle et al. 1994; Brissot et al. 1981).

For paired liver biopsies the stage, grade, degree of steatosis were recorded using each biopsy slide and accompanying pathology report. The degree of steatosis was recorded from the first biopsy in the series. Paired biopsies comprised 48 HCV patients and 11 HBV patients.

Both the virology and histopathology databases were used in the following studies. All studies were approved by the local ethics committee (East London and the City Local Research Ethics Committee – P/ 02/260 and P/02/240).

- 1) Natural History of Chronic Hepatitis C Infection: Life-Long follow-up of an unselected population infected in early childhood
- 2) Factors involved in progression of liver fibrosis in patients with Non-alcoholic steatohepatitis (NASH), hepatitis C and hepatitis B
- 3) Influence of Iron on Fibrosis Progression and Causation of Diabetes Mellitus in a Population with Chronic Hepatitis
- 4) Clinical Significance of Insulin Resistance in Hepatitis C Virus Infection

iv) Patient Details

Patient details such as age, sex, diabetes mellitus or impaired

glucose tolerance, plasma levels of glucose, insulin and C peptide and lipid profile, AST/ALT ratio, ferritin and ethnicity were obtained from the PAS hospital database that has computerized details of each patients details and blood results. If this was not available then patient notes were searched or previous blood samples analysed. Alcohol intake, mode of infection of hepatitis B or C and estimated date of infection were obtained from the medical records and questioning of patients at subsequent clinic attendances. Height which could not be retrieved from the medical records were obtained from telephone calls to the practice nurse of the patients' general practitioner. Body mass index was calculated for each patient by dividing the weight (kilograms) of the patient by their {height (meters)}².

Impaired glucose tolerance was defined by the WHO classification as a fasting plasma glucose less than 7.0 mmol/l and random venous plasma glucose two hours after an oral glucose tolerance test of between 7.8 and 11.1 mmol/l.

Patients were classified as diabetic according to the American Diabetes Association classification criteria -

- 1) Symptoms of diabetes together with a plasma glucose concentration more than or equal to 11.1mmol/l
- 2) Fasting plasma glucose more than or equal to 7.0 mmol/l

3) 2 hour post load glucose of more than or equal to 11.1 mmol/l during an oral glucose tolerance test.

Serum ferritin was defined according to the WHO classification as raised in men if the level was greater than 300 ug/l and in women greater than 200 ug/l.

Alcohol Intake was defined as non-drinker of alcohol, mild/moderate alcohol consumption if <14 units per week for females and <21 units of alcohol for males and heavy alcohol consumption if above these levels per week (Alcohol Concern 1999).

Fasting plasma glucose, ALT and AST were measured using Olympus AU 640 (Olympus UK Limited). However, fasting insulin was analysed using the DPC Immulite 2000 analyser, Diagnostics Products Cooperation. HbA1c was measured by high pressure liquid chromatography using the H1-Auto A1c, HA-8140 A Menarini Diagnostics.

The gold standard for *in vivo* measurement of insulin resistance is the euglycaemic hyperinsulinemic clamp technique (American Diabetes Association. Consensus development conference on insulin resistance 1998). However, as this technique can induce severe hypoglycaemia, and cirrhotic patients are especially at risk

of this, we decided to use the Homeostasis model assessment for insulin resistance (HOMA-IR). This has previously been validated against insulin sensitivity measured directly with the euglycaemic/hyperinsulinaemic clamp technique in both diabetic and non-diabetic subjects (Emoto et al. 1999; Bonora et al. 2000). Using HOMA-IR model, insulin resistance is calculated using the following equation:

$$\text{Homeostasis Model measurement of Insulin Resistance} = \frac{\text{fasting insulin (u U/ml)} \times \text{fasting plasma glucose (mmol/l)}}{22.5}$$

2.2 Statistical Analysis and Patient Selection

i) Natural History of Chronic Hepatitis C Infection: Life-Long follow-up of an unselected population infected in early childhood.

Patients were divided into two groups – Asian patients and Caucasian patients. To begin with, simple univariate relationships between the fibrosis score and other factors were investigated using the Mann Whitney U test (ethnicity and gender) or Spearman's rank correlation coefficients (relationship with age). As both age and ethnicity were associated with the fibrosis score in univariate analyses, and the Asian patients were known to be

older than the Caucasian patients, the relationship between ethnicity and the score was investigated using a linear regression model, after adjusting for age differences between the two populations. This allowed us to isolate the relationship between ethnicity and the fibrosis score that could not be explained by differences in ages between the two populations. The possible confounding effects of other factors, including sex, HCV genotype, alcohol consumption and body mass (BMI), were then removed by incorporating these factors into the same multivariable regression model. The possibility that the relationship between fibrosis score and age differed by either ethnic group or sex was investigated by incorporating interaction terms between ethnic group, sex and age into the multivariable model.

For the purposes of these linear regression analyses, the fibrosis score was taken as the dependent variable in the model. Although the fibrosis score is not strictly quantitative (it is an ordinal variable taking values from 0 to 6), visual assessment of the values confirmed that it would be reasonable to make this assumption for the purposes of our analyses. Inspection of the residuals from the multivariable regression analyses confirmed that this assumption was reasonable. However, as a sensitivity analysis, we also modeled the odds that a patient had cirrhosis

(fibrosis score of 6) using multivariable logistic regression. The conclusions reached from this approach were consistent with those from the linear regression models (results not presented).

Paired biopsies in 48 patients were used to calculate the annual rate of fibrosis progression (the difference in the two scores divided by the time between the biopsies) and these rates were compared between groups using the Mann-Whitney U test. Comparisons of factors in different subgroups of the study population were performed using Chi-squared or Fisher's exact tests (for qualitative variables) or Mann-Whitney U tests (for quantitative variables). Correlations between pairs of quantitative variables were performed using Spearman's rank correlation methods.

Statistical analyses were performed with the assistance of Prof CA Sabin, Department of Primary Care and Population Sciences, Royal Free and University College Medical School, London.

ii) Influence of Iron on Fibrosis Progression and Causation of Diabetes Mellitus in a Population with Chronic Hepatitis C

Due to the heavily skewed nature of ferritin levels, levels were summarized using medians and ranges, and by calculation of the proportion of men with a ferritin level greater than 300 ug/l and

women with a ferritin level greater than 200 ug/l. Comparisons of ferritin levels in different subgroups of the population were performed using Mann-Whitney U tests (for comparisons of the median values) and Chi-squared or Fisher's exact tests (for comparisons of the proportion of men with a ferritin level greater than 300 ug/l and women with a ferritin level greater than 200 ug/l). Relationships between the presence of iron deposition or a diagnosis of diabetes/impaired glucose tolerance and demographic and clinical status were performed in a similar way. Factors independently associated with the stage of fibrosis were identified using multivariable linear regression models, by treating the stage of fibrosis as the outcome in the analysis.

Statistical analyses were performed with the assistance of Prof C Sabin.

iii) Clinical Significance of Insulin Resistance in Hepatitis C Virus Infection

Comparisons of factors in different subgroups of this study population were performed using Chi-squared or Fisher's exact tests (for categorical variables) and Mann-Whitney U tests (for numerical variables). Correlations between pairs of numerical

variables were analysed using Spearman's rank correlation methods.

Factors independently associated with stage of fibrosis were identified using multivariate linear regression analyses. Factors independently associated with sustained virological response were identified using multivariable logistic regression.

All analyses were performed using the Statistical Analysis System (SAS) version 8.02. All p-values are two-sided and were considered to be significant if they were less than 0.05.

2.3 Clinical Significance of Insulin Resistance in Hepatitis C Virus Infection

Methods

a) Patients and Protocol

This was an open labelled study in which consecutive patients with chronic HCV who required antiviral therapy were enrolled. Antiviral treatment for HCV comprises a full course of treatment with pegylated interferon 2a plus ribavirin .

HCV infected patients with a liver biopsy confirming this diagnosis

who were eligible for antiviral therapy were considered for inclusion in the study. Patients were required to have had a recent liver biopsy (within the last six months from starting date of the study) showing mild, moderate or severe disease. Patients with evidence of cirrhosis on biopsy were only accepted for entry into the study if they had compensated cirrhosis (Child-Pugh score A). Entry criteria included patients aged between 18-70 years who were HCV antibody and HCV RNA positive. Patients with other causes of liver disease were excluded from this study. All patients had to be willing to use effective contraception during the duration of the study. Exclusion criteria included patients who were diabetic, pregnant, unable to take effective contraception during the trial, had an abnormal ECG, decompensated cirrhosis (Child-Pugh score of B or C) or any contraindication to combination treatment. Patients who were diabetic according to the American Diabetes Association classification criteria (described previously) were excluded from the study.

Each patient had characteristics such as body mass index (BMI), alcohol intake, ethnicity and HCV genotype recorded. A total of 59 patients were recruited of whom 49 were male and 10 female (Table 1). Ages varied from 34-60 years. 30 patients were Caucasians and 29 patients Asian.

b) Liver Biopsy

Each patient had a liver biopsy that was prepared as previously described. The biopsies were examined as described above. The liver biopsies were scored using the Modified Ishak scoring system and steatosis was assessed.

The majority of patients (85.2%) in this study had stage 1-4 according to modified Ishak scoring on liver biopsy.

c) Laboratory Investigations

Before starting antiviral treatment patients had the following blood tests. They were required to fast for 12 hours and venous blood was then drawn for fasting plasma glucose, insulin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values and glycosylated haemoglobin (HbA1c).

We decided to use the Homeostasis model assessment to measure insulin resistance (HOMA-IR).

The duration of therapy was determined by the genotype of the infecting virus. They received a full course of treatment unless they chose to stop treatment prematurely. If patients were unable to tolerate one of the antiviral treatments then the dose of either the offending pegylated interferon or ribavirin were reduced

respectively to see if this was tolerated better, before considering stopping treatment altogether. The sustained virological response to treatment was determined for all patients completing treatment. All patients began treatment, however seven (11.5%) did not tolerate treatment and withdrew during the first 12 weeks. In these patients we tried first to reduce the dose of the offending antiviral agent, to make it more tolerable to the patient. They still were unable to tolerate treatment and eventually withdrew. This left a total of 52 (88.1%) patients, comprising 27 Caucasians and 25 Asians, who completed treatment.

2.4 Knowledge of Chronic Hepatitis C among East London Primary Care Physicians following the Department of Health's Educational Campaign

Methods

A self-administered questionnaire of eight closed and two open ended questions was devised. Questions comprised the most

important information a GP requires in order to screen, test, diagnose and refer patients with HCV for treatment. All answers to our questions could be found within the DoH Hepatitis C booklet, sent to all GPs in the UK in August 2002. Ten senior practitioners were chosen from primary care trusts in the East End. Dr Comfort Ossonaya chaired the meeting and the questionnaire was reviewed by all members for content and relevance to them. Six of the panel suggested two further open ended questions, which we included.

Permission was obtained from four primary care trusts in the South East sector of London to pilot the study. The 250 South East London GPs were randomly selected from 750 GPs and the revised questionnaire then mailed to 250 GPs in the South East in June 2003. GPs were asked to FAX their replies to a central office.

Following the success of the pilot study we then decided to send out the questionnaire to the original target population of GPs in the North East London. 600 GPs were selected randomly from 650 GPs in the North East and the questionnaire was mailed to the GPs as detailed above.

The majority of GP practices have multiple partners whilst less than 5 % of GP practices were single partners. The South East

region serves an underprivileged area with high levels of unemployment where 30% of the population comprise 2nd and 3rd generation Afro-Caribbeans, whilst the North East region serves a generally deprived area with over 30 % of the population being derived from the Asian continent. Both regions are reported to have people who inject drugs.

GPs who did not reply within the first six weeks were sent a postal reminder with a repeat questionnaire. After a further six weeks if they still had not replied they were telephoned and were offered an appointment for an interviewer to come to the surgery or telephone them at a convenient time to aid filling in the questionnaire.

After 6 months the study was closed.

We randomly selected 10 GPs for face-to-face open-ended interviews derived from the cohort of GPs from the North East of London who had been invited to complete the questionnaire. This was performed in order to try and assess the reasons for GPs lack of knowledge and means by which this knowledge could be improved. The interview was carried out in a standardised manner in the form of a list of structured open ended questions with responses recorded. Selected GPs were telephoned and

asked if they were willing to participate. Only six agreed and a further four were selected. These latter four consented to be interviewed. All ten physicians were sent a letter thanking them for participating and confirming the time and date of the interview.

2.41 Improving General Practitioners Knowledge of Chronic Hepatitis C Infection

Methods

Educational programmes were set up for general practitioners about hepatitis C at Queen Marys School of Medicine and Dentistry in North East London. The educational programmes consisted of 'lunch-and-learn' sessions. Previous studies have shown that for an educational initiative to be successful it requires five key elements (Fischer et al. 2000; Davis et al. 1995):-

- 1) value to healthcare staff (quality and importance of the topic)
- 2) incentives (ie convenience, free lunch and continuing medical education points)
- 3) repeated exposures (multiple opportunities for learning)

4) commitment by key stakeholders at the DH and clinicians

5) an exceptionally well-organised implementation plan

Our group incorporated these elements into our four lunch time meetings. The goal of this project was to provide basic information about the diagnosis and treatment of HCV that was relevant to General Practitioners. A development team including members of the study team (myself and Professor GR Foster) and a practising GP (Dr Comfort Osonnoya) met to ensure that both administrative and clinical perspectives were represented during the design and implementation of the education programmes.

The educational programme for GPs was titled 'Hepatitis C: All You Need To Know', and consisted of 1-hour lunch-and-learn sessions. They were advertised widely to all GPs in East London via the primary care trusts four months in advance. GPs then telephoned or returned a reply slip to book one of the four sessions. If they were unable to attend they were asked to give a reason so that future meetings would take this into account. Eight weeks prior to the sessions they were sent a self-questionnaire composed of eight closed and two open ended questions that had previously been used by our group to assess GPs knowledge of chronic HCV (D'Souza et al. 2003). This assessed participants'

knowledge of basic HCV facts, risk factors, diagnosis, and treatment options and baseline knowledge. To the general practitioners who attended educational meetings all programmes were offered on site, and participants received lunch and continuing medical education credits. The programme was conducted during October 2003 and November 2003. Two experienced presenters with research and clinical experience of HCV presented information on chronic HCV infection and at the end of the session the participants were evaluated and were asked to complete the original questionnaire once more. Participants also completed an anonymous evaluation form at the end of the session to assess whether the meeting was of use and what improvements could be made in the future.

To address the needs of GPs who were unable to attend the meetings we developed a summary sheet that incorporated the key educational messages. This single page information sheet was sent to 200 GPs from East London who were selected on the basis of a poor response to our original questionnaire (all had answered three or more questions incorrectly) and the general practitioners were asked to complete and return the original

questionnaire for the second time after reading the summary sheet.

2.5 Treatment Study

2.51 Study to assess the safety and effectiveness of Phynova-17 (PYN-17) in the treatment of Hepatitis C Virus related symptoms

This was a double blind, randomised, placebo-controlled, parallel group study of PYN-17 - a combination of traditional Chinese herbal medicines (TCM) for the treatment of chronic hepatitis C. The effectiveness of herbal treatment was assessed using quality of life data (symptom improvement), liver function tests and HCV viral load. Male and female subjects aged between 18 and 70 with biopsy proven hepatitis C who were refractory to, unwilling or unable to take interferon/ ribavarin therapy were recruited.

STUDY OBJECTIVES

The primary objective of this study was to indicate whether chronic hepatitis C patients on active treatment showed significant improvement in the SF36 quality of life questionnaire scores when compared to the placebo group.

Secondary objectives were:

- to indicate whether patients on active treatment showed significant reduction in ALT when compared to the placebo group
- to indicate whether patients on active treatment showed significant reduction in AST when compared to the placebo group
- to determine whether treatment reduced serum viraemia
- to demonstrate the clinical safety of the product.
- to show an improvement in Fatigue Symptom Score (FSS)

INCLUSION CRITERIA

Subjects met all of the following criteria at screening in order to be considered for this study.

1. Male or female aged 18 – 70 inclusive on date of screening
2. HCV RNA and antibody positive
3. HbsAg negative
4. HIV antibody negative
5. Liver biopsy within the last 5 years which showed features of chronic hepatitis C without cirrhosis
6. Normal thyroid function tests
7. Serum ferritin within 2x upper limit of normal

8. Normal serum albumin
9. Normal prothrombin index
10. Refractory to, unwilling or unable to take interferon/ ribavarin therapy
11. Showed symptoms typically associated with chronic hepatitis C infection
12. Sterilized, used hormonal contraception or prepared to use barrier forms of contraception
13. Full understanding of the study requirements and the need to comply with the protocol
14. Signed Informed Consent Form

EXCLUSION CRITERIA

Subjects who met any of the following criteria at screening were excluded from study participation:

1. Ongoing chronic illness that required regular medication including unstable diabetes, hypertension and asthma
2. History of significant cardiovascular disease/disorder
3. Administration of antiviral therapy within the 6 months prior to screening

4. Concurrent administration of any other investigational drug or participation in a research study within the last three months
5. Sexually active females not employing reliable contraceptive methods
6. Pregnant women and nursing mothers
7. A history of recent (within 6 months) alcohol or drug abuse
8. Clinically significant abnormal haematological or biochemical parameters other than those associated with hepatitis.

WITHDRAWAL FROM THE STUDY

Participation in the study was strictly voluntary. Subjects could withdraw from the study at any moment for any reason. This would not in anyway affect their further medical care. The investigator had the right to terminate participation of any subject at any time if it was in the subject's best interest or if the protocol was violated. The reason and circumstances for premature discontinuation were documented.

METHODS:- STUDY DESIGN AND PLAN

Study medication were taken over a 24-week period, and patients attended a follow-up visit at four weeks after the final dose was taken.

ASSESSMENT (See Table 4)

Table 4 Assessments to be performed during the study

SCREENING VISIT & BASELINE ASSESSMENTS (VISIT 1)	Demographics & vital signs Review of concomitant medication Laboratory blood and urine sampling Pregnancy tests (females only) SF36 Quality of life questionnaire & FSS Physical examination Significant Medical history & Concurrent disease Quantitative PCR for HCV RNA Dispense medication for first 2 weeks treatment
VISITS 2 – 5 (after 2, 6, 12 and 16 weeks treatment)	Review of Adverse events and concomitant medication Laboratory blood and urine sampling Measurement of vital signs, and weight Pregnancy test (females only) Dispense medication to last until the next visit. NB – at week 12 (visit 4) thyroid function will also be assessed
VISIT 6: (after 24 weeks treatment)	Review of Adverse events and concomitant medication Laboratory blood and urine sampling

	<p>Measurement of vital signs, and weight</p> <p>SF36 Quality of life questionnaire & FSS</p> <p>Physical examination</p> <p>Quantitative PCR for HCV RNA</p>
<p>Patients discontinued their trial medication at this stage and four weeks later a final assessment was made involving:</p>	
<p>COMPLETION</p> <p>VISIT 7:</p>	<p>Review of Adverse events and concomitant medication</p> <p>Laboratory blood and urine sampling</p> <p>Measurement of vital signs, and weight</p> <p>Physical examination</p> <p>Quantitative PCR for HCV RNA</p>
<p>STUDY ENDPOINTS:</p>	<p>Primary Endpoint: To alleviate the impairment in quality of life associated with chronic hepatitis C infection by improving the symptoms of fatigue and poor concentration associated with Hepatitis C infection.</p> <p>Secondary Endpoints: To reduce hepatic inflammation (measured as an improvement in liver function tests) in patients with chronic hepatitis C and to reduce circulating viral load.</p>
<p>SAFETY AND</p> <p>TOLERABILITY:</p>	<p>Adverse events</p> <p>General well being</p> <p>Laboratory parameters</p>
<p>DURATION OF</p>	<p>Total duration: 18 months</p>

STUDY:	
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STUDY PROCEDURES

At the first meeting, the subjects were informed about the study and the randomisation process; and provided with information about PYN-17. They received the study information leaflet that described in more detail the risks and benefits implicit in taking part. Subjects were then encouraged to consider the implications of participating, to discuss the possibility with, if appropriate their partner, their general practitioner, and to ask for any further advice as necessary. The investigator and/or research nurse were available to answer any additional questions. Subjects who wished to take part attended a study screening visit.

Screening Visit

Subjects who agreed to take part were asked to sign a consent form in the presence of the investigator for the study. The general practitioner was informed if the subject agreed.

At the screening visit, the following information were collected and assessments performed:

- subject's initials and demographics
- age, weight and height
- vital signs
- Physical exam
- medical history of significant or severe past illnesses)
- method of contraception and pregnancy test for females
- understanding of TCM principles regarding treatment of hepatitis
- concomitant diseases and hypersensitivities
- concomitant medication
- quality of life questionnaire
- fatigue symptom scale

Laboratory blood and urine tests were carried out which included the following:

- Quantitative PCR for HCV RNA
- Liver function tests
- Renal Profile
- Thyroid function tests

Subjects who met the entry criteria were randomised on a 1:1 basis to an Active (PYN-17) or Control (placebo) group. The active group received 1.41 gm of PYN-17 twice daily in the form of sachets of dry powder reconstituted to form a suspension. The placebo preparation was a similar dry powder that was dissolved in water with a similar taste.

Visits 2 - 5

Subjects were seen within approximately 2, 6, 12 and 16 weeks after start of treatment at which times the following procedures were carried out:

- Review of Adverse events and concomitant medication
- Laboratory blood and urine sampling

- Measurement of vital signs and weight
- Physical exam
- Pregnancy test (females only)
- Dispense medication to last until next visit

Laboratory blood and urine tests were performed as follows:

- Liver function tests
- Full blood count
- Renal profile

At week 12, thyroid function was assessed.

Visit 6

Subjects were seen at 24 weeks after start of treatment for Visit 6.

This visit included

- Review of Adverse events and concomitant medication
- Laboratory blood and urine samples
- Measurement of vital signs, and weight
- Quality of life questionnaire
- FSS
- Physical examination
- Pregnancy test (females only)

Laboratory blood and urine tests were performed as follows:

- Quantitative PCR for HCV RNA
- Liver function tests
- Full blood count
- Renal profile
- Thyroid function tests

Medication were not dispensed at this visit.

Follow-up visit

Subjects were asked to return four weeks after stopping study medication for a follow-up visit. At this visit the following procedures were carried out:

- Review of Adverse events and concomitant medication
- Laboratory blood and urine sampling
- Measurement of vital signs and weight
- Physical examination
- Quantitative PCR for HCV RNA
- Pregnancy test (females only)

Final laboratory samples were taken for the following tests:

- Quantitative PCR for HCV RNA
- Liver function tests
- Full blood count
- Renal profile

The PYN-17 was designed by the herbal company and was claimed to reduce fatigue and tiredness in chronic hepatitis C patients and normalise liver function i.e. AST and ALT. The formula comprises four herbs:- Astragalus root, Milk thistle, Schisandra fruits and Salvia root made up into a dry powder (1.41g) that can be reconstituted in 15-25 ml of a taste masked suspension (See Table 5). The herbal company also provided the placebo as a sachet of dry powder that can be reconstituted into 15-25 ml of a taste masked suspension.

No significant side effects had been observed in the clinical studies conducted for milk thistle fruit and astragalus root; Schisandra fruit had been made into drinks and was available for sale in the UK; *Salvia miltiorrhiza* root as one of the three ingredients in a different herbal formula had obtained an indication from the FDA.

Table 5: Properties of the ingredients of PYN 17 (based on Chinese, German and Russian Pharmacopoeia).

Name	Actions	Indications
Astragalus root	Liver protection – Prevent decrease of hepatic glycogen and raised levels of total serum protein and albumin Immunorestorative activity – Increased phagocytic activity	Chronic hepatitis Cancer
Milk thistle	Liver protector – antioxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, immunomodulating and liver regenerating effects	Toxic liver damage, supportive treatment in chronic inflammatory liver disease and hepatic cirrhosis, ischemic injury, radiation toxicity and viral hepatitis
Schisandra fruits	Tonic Tranquillising Liver protector- Lowers elevated SGPT levels Reduces/ inhibits liver damage Anti-oxidant activity CNS action- Increased concentration, fine coordination, sensitivity and endurance	Hepatitis A, B or C
Salvia root	Various Cardiovascular effects Liver protector –	Acute liver damage Chronic hepatitis

	Regulates liver enzyme production, reduces/ reverses fibrosis Lowers elevated SGPT levels Immune regulator – Increased macrophage activity	
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SELECTION OF STUDY POPULATION

The SF36 questionnaire measured 8 different health modalities. These was combined to provide a physical and mental health summary scale and changes of greater than 5 points in the summary score were regarded as clinically significant. Assuming a p value of 0.05 to be significant 33 patients in each group were required to detect a difference between the two treatments with a power of 80%. To allow for treatment withdrawals we aimed to recruit a total of 76 patients.

BLINDING

This was a double blind study, neither the investigators nor the subjects were aware who was on active treatment or placebo during the trial. Active preparations and placebo were indistinguishable by appearance. Knowledge of the randomisation list was limited to the pharmacist responsible for creation of the

randomisation list, preparation of the random code envelopes and preparation of the study medication. Once treatment of the last subject (i.e. final examination of the last subject), and entry of the case report forms into the database were completed, then the randomisation list was unblinded.

The randomization list allocated each treatment number to one of the two possible groups. The randomization list was initially drawn up for 100 treatment numbers in order to provide for drop-outs and will be computer generated. Randomization was not stratified for age or other criteria.

Together with the study medication the investigator received a sealed random code envelope for each individual subject number. The sealed random code envelope was opened only in case of emergency when knowledge of the actual treatment became medically necessary.

PRIOR AND CONCOMITANT THERAPY

The use of any new medication, other than study medication, was avoided where possible during the double-blind phase

TREATMENT COMPLIANCE

The subjects returned all used and unused medication containers and any remaining study drug at the next scheduled visit, where the returns were counted and documented.

EFFICACY AND SAFETY VARIABLES

Safety was measured and assessed by means of Adverse Event recording, pregnancy tests (females only), laboratory results, and patients general health.

Every randomised subject who received at least one dose of study medication was included in the safety analysis. Quality of Life Questionnaire scores were recorded and summarised by treatment group. Laboratory parameters taken at each visit were recorded and summarised by treatment groups. All adverse events were recorded and summarized by treatment group.

Serious and non-serious adverse events were reported to Phynova Products on an ongoing basis.

ADVERSE EVENTS

DEFINITIONS

An adverse event was any undesirable experience that occurred in a subject during the clinical trial, regardless of whether or not it was considered to be related to the investigational product.

With respect to intensity, adverse events were classified as follows:

- Mild** Some awareness of symptoms, but easily tolerated.
- Moderate** Symptoms that caused enough discomfort to interfere with usual activity.
- Severe** Incapacitating event that caused inability to work or to perform usual activity.

ASSESSMENT OF CAUSALITY

The following criteria were used for the assessment of the causal relationship to the test medication.

None (Unrelated, Not related, No relation): The time course between administration of the study drug and occurrence or worsening of the adverse event ruled out a causal relationship

and another cause (concomitant drugs, therapies and complications etc.) was confirmed

Remote (Unlikely, doubtful, Improbable): The time course between administration of the study drug and occurrence or worsening of the adverse event made a causal relationship unlikely and another cause (concomitant drugs, therapies and complications etc.) was probable.

Possible: The time course between administration of the study drug and occurrence or worsening of the adverse event was consistent with a causal relationship but another cause (concomitant drugs, therapies and complications etc.) could not be ruled out. Or where the time course between administration of the study drug and occurrence or worsening of the adverse event was not consistent with a causal relationship but other cause was denied.

Probable: The time course between administration of the study drug and occurrence or worsening of the adverse event was highly consistent with a causal relationship and other cause (concomitant drugs, therapies and complications etc.) was denied.

Certain (Definite, very likely): The time course between administration of the study drug and occurrence or worsening of the adverse event was highly consistent with a causal relationship and other cause (concomitant drugs, therapies and complications etc.) was denied.

INDEPENDENT ETHICS COMMITTEE (IEC) and REGULATORY APPROVAL

Prior to the start of the study, the East London Local Research Ethics Committee (LREC) approved the study protocol (P/02/241) and were informed of any changes to the protocol. Prof. Foster obtained a Doctors and Dentists Certificate of Exemption (DDX) from the Medicines Control Agency (MCA) for this study.

ETHICAL CONDUCT OF THE STUDY

This study was performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments) and local legal and regulatory requirements.

MY ROLE IN THE STUDY

The Herbal study was originally designed by Professor Foster and initiated three years ago. Owing to the bankruptcy of the original company the trial was suspended and recommenced in 2003 when Phynova assumed financial responsibility. I was responsible for the re-initiation of the study and enrolled and supervised the care of all patients at The Royal London. I collected data from the other centre, St Marys Hospital, London and analysed the results and prepared the final report. The trial was formally monitored by Dr E. Blair, Phynova.

CHAPTER 3: RESULTS - Natural History Of Hepatitis C Infection In The North-East Of London

3.1 Natural History of Chronic Hepatitis C Infection: Long Term Follow-up of a Population Infected in Early Childhood

To this date studies on prolonged (>30 years) follow up of chronically infected patients has not been performed and therefore it is not known whether the proportion of patients with severe liver disease will increase with increasing length of infection. Studies of the natural history of chronic HCV infection in untreated patients infected for over 50 years are still awaited.

At the time of this study it was recognized that the large population of elderly Asian patients with childhood HCV infection in North-East London provided an ideal opportunity to study the life long natural history of chronic HCV infection in a population of patients attending local hospitals. We included a control group of non-Asian patients managed in an identical fashion.

RESULTS

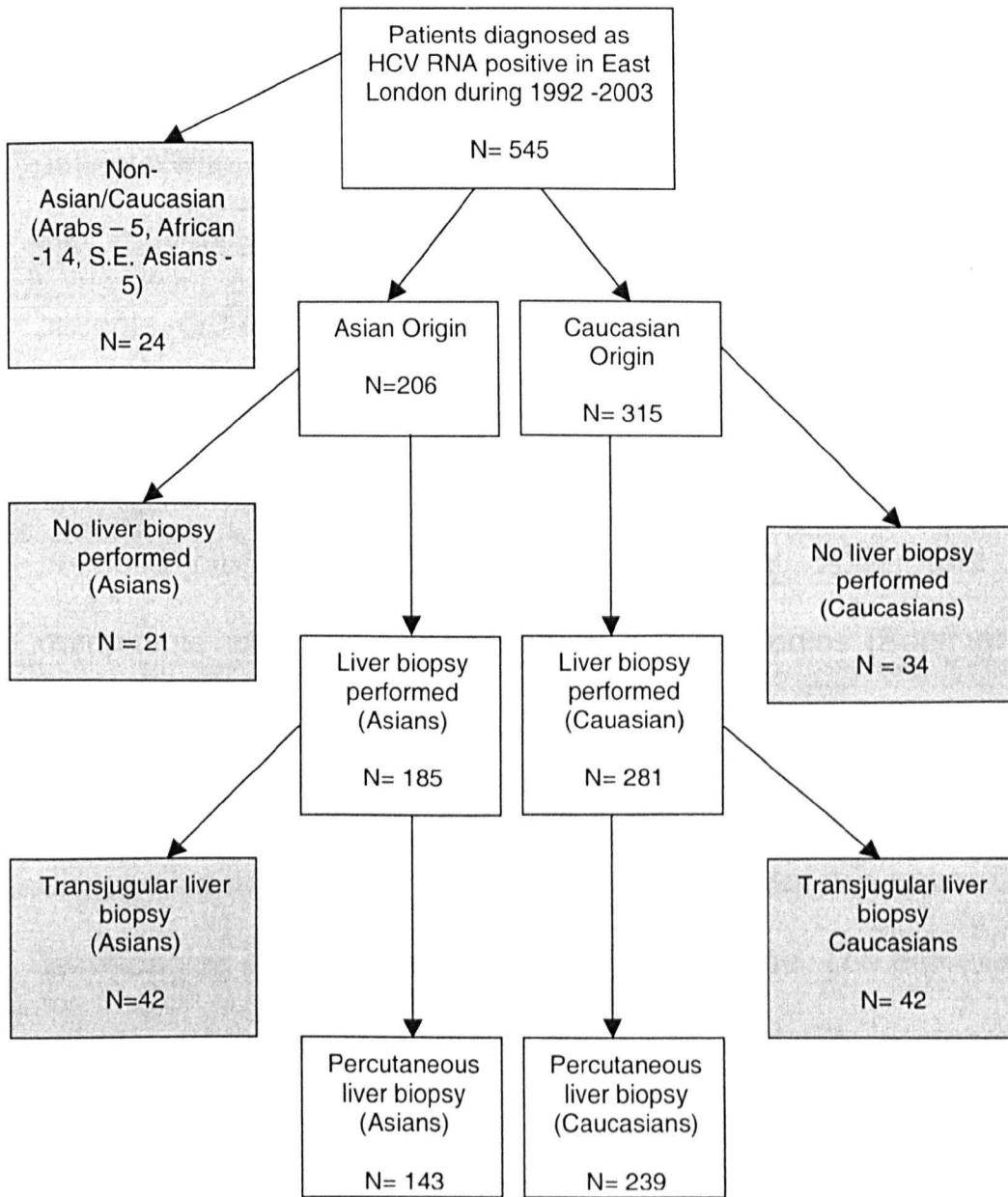
3.11 Patients Demography

A total of 545 patients had a diagnosis of chronic HCV infection (HCV antibody and HCV PCR positive) (Figure 3). 24 HCV infected patients were from other ethnic minorities and were excluded from this study. The demographic details of the remaining 521 Asian and Caucasian patients are shown in Table 6. The median age was 50 years (range 25-80 y). Asian patients (median age, 56 y; range, 26-80 y) were significantly older than Caucasian patients (median age, 47 y; range, 25-79 y; $p=0.0001$). The majority of patients (369) were diagnosed during the later part of the study, 1997-2003). A percutaneous liver biopsy was performed in 382 patients (Table 6, Figure 3). A minority of patients (84 - 16%) underwent a transjugular liver biopsy – either because they had a significant clotting disorder (usually haemophilia A) or signs of decompensated cirrhosis and a small proportion of patients (55 – 11%) did not undergo a liver biopsy. For the purpose of this research patients who had not undergone liver biopsy or a transjugular liver biopsy were excluded from this study.

Table 6 Demography of the population studied and proportion of patients with HCV related cirrhosis in the different study groups.

		Caucasian Patients			Asian Patients		
All HCV Positive Patients		20-40 yrs HCV RNA +ve,	41-60 yrs HCV RNA +ve,	>61 yrs HCV RNA +ve,	20-40 yrs HCV RNA +ve,	41-60 yrs HCV RNA +ve,	>61 yrs HCV RNA +ve,
Number of patients	Men	46	109	49	21	57	43
	Women	35	52	24	11	41	33
	All	81	161	73	32	98	76
Patients with cirrhosis N (%)	Men	2 (4%)	18 (17%)	9 (18%)	5 (24%)	29 (51%)	30 (70%)
	Women	0 (0%)	3 (6%)	9 (38%)	0 (0%)	12 (29%)	24 (73%)
	All	2 (2%)	21 (13%)	18 (25%)	5 (16%)	41 (42%)	54 (71%)
Percutaneous Liver Biopsy		20-40 yrs Percutaneous liver biopsy	41-60 yrs Percutaneous liver biopsy	>61 yrs Percutaneous liver biopsy	20-40 yrs Percutaneous liver biopsy	41-60 yrs Percutaneous liver biopsy	>61 yrs Percutaneous liver biopsy
Number of patients	Men	30	81	38	9	40	27
	Women	28	45	17	9	30	28
	All	58	126	55	18	70	55
Patients with cirrhosis N (%)	Men	1 (3%)	18 (22%)	6 (16%)	2 (22%)	16 (40%)	21 (77%)
	Women	0 (0%)	1 (2%)	8 (47%)	0 (0%)	7 (23%)	22 (79%)
	All	1 (2%)	6 (5%)	14 (25%)	2 (11%)	23 (33%)	43 (78%)
Transjugular Liver Biopsy		20-40 yrs Transjugular biopsy	41-60 yrs Transjugular biopsy	>61 yrs Transjugular biopsy	20-40 yrs Transjugular biopsy	41-60 yrs Transjugular biopsy	>61 yrs Transjugular biopsy
Number of patients	Men	11	17	4	9	14	9
	Women	4	4	1	2	5	3
	All	15	22	5	11	19	12
Patients with cirrhosis N (%)	Men	1 (9%)	13 (76%)	3 (75%)	3 (33%)	13 (93%)	9 (100%)
	Women	0 (0%)	2 (50%)	1 (100%)	0 (0%)	5 (100%)	2 (67%)
	All	1 (7%)	15 (68%)	4 (80%)	3 (27%)	18 (95%)	12 (100%)
No Liver Biopsy Performed		20-40 yrs	41-60 yrs	>61 yrs	20-40 yrs	41-60 yrs	>61 yrs
Number of patients	Men	6	8	9	2	5	6
	Women	2	5	4	1	4	3
	All	8	13	13	3	9	9
	% not biopsied	10%	8%	18%	9%	9%	12%

Figure 3: Proportion of total HCV infected Asian and Caucasian patients that have had a liver biopsy



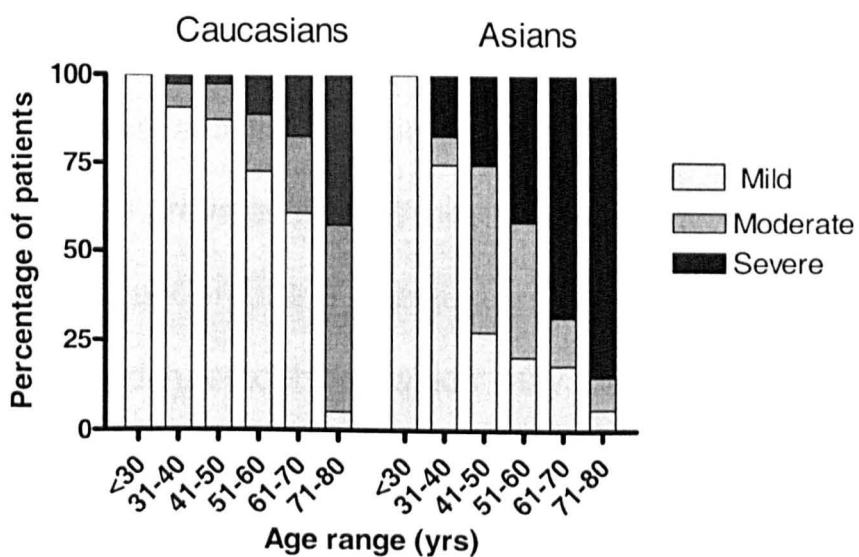
3.12 Disease progression in patients with a single liver biopsy

Table 6 shows the details of the liver biopsy and percentage of patients who had cirrhosis in the entire population (Figure 4). In the Caucasian population cirrhosis was very rare in young patients (20-40); uncommon in middle-aged patients and the prevalence increased in elderly patients. 25% of Caucasians over the age of 61 had cirrhosis. These data are very similar to previous cross sectional studies (Seeff et al. 2002) and are compatible with prospective natural history studies (Seeff et al. 2002) and suggest that our methodology did not lead to significant over-representation of patients with advanced liver disease, as might occur in a tertiary referral center that specializes in managing patients with advanced liver disease. The prevalence of cirrhosis in patients of Asian origin was markedly increased in all age ranges. However the proportion of Asian patients who were HCV RNA positive and underwent liver biopsy (185 from 206) was not significantly different from the proportion of Caucasian patients who underwent liver biopsy (281 from 315 - $p =$ non-significant, Chi Square) suggesting that selection of Asian patients with advanced disease for liver biopsy was not

responsible for the increased prevalence of cirrhosis in this group (Table 5).

Our study revealed that there was a very high prevalence of cirrhosis in elderly Asian patients – 71% of Asian patients over the age of 61 had cirrhosis, compared to 25% of Caucasian patients of a similar age. One reason for the high prevalence of cirrhosis could be due to the number of liver biopsies performed in the different age groups such that elderly patients were less likely to undergo biopsy than younger patients. However, there were no significant difference in the frequency of liver biopsies in young (20-40 yrs : 102 from 113 patients) compared to older patients (>61 years : 127 from 149 patients) ($p= 0.34$, Chi squared test). To determine whether lesser degrees of fibrosis were also present in increased numbers of Asian patients the proportion of patients with moderate fibrosis (fibrosis score of 3,4) and mild fibrosis (fibrosis scores of 1, 2) were analysed. The proportion of patients with moderate fibrosis increased with age whilst patients with mild fibrosis decreased with age (Figure 4).

Figure 4: Prevalence of different stages of fibrosis in Asian and Caucasian patients (Mild =F1, F2; Moderate = F3, F4; Severe = F5, F6)



3.13 Factors associated with cirrhosis

To examine factors associated with cirrhosis and to try and ascertain the mechanism underlying the high prevalence of cirrhosis in Asian patients we studied patients who had undergone a percutaneous liver biopsy and compared Asian and Caucasian subjects using univariate and multivariate analyses. We excluded from these analyses patients who did not undergo liver biopsy (unknown prevalence of cirrhosis) and we also excluded patients who had undergone a transjugular liver biopsy as these patients either had haemophilia (which may be associated with atypical progression of fibrosis) or advanced liver disease (which may introduce an element of referral bias into the analysis). A total of 382 unselected patients who had undergone a percutaneous liver biopsy for chronic HCV infection were studied further.

The median (range) fibrosis score was 2 (0-6). In univariate analyses, there was a significant positive correlation between the score and increased age (correlation coefficient=0.59, $p=0.0001$) and scores were significantly higher in Asians than Caucasians (Asians: 4 (0-6), Caucasians: 1 (0-6), $p=0.0001$, Mann-Whitney U

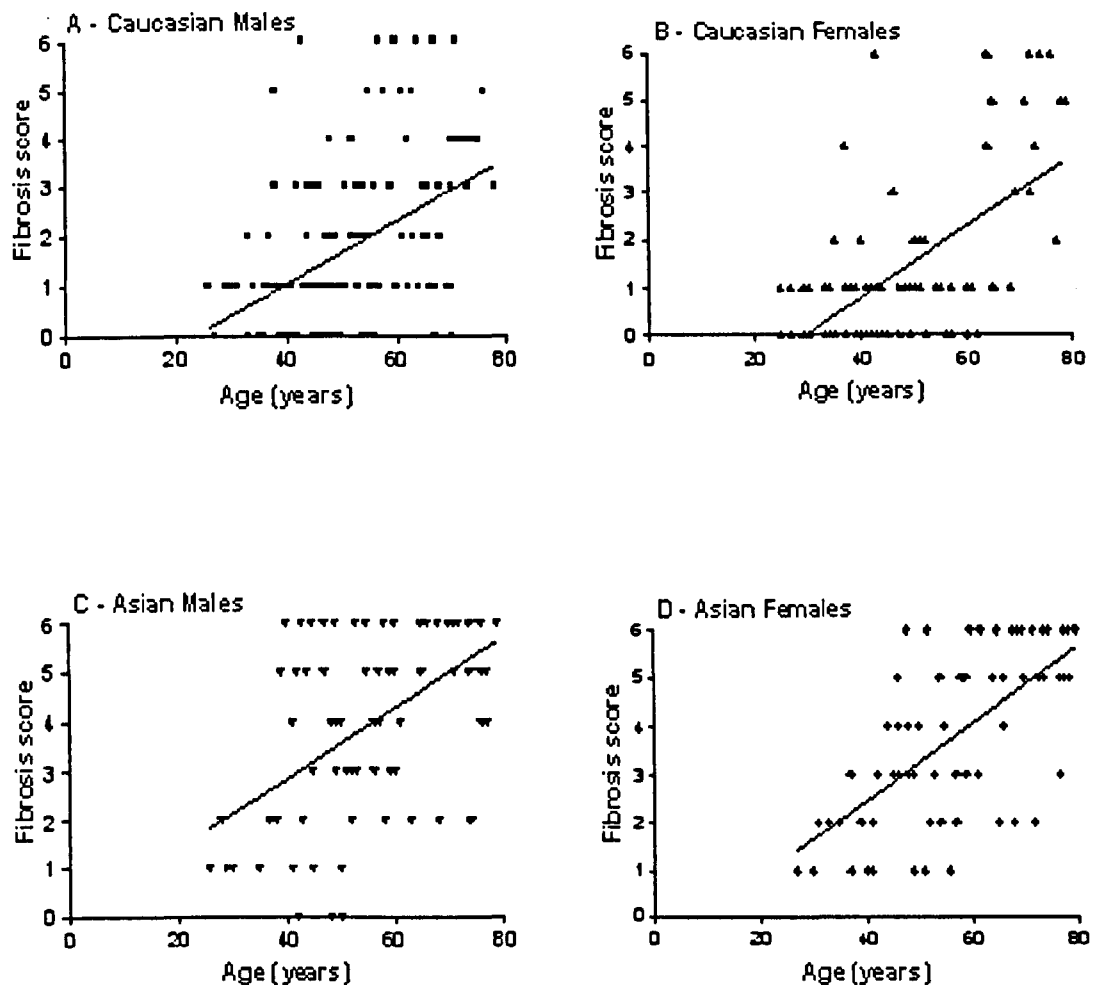
test). There was no significant difference in fibrosis scores between males and females ($p=0.46$) and the majority of female patients were older than 40. Thus unselected, elderly, Asian patients with chronic HCV infection had a high prevalence of cirrhosis. In Asian patients this may be due to either a longer duration of infection or to an increase in fibrosis progression or a combination of these two factors.

3.2 Disease progression in Asian and Caucasian patients

To determine whether fibrosis progression was increased in Asian patients we examined the relationship between fibrosis scores and age separately for the different ethnic and sex groups (Figure 5). Whilst hepatic fibrosis was strongly correlated with age in all four groups, the slopes of the individual regression lines suggested that the relationship between fibrosis progression and age did not differ between those of Asian and Caucasian ethnicity i.e. the disease progressed at similar rates in both Asian and Caucasian patients. However, the points at which these lines crossed the x-axis (may give an indication of the mean age of infection in each group) were lower in patients from Asia than in Caucasians.

Figure 5 - Linear regression analysis of extent of hepatic cirrhosis vs age. Liver biopsies from Asian and Caucasian patients with chronic HCV infection were examined and the fibrosis score (assayed as recommended by Ishak and colleagues) plotted against patient age at biopsy – note that many points represent multiple patients.

Linear regression analysis was performed (PRISM GraphPad) and the regression lines are shown. The gradient of the slopes were similar for all the different groups - Caucasian Males gradient = 0.064, Caucasian females = 0.075, Asian males = 0.071 and Asian females = 0.079 but the intercepts differed – the X axes intercepts were 23.5yrs for Caucasian males, 29.4yrs for Caucasian females, -0.3 yrs for Asian men and 9 yrs for Asian females. For all the analyses the slope of the regression plot was significantly different from zero ($p < 0.0001$).



In Figure 5 liver biopsies from Asian and Caucasian patients with chronic HCV infection were examined and the fibrosis score (assayed as recommended by Ishak and colleagues 1998) plotted against patient age at biopsy – note that many points represent multiple patients.

Linear regression analysis was performed (PRISM GraphPad) and the regression lines are shown. The gradient of the slopes were similar for all the different groups - Caucasian Males gradient = 0.064, Caucasian females = 0.075, Asian males = 0.071 and Asian females = 0.079 but the intercepts differed – the X axes intercepts were 23.5yrs for Caucasian males, 29.4yrs for Caucasian females, -0.3 yrs for Asian men and 9 yrs for Asian females. For all the analyses the slope of the regression plot was significantly different from zero ($p < 0.0001$).

3.21 Fibrosis Severity in Asian and Caucasian patients.

When considering the patients as a single group, simple linear regression models confirmed that there was a significant relationship between fibrosis score and increased age and that Asians had higher fibrosis scores than Caucasians. To further

examine this relationship we performed a multivariable analysis. This showed that both age and ethnicity were significantly associated with the fibrosis score ($p=0.0001$ for each) and confirmed the visual impressions noted above - the mean fibrosis score was 1.81 (95% CI 1.51-2.11) units higher in Asians than in similarly aged Caucasians and the fibrosis score increased by 0.72 (95% CI 0.61-0.82) units for each 10-year increase in age. An interaction term between ethnic group and age was non-significant ($p=0.64$) suggesting that the relationship between the fibrosis score and age was not modified by ethnicity. As in the earlier analyses, neither fibrosis scores themselves ($p=0.22$) nor the relationship with age ($p=0.34$) were modified by gender. Hence, liver fibrosis advanced at a similar rate in both Asian and Caucasian populations, therefore it was postulated that the increase in the degree of fibrosis in elderly Asian patients was due to their being infected for a longer period of time than the Caucasians rather than to an increase in the rate of fibrosis progression in Asian patients.

3.22 Disease progression in paired liver biopsies

To further examine the rate of disease progression in Asian and Caucasian patients we analysed 48 paired liver biopsies from untreated patients whose initial liver biopsy showed mild or moderate disease. These patients all had mild disease which did not reach the UK criteria for the introduction of anti-viral therapy or had declined to receive treatment for their infection. This sub-sample of 48 patients had repeat biopsy samples over a median (range) period of 5 (1-16) years and allowed us to compare rates of fibrosis progression in Asian and Caucasian patients in a prospective manner. The median (range) scores were 0.4 (0-4) at the time of the first biopsy and 0.9 (0-5) at the time of the second. The median (range) time between the two biopsies was 5 (1-16) years and the median (range) rate overall was 0.04 (0-3) per year. Among the 29 Caucasians the median (range) rate was 0.04 (0-2) which did not differ from the rate (0.04, range 0-3) in the 19 Asians ($p=0.45$, Mann-Whitney U test) (Figure 6). Hence prospective studies on fibrosis progression in a small subset of patients also indicated that the rate of disease progression is similar in both Asian and Caucasian patients.

3.3 Factors influencing fibrosis progression

3.31 Viral and host factors

To identify factors that might be responsible for the increased prevalence of cirrhosis in elderly Asians with HCV we studied a number of viral and host factors. Viral genotype was available for 243 patients (187 Caucasians and 56 Asians). Of these 243 patients :-

Genotype 1	97 (39.9%)
Genotype 2	21 (8.6%)
Genotype 3	120 (49.4%)
Genotype 4	4 (1.7%)
Genotype 5	1 (0.4%)

For the purposes of our analysis the genotypes were combined into three groups: genotype 1, genotype 3 and others. Among Caucasians, the numbers (%) with genotype 1, 3 and other were 84 (44.9%), 21 (11.2) and 82 (43.9) respectively, compared to 13 (23.2), 38 (67.9) and 5 (8.9), respectively among Asians ($p=0.006$, Chi-squared test). In multivariable analyses, after controlling for ethnicity and age, genotype 3 was associated with a significantly lower score than genotype 1 (mean fibrosis score was 0.48 (0.07 - 0.88) lower than those with genotype 1, $p=0.02$). Other

genotypes were not associated with any change in the score ($p=0.77$). After controlling for the different distribution of genotypes in the two populations, both age and ethnicity remained significantly associated with the fibrosis score. The age effect remained similar to that reported in our earlier analysis, whereas the ethnicity effect was, if anything, stronger (with Asians having a score that was 2.36 (1.91-2.81) higher than that of similarly-aged Caucasians infected with the same HCV genotype ($p=0.0001$)).

3.32 Alcohol

Information on alcohol consumption was available for 336 patients (207 Caucasians, 129 Asians) and is shown in Table 6. In multivariable analyses, heavy consumption (>14 units per week for females and >21 units per week for males) was associated with an increase in the fibrosis score (increase in mean fibrosis score of 0.66 (95% CI 0.23-1.09), $p=0.003$). However, neither the relationship between fibrosis score and age, nor that between fibrosis score and ethnicity were moderated after adjusting for alcohol consumption.

3.33 Acquisition of HCV

Elderly Asian patients have a high prevalence of cirrhosis that cannot be accounted for by an increase in the rate of fibrosis progression. Multivariable analyses of factors known to be associated with rapidly progressive fibrosis did not identify any variable that could account for the increased prevalence of cirrhosis in elderly Asians. However extrapolation of the linear regression analysis (Figure 5) suggested that Asian patients were infected at a younger age than Caucasian patients and suggests that the increase in elderly Asian patients with HCV induced cirrhosis may be due to a longer duration of infection in these patients. To confirm that Asian patients were infected at an earlier age than Caucasians, we studied the route and probable time of infection in these patients (Table 7).

The majority of Caucasian patients acquired HCV infection in adult life from blood transfusions or drug use. In contrast, Asian patients were commonly infected by blood transfusion before the age of 20 (91 patients) or had no obvious source of infection.

Among the Asian patients with no obvious route of infection, 14 out of 24 had been born or lived in Asia during childhood.

Although the precise age of infection is unknown for the majority

of our Asian patients most (105/134, 78.4%) had a single risk factor for infection in childhood compared to only 13 Caucasian patients (13/236, 5.5%) who were at risk of infection before the age of 20. Thus the available evidence indicates that Asian patients were infected in childhood and that elderly Asian patients had been infected with HCV for many decades. Taken together these data indicate that life long infection with HCV leads to cirrhosis in a large proportion of those who are infected.

TABLE 7 - Mode of acquisition and alcohol consumption in Caucasian and Asian patients. The mode of acquisition and alcohol intake at the time of the liver biopsy were identified from a study of the patients records. Alcohol consumption was scored as nil/moderate if less than 14 units per week in a female (21 units for a man) and excessive if greater than these amounts.

	Total N (% of total)	Caucasian N (% of Caucasians)	Asian N (% of Asians)
Mode of Acquisition of HCV			
Number studied	382	239	143
Illicit drug use in adults	156 (40.8)	148 (61.9)	8 (5.6)
Blood product before 20 yrs old	104 (27.2)	13 (5.4)	91 (63.6)
Blood product after 20 yrs old	72 (18.8)	54 (22.6)	18 (12.6)

Sexual/occupational	5 (1.3)	3 (1.3)	2 (1.4)
Unknown	45 (11.8)	21 (8.8)	24 (16.8)
Alcohol Consumption			
Number with available information	336	207	129
Nil/moderate	287 (85.4)	166 (80.2)	121 (93.8)
Excessive	49 (14.6)	41 (19.8)	8 (6.2)

3.34 Steatosis, obesity and BMI

Single Biopsies:-

Patients from Asia have a high prevalence of diabetes and obesity. One possible explanation for the high prevalence of HCV related cirrhosis in Asian patients is that they may have non-alcoholic steatohepatitis (NASH) in addition to their HCV. If this were the case, then the interaction between NASH and HCV may lead to accelerated disease. Information on steatosis was available for 382 patients (160 grade 0; 177 grade 1; 36 grade 2; 9 grade 3). Multivariable analyses revealed no independent relationship between steatosis and fibrosis score, however Asian ethnicity remained significantly associated with a higher fibrosis score ($p < 0.0001$). Information on body mass index (BMI) was available for 357 patients (median 28.3, range 20.2-41.5 kg/m²). As expected, BMIs were significantly lower in Caucasians

(n=222, 27.2 (20.2-39.6) kg/m²) compared to Asians (n=135, 29.4 (24.0-41.5) kg/m², p=0.0001, Mann-Whitney U test) and there was a significant correlation between BMI and the fibrosis score (Spearman's correlation coefficient = 0.20, p=0.0002). However, after controlling for ethnicity and age in a multivariable analysis, BMI did not remain significantly associated with the fibrosis score (p=0.27), and adjusting for BMI did not modify the relationship between the fibrosis score and either ethnicity or age.

Paired Liver biopsies:- the Role of Steatosis

The characteristics of the 48 HCV-infected patients who had paired biopsies is shown in Table 8. This comprised 28 Caucasians and 20 Asians. Steatosis was present in 81% of the paired biopsies.

There appears to be a moderate positive correlation between the rate of fibrosis progression and the change in steatosis between the two biopsies (Table 9). The rate of fibrosis progression was more rapid in males than females (p=0.002) and in those with higher levels of steatosis in their first biopsy (p =0.004). Moderate and severe steatosis in paired liver biopsies is a major risk factor for fibrosis progression as compared to mild steatosis.

Table 8: Characteristics of patients included in study

Number of patients		48	(100.0)
Genotype:	1	23	(46.9)
	2	7	(14.3)
	3	19	(38.8)
Median (range) stage at biopsy 1		0.25	(0 to 4)
Median (range) stage at biopsy 2		0.75	(0 to 5)
Median (range) years between paired biopsies		5	(1 to 16)
Median (range) rate of fibrosis progression/year		0.04	(0 to 3)
Steatosis:	0	9	(18.4)
	1	23	(46.9)
	2	13	(26.5)
	3	4	(8.2)
Ethnicity:	White	28	(59.2)
	Asian	20	(40.8)
Sex:	Male	25	(51.0)
	Female	23	(49.0)
Median (range) BMI		25	(23 to 33)
Median (range) change in grade		0.5	(-4 to +4)
Median (range) age (years)		51	(30 to 68)

Unfortunately, the small number of patients in the study, and the rather non-Normal distribution of the rates of fibrosis progression, mean that it is not possible to run multivariable analyses to assess whether any of these factors are independently associated with fibrosis progression.

Table 9: Associations between rate of fibrosis progression and other categorical measurements

		Median	Range	p-value
Steatosis:	0	0	(0 to 2)	
	1	0	(0 to 2)	
	2	0.09	(0 to 3)	
	3	0.1	(0.02 to 1)	0.02
Steatosis (combined groups):	None/Mild	0	(0 to 2)	
	Moderate/Severe	0.1	(0 to 3)	0.004
Genotype:	1	0.06	(0 to 3)	
	2	0	(0 to 0.05)	
	3	0.02	(0 to 2)	0.13
Ethnicity:	White	0.04	(0 to 2)	
	Asian	0.03	(0 to 3)	0.56
Sex:	Male	0.1	(0 to 3)	
	Female	0	(0 to 1)	0.002

3.36 Influence of Iron on Fibrosis Progression and Causation of Diabetes Mellitus in a Population with Chronic Hepatitis C.

339 HCV patients in total were included in the study (Table 10). The median age of the patients was 50 years (range 25-79) and 56% were male. Of the 339 patients, 131 (38.6%) had mild fibrosis, 68 (20.1%) severe fibrosis and 87 (25.7%) cirrhosis. 215 out of 339 HCV patients had genotype data available of which 87 were genotype 1, 24 genotype 2, 99 genotype 3, 4 genotype 4 and 1 genotype 5. HFE genotyping was performed on seventy-two patients of whom 68 were negative and four heterozygotes.

Serum ferritin levels ranged from 3 to 782 (median 86) ug/L (Table 11). Ferritin levels were significantly different among the fibrosis stages ($p= 0.004$). This can be explained largely as a consequence of lower values in individuals with severe fibrosis and cirrhosis (Table 11), as ferritin levels in those with no fibrosis and mild fibrosis were similar. Caucasians had significantly higher ferritin levels (median 139, range [13-782]) than Asians (71 [3-782], $p=0.0001$). Males also had significantly higher ferritin levels (172 [18-782]) as compared to females (61 [3-365], $p=0.0001$). The proportions of males (ferritin levels > 300 ug/l)

and females (ferritin levels > 200 ug/l) were not significantly different (6.3% of males and 2.0% of females, $p = 0.10$, Chi-squared test). When stratified by sex, the relationship between stage of fibrosis and ferritin levels was only significant in men ($p=0.0001$, Table 11). There was no significant relationship between ferritin levels and BMI.

Hepatic iron deposition was assessed on the basis of a positive iron stain on liver biopsy using Perls' Prussian blue. This was graded by the histopathologist on a scale from 0 to 4 (Searle et al. 1994). 28 patients (8.3%) had grade 1 (minimal deposition of iron), 5 patients (1.5%) had grade 2 and 4 patients (1.2%) had grade 3 positive staining (intermediate deposition). This resulted in 37 (10.9%) patients having some level of iron deposition. No patients had massive deposition of iron (grade 4). Iron deposition was more common in men ($n=33$; 17.3%) than women (4; 2.7%, $p=0.0001$), and in Caucasians (30; 15.0%) than Asians (7; 5.0%, $p=0.006$). No relationship was found between the presence of iron deposition and either age ($p=0.73$), BMI ($p=0.11$) or the stage of fibrosis ($p=0.44$). Iron deposition was more common, however, in those with a high ferritin. All 15 (100.0%) patients with a high

ferritin level had iron deposition compared to only 22 (6.8%) of those with lower ferritin levels ($p=0.0001$, Fisher's exact test).

Univariable analyses revealed that lower alcohol consumption ($p=0.0001$), Asian ethnicity ($p=0.0001$), older age ($p=0.0001$) and higher BMI ($p=0.007$) were all associated with an increased fibrosis stage. However, in multivariable linear regression analyses, only Asian ethnicity and age remained significantly associated with an advanced stages of fibrosis. For example, those of Asian ethnicity had a mean fibrosis score that was 1.72 (95% confidence interval: 1.40 to 2.04) units higher, on average, than that seen in Caucasians. A 10-year increase in age was associated with an average increase in fibrosis score of 0.75 (95% confidence interval: 0.63 to 0.86). Once these two variables were controlled, there were no significant associations between the stage of fibrosis and either ferritin levels or iron deposition. When stratifying for sex, multivariable analyses gave similar results with the exception that a diagnosis of diabetes was independently associated with a worse stage of fibrosis in women (effect on mean score: 0.82, 95% confidence interval: 0.22 to 1.43, $p=0.009$) but not in men (-0.03, -0.54 to 0.47, $p=0.89$).

Sixty-four patients (19.2%) had a diagnosis of diabetes mellitus or impaired glucose tolerance. The number of patients with a diagnosis of diabetes mellitus or impaired glucose tolerance did not vary by stage of fibrosis (11.3%, 12.2%, 11.8% and 8.1% of those with no fibrosis, mild fibrosis, severe fibrosis and cirrhosis respectively, $p=0.75$, Chi-squared test) and was not associated with the presence of iron deposition (25; 9.3% of those without diabetes had iron deposition compared to 11; 17.2% of those with diabetes, $p=0.11$). However, ferritin levels were found to be significantly higher in those with diabetes (141.5 [13-514] ug/L) compared to those without diabetes (83 [3-782] ug/L, $p=0.02$, Mann-Whitney U test).

Table 10: Characteristics of patients included in study

Number of patients	339	(100.0)
Male: n (%)	191	(56.3)
Ethnicity: n (%)		
Caucasian	200	(59.0)
Asian	139	(41.0)
Age (years): median (range)	50	(25-79)
Fibrosis stage: Median (range)	2	(0-6)
No fibrosis (stage 0)	53	(15.6)
Mild fibrosis (stage 1-2)	131	(38.6)
Severe fibrosis (stage 3-4)	68	(20.1)
Cirrhosis (stage 5-6)	87	(25.7)
BMI (kg/m²): median (range) (n=311)	28	(22-40)
Alcohol consumption*:		
None	104	(37.8)
Mild/moderate	138	(50.2)
High	33	(12.0)
Diabetes mellitus/impaired glucose tolerance*:	64	(19.2)

BMI, Body Mass Index

* Information on alcohol consumption available for 275 patients; information on diabetes/impaired glucose tolerance available for 334 patients.

	All patients			Men			Women		
	N	Median (range)	n (>160 ug/L) (%)	N	Median (range)	n (>160 ug/L) (%)	N	Median (range)	n (>160 ug/L) (%)
All patients	339	86 (3-782)	110 (32.5)	191	172 (89.5)	106 (55.5)	148	61 (3-365)	4 (2.7)
Fibrosis stage									
No fibrosis	53	113 (13-646)	20 (37.7)	29	189 (87-646)	20 (69.0)	24	53 (13-86)	0 (-)
Mild fibrosis	131	112 (13-782)	47 (35.9)	72	185 (19-782)	45 (62.5)	59	65 (5-365)	2 (3.4)
Severe fibrosis	68	94.5 (9-611)	25 (36.8)	41	183 (18-611)	25 (61.0)	27	61 (9-132)	0 (-)
Cirrhosis	87	71 (3-432)	18 (20.7)	49	84 (21-432)	16 (32.7)	38	53 (3-317)	2 (5.3)
p-value*		0.004	0.06		0.0001	0.002		0.27	0.69

Table 10: Serum ferritin levels (ug/L) overall and stratified by stage of fibrosis and sex

* p-value obtained from Mann-Whitney U test for comparison of medians and Chi-squared test for comparison of proportions

3.37 The Role of Diabetes Mellitus in Fibrosis Progression

In the original main study we determined whether diabetes mellitus was a pre-requisite for the development of cirrhosis in Asian patients. We examined the proportion of Asian patients who were known to have diabetes or who had had investigations at the time of the liver biopsy to exclude it (fasting glucose assessment). All 143 Asian patients were assessed of whom 34 (23.8 %) had diabetes mellitus. The prevalence of diabetes in patients with mild fibrosis (stage 1-4, 21 from 75 had cirrhosis) was not significantly different from the prevalence in Asian patients with moderate/severe fibrosis (fibrosis score 5-6 – 13 from 68 had cirrhosis), $p =$ non-significant, Chi square, suggesting that co-existing diabetes mellitus is not responsible for the high prevalence of cirrhosis in elderly Asian patients.

3.38 Clinical Significance of Insulin Resistance in Fibrosis Progression in Hepatitis C Virus Infection.

Recently, there is increasing evidence that insulin resistance can contribute to fibrosis progression in chronic hepatitis C infection

(Hui et al. 2003; Furantani et al. 2003). With this in mind we sought ethical approval for a follow up study to assess insulin resistance on a population of HCV–infected patients. All these patients were enrolled in the study if they had a liver biopsy within the last six months and were eligible for antiviral therapy. Some of these patients were from the previous study but the majority of patients were newly referred patients to the department.

RESULTS

Anthropometric, biochemical and histological findings by ethnicity

The median age of the 59 patients was 47 (range 34-60) years; 51 (83.6%) of them were male, and the median BMI was 26 (23-30) kg/m² (Table 12).

30 Caucasian and 31 Asian patients were entered into this study. None of the following factors such as sex, age, BMI, alcohol consumption, liver function tests, fibrosis stage, portal and lobular scores nor grade of steatosis differed significantly between the two groups (Table 12). Equal numbers of Asian patients were infected with HCV genotype 1/2 and 3, whilst Caucasian patients

were more likely to be infected with genotype 1/2 ($p=0.04$). Both fasting insulin and insulin resistance as measured using the HOMA model were significantly higher in Asians than in Caucasians ($p=0.007$ and $p=0.004$ respectively). Asians also had higher glycosylated haemoglobin levels than Caucasians ($p=0.03$). The characteristics of those infected with genotype 1/2 or genotype 3 were similar (data not shown).

Table 12: Characteristics of 59 patients included in study, overall and stratified by ethnicity. Table entries are n (%) or median (range), as appropriate.

	All patients	Caucasians	Asians	P-value*
No. of patients+	59 (100.0)	30 (100.0)	29 (100.0)	
Male sex	49 (83.1)	25 (83.3)	24 (82.8)	1.00
Age (years)	47 (34-60)	47.5 (34-59)	47 (36-60)	0.70
BMI (kg/m ²)	26 (23-30)	26 (23-30)	26 (25-30)	0.27
HbA1c	5.3 (4.0-8.0)	5.1 (4.0-6.2)	5.7 (4.0-8.0)	0.03
AST (IU/l)	37 (18-124)	37 (19-99)	38 (18-124)	0.73
ALT (IU/l)	47 (15-213)	41.5 (15-213)	52 (16-146)	0.33
AST:ALT ratio	0.88 (0.45-2.54)	0.88 (0.45-2.54)	0.87 (0.49-1.69)	0.34
Fasting insulin (μ mol/l)	12.4 (4.2-19.7)	9.6 (4.2-19.7)	15.9 (7.9-19.2)	0.004
Fasting glucose (mmol/l)	5.6 (4.3-7.2)	5.6 (4.3-7.2)	5.9 (4.6-7.2)	0.31
HOMA-IR	2.91 (0.98-5.83)	2.58 (0.98-5.19)	3.67 (1.90-5.83)	0.004
HCV genotype				
1-2	37 (62.7)	23 (76.7)	14 (48.3)	
3	22 (37.3)	7 (23.3)	15 (51.7)	0.02
Fibrosis stage				
1	9 (15.3)	5 (16.7)	4 (13.81)	
2	15 (25.4)	7 (23.3)	8 (27.6)	
3	14 (23.7)	8 (26.7)	6 (20.7)	
4	13 (22.0)	7 (23.3)	6 (20.7)	
5	5 (8.5)	2 (6.7)	3 (10.3)	
6	3 (5.1)	1 (3.3)	2 (6.9)	0.73
Portal score				
1	44 (77.2)	23 (79.3)	21 (75.0)	
2	13 (22.8)	6 (20.7)	7 (25.0)	0.94
Lobular score				
1	21 (84.0)	13 (86.7)	8 (80.0)	
2	4 (16.0)	2 (13.3)	2 (20.0)	1.00
Steatosis				
None	10 (17.5)	6 (20.7)	4 (14.3)	
Mild	36 (63.2)	17 (58.6)	19 (67.9)	
Moderate	11 (19.3)	6 (20.7)	5 (17.9)	0.75
Alcohol				
None	29 (50.0)	14 (46.7)	15 (53.6)	
Mild/ Moderate	13 (22.4)	6 (20.0)	7 (25.0)	
High	16 (27.6)	10 (33.3)	6 (21.4)	0.59

* P-values obtained from Mann-Whitney U test (numerical variables) or Chi-squared test (categorical variables). Fibrosis score treated as a numerical variable for the purpose of calculating p-values.

+ Missing values: HbA1c (n=14), portal score (n=2), lobular score (n=34), steatosis (n=2), alcohol consumption (n=1)

Insulin Resistance (HOMA-IR) and Anthropometric, Biochemical and Histological Findings

There was a strong positive correlation between the patient's BMI and the value of HOMA-IR (Spearman's $r = 0.53$; $p=0.001$). On stratifying by ethnicity, this correlation was slightly stronger in Caucasian (0.58 ; $p=0.007$) than in Asian (0.43 ; $p=0.01$) patients. In addition, the level of HOMA-IR was positively correlated with fasting insulin (Spearman's $r=0.93$; $p=0.005$), glucose (0.36 ; $p=0.005$) levels, HbA1c (0.30 ; $p=0.05$), AST (0.31 ; $p=0.01$) and ALT (0.34 ; $p=0.007$). HOMA-IR levels were also higher in those with high (median 3.52 ; range $0.98-5.83$) alcohol consumption, compared to those with mild/moderate (2.45 ; $1.10-5.51$) or no (2.87 ; $1.25-5.16$) alcohol consumption ($p=0.04$, Kruskal-Wallis test). In addition, HOMA-IR levels were higher in those with portal scores of 2 (4.13 ; $2.30-5.23$) compared to those with scores of 1 (2.81 ; $0.98-5.83$, $p=0.03$). There were no significant relationships seen between HOMA-IR and age, sex, HCV genotype, lobular score, steatosis grade or the AST:ALT ratio.

Fibrosis and Anthropometric, Biochemical and Histological Findings.

In univariable analyses the following factors such as age, BMI, fasting insulin and glucose, HOMA-IR, glycosylated haemoglobin, AST and ALT were all positively correlated with fibrosis stage (Table 13). If cirrhotic patients (fibrosis stage 5 or 6) were excluded from the analysis this did not change the association between HOMA-IR and fibrosis stage (Spearman's correlation after excluding these patients: 0.69; $p=0.001$). No significant relationships were seen between the fibrosis stage and either sex, HCV genotype, steatosis, alcohol consumption, fasting glucose or the AST:ALT ratio. In multivariable analyses, HOMA-IR was still significantly associated with fibrosis stage, even after adjusting for all other variables associated with fibrosis stage in univariable analyses (age, fasting glucose, HbA1c, AST, ALT, BMI and high alcohol consumption). In this multivariable model, a 1-unit increase in HOMA-IR was associated with an increase in the estimated fibrosis score of 0.85 (95% confidence interval: 0.58 to 1.12; $p=0.001$). In this multivariable model, older age was the only other factor found to be independently associated with a worse fibrosis stage.

Table 13: Univariable relationships between each variable and fibrosis score on liver biopsy

		Median (range) fibrosis score or Spearman's correlation coefficient	p-value*
Sex:	3	3 (1-6)	
	Female	3 (1-4)	0.28
HCV Genotype:	1-2	3 (1-6)	
	3	3 (1-6)	0.99
Steatosis:	None	3 (1-5)	
	Mild	3 (1-6)	
	Moderate	4 (2-6)	0.09
Alcohol:			
	None	3 (1-5)	
	Mild/moderate	3 (1-5)	
	High	4 (1-6)	0.16
Spearman's rank correlation between fibrosis score and:			
	Age	0.42	0.0009
	Fasting insulin	0.73	0.0001
	Fasting glucose	0.20	0.13
	HOMA-IR	0.75	0.0001
	HbA1c	0.31	0.04
	AST	0.30	0.02
	ALT	0.25	0.06
	AST:ALT ratio	-0.09	0.50
	BMI	0.39	0.003

* P-value obtained from Mann-Whitney U test or from a test of the correlation coefficient

3.4 Clinical Significance of Insulin Resistance in Response to Antiviral Therapy.

Insulin resistance precedes diabetes mellitus by 10-20 years. In the second part of this study we assessed whether insulin resistance plays a part in determining which patients respond to antiviral therapy.

Results

We assessed the same group of 59 patients previously assessed for insulin resistance in the small pilot study on fibrosis progression. Their characteristics are described in Table 12. Although all patients started treatment, seven (11.5%) did not tolerate the treatment and withdrew from treatment during the first 12 weeks .

Factors associated with Sustained Virological Response

Overall, 52 patients completed a full course of antiviral treatment. Of these 29 (55.8%) experienced a sustained virological response. In univariable analyses (Table 14), patients with a sustained virological response were less likely to be male ($p=0.03$) and had lower fasting insulin ($p=0.01$),

fasting glucose ($p=0.0007$), HOMA-IR ($p=0.0009$), AST ($p=0.01$), ALT ($p=0.003$) levels but higher AST:ALT ratios ($p=0.004$). Also those with a sustained virological response tended to have lower BMIs ($p=0.005$) and more likely to be infected HCV genotype 3 ($p=0.05$) compared to those patients without a sustained virological response. In multivariate regression analyses (Table 14), three different models gave a very similar fit to the data. In each model, HCV genotype 3 was found to be independently associated with an increased likelihood of sustained viral response (with odds ratios ranging from 9.9 to 29.2 depending on which model was chosen). In contrast, higher fasting glucose levels and higher AST levels were both independently associated with a poorer virological response to treatment. After adjusting for these three variables, Asian ethnicity, higher fasting insulin levels and higher HOMA-IR levels were all independently associated with a poorer virological response to treatment. However, due to the strong correlations between these variables, it was not possible to tease out the independent effects of these three variables.

There was a strong relationship between the sustained virological response and HCV genotype 3. The analyses was

therefore repeated after excluding those patients with genotype 3. Although the power to consider factors associated with sustained virological response was much reduced in this subgroup, the association between fasting glucose, Asian ethnicity, fasting insulin and HOMA-IR levels and sustained virological response still remained significant.

Table 14: Results from univariable analyses of factors associated with sustained virological response to antiviral treatment (n=52)

		No response	Response	p-value*
No. of patients		23 (100.0)	29 (100.0)	
Age (years)		46 (36-55)	48 (34-59)	0.14
Sex:	Male	22 (95.7)	21 (72.4)	
	Female	1 (4.4)	8 (27.6)	0.03
Ethnicity:	White	8 (34.8)	19 (65.5)	
	Asian	15 (65.2)	10 (34.5)	0.05
BMI (kg/m ²)		27 (25-30)	26 (23-29)	0.005
Fasting insulin (umol/l)		15.2 (6.5-19.2)	10.4 (4.2-18.8)	0.01
Fasting glucose (mmol/l)		6.4 (5.2-7.2)	5.4 (4.3-7.2)	0.0007
HOMA-IR (units)		4.25 (2.05-5.46)	2.45 (0.98-5.83)	0.0009
HbA1c (units)		5.2 (4.0-7.0)	5.3 (4.0-8.0)	0.55
AST (IU/l)		45 (18-100)	32 (19-115)	0.01
ALT (IU/l)		58 (16-213)	31 (15-146)	0.003
AST:ALT ratio		0.81 (0.45-1.13)	0.92 (0.49-2.54)	0.004
HCV genotype:	1-2	18 (78.3)	14 (48.3)	
	3	5 (21.7)	15 (51.7)	0.05
Fibrosis score		3 (1-6)	3 (1-6)	0.40
LB portal:	1	18 (78.3)	23 (82.1)	
	2	5 (21.7)	5 (17.9)	0.74
LB lobular:	1	7 (77.8)	10 (83.3)	
	2	2 (22.2)	2 (16.7)	1.00
Steatosis:	None	4 (17.4)	5 (17.2)	
	Mild	14 (60.9)	18 (64.3)	
	Moderate	5 (21.7)	5 (17.2)	1.00
Alcohol:	None	9 (40.9)	16 (55.2)	
	Mild/ Moderate	4 (18.2)	8 (27.6)	
	High	9 (40.9)	5 (17.2)	0.17

* p-value obtained from Mann-Whitney U or Kruskal-Wallis tests (numerical variables) or Chi-squared test (categorical variables) as appropriate.

Table 15: Factors independently associated with treatment response from multivariable logistic regression analysis.

	Odds ratio	95% CI	p-value
Model 1			
HCV genotype 3	29.24	2.80-305.11	0.005
Fasting glucose (per mmol/l higher)	0.10	0.02-0.47	0.003
Asian ethnicity	0.05	0.006-0.43	0.006
AST (per IU/l higher)	0.96	0.93-1.00	0.02
Model 2			
HCV genotype 3	9.91	1.34-73.12	0.02
Fasting glucose (per mmol/l higher)	0.12	0.03-0.46	0.002
Fasting insulin (per μ mol/l higher)	0.79	0.65-0.96	0.02
AST (per IU/l higher)	0.96	0.93-1.00	0.04
Model 3			
HCV genotype 3	10.16	1.37-75.13	0.02
Fasting glucose (per mmol/l higher)	0.19	0.06-0.67	0.009
HOMA-IR (per unit higher)	0.44	0.22-0.88	0.02
AST (per IU/l higher)	0.96	0.93-1.00	0.05

SUMMARY

In this chapter I have explored the natural history of hepatitis C in the North-East of London. Studies on prolonged (> 30 years) follow-up of chronically infected patients have not been performed and will not be performed in the future due to universal use of antiviral therapy. We had a unique opportunity to study an untreated elderly population of first and second generation Bengali and Pakistani patients. These patients had been infected with HCV at a young age whilst living in their country of origin. We were able to carry out a prolonged (>30

years) follow up of chronically infected patients. Fibrosis progression measured in several hundred single biopsies and in nearly 50 paired liver biopsies was the same in Asian and Caucasian patients. We found the longer the exposure to hepatitis C virus the greater the degree of fibrosis progression. We feel that as fibrosis progression was the same in Asians as Caucasians that the same rates of fibrosis progression would be expected in Caucasians after the same duration after infection.

We confirm that factors such as age and alcohol excess as reported by Poynard et al. (1997) are associated with faster fibrosis progression. There was no association between fibrosis stage and sex, which may be due to the large majority of postmenopausal women in the cohort. Despite the small sample of paired biopsies moderate to severe steatosis was associated with rapid fibrosis progression. Insulin resistance was associated with fibrosis progression, however serum ferritin and hepatic iron played no role.

CHAPTER 4: RESULTS - Management Of Chronic Hepatitis C Among East London Primary Care Physicians.

4.1 Knowledge of Chronic Hepatitis C among East London Primary Care Physicians following the Department of Health's Educational Campaign

Chronic hepatitis C is an important public health problem with the development in time of advanced liver disease in a large proportion of those who are infected. General practitioners are often the first health care professionals to see infected patients and it is paramount that they are able to investigate, diagnose and refer these patients for treatment. We report our findings on the effectiveness of the Department of Health Hepatitis C information campaign amongst East London GPs ten months following the campaign. Because of the pivotal role of GPs in the identification and screening of patients at risk we have specifically asked about risk factors

and indications for hepatitis C testing in our questionnaire.

RESULTS

FACE-TO-FACE INTERVIEWS

Ten GPs were randomly selected for face-to-face open-ended interviews from the cohort of GPs from the East End of London. They had previously been invited to complete the questionnaire. The selected GPs were then telephoned and asked if they were willing to participate. Only six agreed and a further four were randomly selected. These latter four consented to be interviewed. GPs were interviewed in their surgery after confirming a time and date of the interview on the telephone. The four practitioners who decided not to take part declined because of time constraints or understaffing in their practices. In the interviews we discussed opinions and beliefs about hepatitis C, including transmission, screening, treatment, and ways of improving services. The general practitioners were encouraged to speak freely, raise issues important to them and give examples from their clinical practice.

Each general practitioner saw an average of 4.2 hepatitis C infected patients per year (range 0-15). All ten GPs were aware that infection was common in drug users and could be prevented by meticulous hygiene. Three practices had access to needle exchange facilities. However, four practices were unaware of these facilities and three did not refer patients as they believed it encouraged them to abuse drugs. Therefore only 30% actively facilitated access to needle exchange programmes. All ten GPs were aware of local drug services and nine believed that they were effective.

Although our entire panel recognized that drug use was a major route of transmission, knowledge of other modes of transmission were limited. The majority (90%) believed that materno-fetal transmission was common (in fact transmission occurs in 5% (Zanetti et al. 1995)) and pregnant women were advised wrongly that there was a 50:50 chance of transmission to their baby. All of our GPs told breast feeding mothers not to breast feed due to the risk of HCV transmission. This is wrong, as is the view of six practitioners who believed that blood transfusion until the end of the 1990s carried a high risk of transmitting the virus.

Diagnosis and screening of hepatitis C were reviewed in the practitioners surgery. Two general practitioners believed that the presence of antibodies against hepatitis C indicated that patients had eradicated the virus and had immunity from further infection. The majority of primary care physicians (90%) did not screen active intravenous drug users until they believed that they were "clean". In their opinion if the first test was negative, unreformed drug addicts remained at high risk of re-infection and therefore screening was futile. They claimed that, in their experience 'these patients' (i.e those currently using intravenous drugs) consistently do not turn up for their appointments and are not compliant with treatment. They strongly felt that these patients should take responsibility for their own health and show a commitment to being 'clean' prior to seeking testing or treatment. Two (20%) general practitioners do not screen patients at high risk for hepatitis C as resources in their practice are poor. Two doctors (20%) indicated that all homosexuals should be screened. All of our panel of GPs wanted routine screening of all pregnant women for HCV introduced.

All of our panel of GPs would welcome open access clinics manned by hospital staff as they had insufficient time to counsel patients prior to hepatitis C testing. In their opinion antibody positive patients should be followed-up by hospitals and should be referred directly by the virology department. Only two of the ten general practitioners routinely vaccinate hepatitis C infected patients for hepatitis A and B and all advise their infected patients to cease all alcohol intake as this worsens progression of liver disease.

Knowledge about treatments for hepatitis C was limited, and this probably reflects the rapidity of progress in this field. GPs were unaware of the availability of pegylated interferon (which is now the treatment of choice for HCV-infected patients (Manns et al. 2001; Fried et al. 2002)) and only 3 were aware of the value of combination therapy (ribavirin and interferon). Five general practitioners believed that chronic hepatitis C should be treated with standard interferon monotherapy, and two suggested combination treatment with lamivudine and interferon (lamivudine is recommended for therapy for HIV and hepatitis B). Three (30%) primary care physicians agreed that treatment is effective in more than 50% of patients and four,

who recommended therapy with interferon and ribavirin, were aware of the main side effects of this combination therapy. Three GPs selected patients for referral based on their assessment of their fitness for antiviral therapy.

Knowledge of the complications of hepatitis C were discussed - 60% of practitioners believed that 50% of patients develop cirrhosis from their HCV in 20 years (current opinion suggests that 20% develop cirrhosis after 20 years (Poynard et al. 1997)) and 20 % only refer patients with cirrhosis who have decompensated liver disease for treatment (i.e. patients with ascites, encephalopathy or variceal bleeding). The primary care physician followed up patients with compensated cirrhosis but none of these doctors screened these patients for hepatocellular carcinoma by either ultrasound or alpha-fetoprotein evaluation. Our practitioners believed that the Department of Health booklet was good to refer to, but was too detailed and long. A short checklist with bullet points would have been much better. More investment was promised in the document but is still awaited.

Primary care physicians were of the view that more should be done to improve education in hepatology. Their suggestions

comprised hepatitis C workshops and liver clinics in primary care which they felt should be set up by hepatologists. There should be training of primary care physicians with a specialist interest in hepatology. General practitioners complained that they are often not involved in liver meetings and wish to have more involvement. They also saw a need for more public education and were concerned that ethnic minorities were particularly poorly informed about hepatitis C.

POSTAL QUESTIONNAIRE

The pilot study was performed in the South-East of London and comprised 250 GPs who were randomly selected from 750 GPs. The main study selected 600 GPs from a total of 650 GPs in the North East of London.

The questionnaire is shown below (Table 16). It was posted to 850 GPs in total with 482 responses. This comprised one hundred and forty one out of 250 general practitioners in South East London (56%) and 341 of 600 (59%) practitioners from North East London completed the questionnaire. There

were no clear differences between the two groups and Table 16 summarises the responses.

Table 16 GP Questionnaire

1.	Patients who are hepatitis C antibody positive no longer have active disease	Yes	No
2.	Hepatitis C is common (> 40 %) in those who have ever used intravenous drugs	Yes	No
3.	The prevalence of HIV is higher than hepatitis C in the East End	Yes	No
4.	More than 50 % of pregnant women infect their children	Yes	No
5.	Blood transfusion in the 1990s carries a high risk of transmitting hepatitis C	Yes	No
6.	In patients using intravenous drugs, transmission of hepatitis C can be prevented by meticulous hygiene and non-sharing of all paraphernalia	Yes	No

7. Therapy for hepatitis C is effective in more than 50% of patients treated Yes No

8. Out of 100 patients with chronic hepatitis C which proportion of patients will develop cirrhosis in 30 years

5 10 20 50 70

9. Where do you refer your patients with hepatitis C

.....
.....
.....

10. What changes do you feel would improve the services offered to your patients with hepatitis C in your area

.....
.....
.....
.....

The majority of general practitioners (> 86%) were aware that hepatitis C was common in drug users and that the prevalence is higher than HIV. Yet 14 % believed that the presence of antibodies to hepatitis C indicated that the patient no longer had active disease. Another common misconception was that 49 % thought that materno-fetal transmission occurred in more than 50% of infected women (transmission occurs in around 5%) whilst 50 % of practitioners believed that blood transfusion in the 1990s carried a high risk of infection.

Knowledge of the natural history of HCV was limited - only 23 % of general practitioners knew that 20% of patients develop cirrhosis after 20 years and only 58% were aware that treatment was effective in more than 50% of cases.

In response to our open-ended questions every doctor who responded could name either a district general hospital or tertiary referral centre that they referred to. The majority screened some patients for hepatitis C (23% did not) and of those who screened 14% tested homosexuals or patients with learning difficulties. Every general practitioner wanted more information about hepatitis C and 71 % believed that there should be more public education, together with linguistically

and culturally appropriate patient information for the different ethnic groups in the East End. Links between primary care physicians and hepatologists should be strengthened, with representatives from the primary care trusts invited to hospital hepatology meetings.

Table 17: Percentage of correct responses

	S-E LONDON STUDY	N-E LONDON STUDY	AVERAGE
1. Patients who are hepatitis C antibody positive no longer have active disease	84%	88%	86%
2. Hepatitis C is common (> 40 %) in those who have ever used intravenous drugs	85%	90%	88%
3. The prevalence of HIV is higher than hepatitis C in the East End	86%	88%	87%
4. More than 50 % of pregnant women infect their children	43 %	55 %	49%
5. Blood transfusion in the 1990s carries a high risk of transmitting hepatitis C	44%	55%	50%
6. In patients using intravenous drugs, transmission of hepatitis C can be prevented by meticulous hygiene and non-sharing of all paraphernalia	85%	95%	90%
7. Therapy for hepatitis C is effective in eradicating the virus in more than 50% of patients treated	64%	52%	58%
8. Out of 100 patients with chronic hepatitis C which proportion of patients will develop cirrhosis in 30 years	5 10 <u>20</u> 50 70		
	25%	20%	23%

4.2 Improving GPs knowledge by educational programmes or learning via a postal service

A Hepatitis C educational programme

A total of 43 GPs attended the four sessions of which 13 were GP trainees. All those who registered to attend did so.

Circulars had been sent out to 150 GPs by the PCP Trusts.

Attendance was poor however, a large proportion of the GPs (91) who did not attend replied and explained that they were unable to get cover and time off due to work constraints.

The evaluation forms were very positive. Almost all the attendees rated speaker knowledge, speaker effectiveness, and the overall programme as good or excellent. Participants felt the most important things they learned about included a general overview of the topic, how to diagnose and test for HCV, risk factors and treatment methods.

Due to the poor attendance the information sheet on HCV with the post test questionnaire was sent out to 200 GPs with the worst scores on the questionnaire. 123 replied back within the next four weeks; and a further 41 were retrieved after several

telephone calls and in some cases travelling to the surgery. All GPs were unaware that they had answered the pre-test questionnaire poorly. All felt the information sheet was beneficial.

The pre- and post-tests questionnaires show substantial improvement in basic HCV knowledge and includes the educational meeting and postal information sheet results separately (Table 18). All eight questions had a percentage of correct responses of more than 90%. GPs were also able to list more than three groups that they would screen compared to pre-test where only one or a wrong group such as patients with learning difficulties were listed. They were also now aware of ways in which the DH is improving HCV services such as increased drug counselling facilities, needle exchange services and provision of harm minimisation information and activities to prevent transmission of HCV. All GPs requested further information on HCV. Considerable frustration was expressed regarding access to reliable information about treatment outcomes and to linguistically and culturally appropriate patient information.

Table 18: Percentage of correct responses

	Educational Meeting		Postal Survey	Information Sheet
	Pre-Test	Post-Test	Pre Test	Post-Test
1. Patients who are hepatitis C antibody positive no longer have active disease	65%	100%	45%	96%
2. Hepatitis C is common (> 40 %) in those who have ever used intravenous drugs	80%	100%	72%	98%
3. The prevalence of HIV is higher than hepatitis C in the East End	58%	92%	47%	89%
4. More than 50 % of pregnant women infect their children	55 %	94%	35%	90%
5. Blood transfusion in the 1990s carries a high risk of transmitting hepatitis C	55%	96%	41%	92%
6. In patients using intravenous drugs, transmission of hepatitis C can be prevented by meticulous hygiene and non-sharing of all paraphernalia	85%	100%	81%	100%
7. Therapy for hepatitis C is effective in eradicating the virus in more than 50% of patients treated	52%	100%	43%	98%
8. Out of 100 patients with chronic hepatitis C which proportion of patients will develop cirrhosis in 30 years	20%	86 %	10%	94%
	5 10 <u>20</u> 50 70			

SUMMARY

In the second part of the thesis we assess the knowledge of General Practitioners (GPs) in the East End of London following the Department of Health campaign. The knowledge from the two regions in the East End of London were similar. Therefore it is likely to assume that this is a good reflection of the knowledge in the UK. Our survey clearly revealed that significant deficits in knowledge regarding hepatitis C among GPs continue to exist despite the provision of a 45 page booklet discussing all aspects of HCV. This booklet was sent to every GP in the United Kingdom by the Department of Health approximately 10 months prior to our questionnaire. Several factors may contribute to the knowledge deficits regarding hepatitis C among GPs: the rapid evolution of new information, the lack of established guidelines catered for GPs, the need for GPs to keep up with advances in many diverse areas of medicine, and the possible ineffectiveness of current educational resources.

We endeavored to improve GPs knowledge by inviting them to educational meetings on HCV or by improving their knowledge via a postal service. This strategy has met with success, although an immediate post-test does not address knowledge

retention. The feasibility and pick up rates of open access confidential hepatitis C testing clinics were ascertained. These clinics were found to be labour intensive and, in our experience unlikely to provide a cost effective solution to the identification of people with this treatable and sometimes fatal infection. GPs are the 'gate-keepers' to the NHS and will be the first health care professionals to have the opportunity to screen high risk patients for HCV. Thus, educational initiatives and guidelines specifically designed for GPs are needed to assist them in identifying patients at risk, conducting initial diagnostic evaluation, and initiating appropriate referrals, so patients with hepatitis C can be correctly diagnosed and treated while they are still in the early stages of disease. The best method of providing information should be provided and GPs may wish to become more directly involved in shared care arrangements with specialists that will broaden education and treatment opportunities for HCV-infected patients. The Department of Health has launched a second campaign for tackling hepatitis C in the UK. This includes an information pack for general practitioners. This will assist professionals recognise the main risk factors, advise on how to avoid infection, discuss testing with patients, if appropriate, and

follow-up if they discover patients are infected. This will be followed by education of the public about avoiding the risk of infection and considering testing where appropriate.

CHAPTER 5: RESULTS - Management Of Hepatitis C In The North-East Of London

5.1 Study to assess the safety and effectiveness of ONP-17 for the treatment of hepatitis C

Enrolment for the herbal study began on the 25 th of February 2004 and continued until the 1st October 2004 when enrolment was terminated as the herbal company were worried about the efficacy of the herbs.

Enrolment was very difficult. In the first four months enrolment took place at a single centre, the Royal London Hospital with 36 patients recruited from clinic. However this tailed off and as we had previous ethical approval at St Marys Hospital we gained permission from our local ethical authority to use St Marys as a second site. This resulted in a further 4 patients. However we were still struggling to achieve our target of 66 patients. We therefore sought ethical approval to advertise in the local paper about the herbal study. We gained ethical approval but this increased interest in the study; however only 2 patients fitted the inclusion criteria.

Finally by the 1st October 2004 45 patients were entered into the study. This comprised 26 men and 17 women. The large majority (31) were unable to tolerate antiviral therapy or were non-responders to treatment. However nine patients had mild liver disease and were being monitored with serial liver biopsies every four to five years; whilst five patients only wished to take herbal treatments.

Only 26 patients completed the course of treatment and the 4 week washout period. Of these 12 patients (6 male, 6 female) (mean age 45.9) were taking placebo and 14 patients (9 male, 5 female) (mean age 50.5) were on the herbal preparation (Figure 7).

Figure 7: Demographics

	N	Age	Sex (M/F)
Placebo (Total)	20	46.6	11/9
Placebo (Completed)	12	45.9	6/6
Herbs (Total)	25	49.7	15/8
Herbs (Completed)	14	50.5	9/5

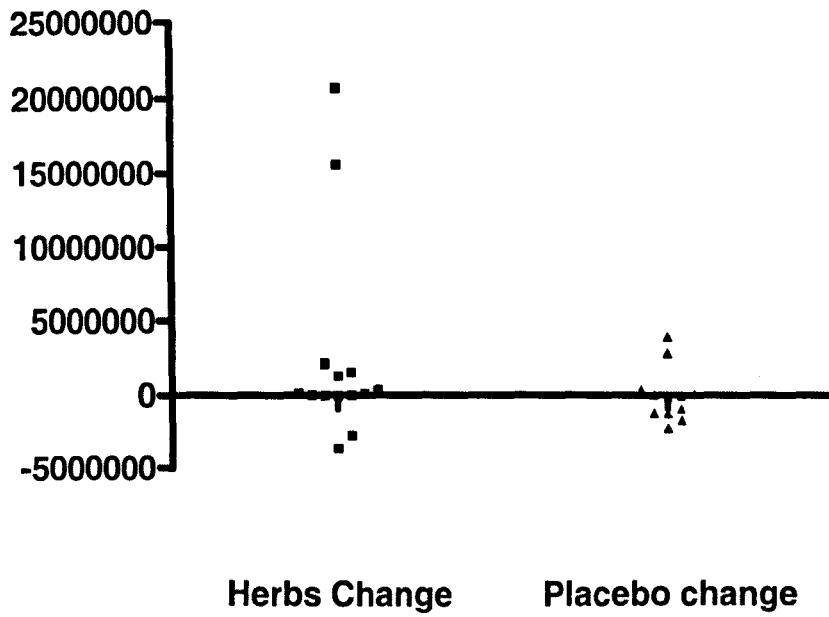
All patients had symptoms which could be related to their hepatitis C and the majority of patients (36) had liver enzymes (ALT or AST) twice the upper limit of normal.

There have been three adverse events, for which the patients were withdrawn from the study and unblinded. One patient was on placebo and two on active treatment. The patient on placebo was withdrawn as he developed acute pancreatitis whilst abroad (thought to be due to hyperlidaemia). The two other patients on active treatment were withdrawn as one lady with a past history of osteoarthritis of her left knee developed a large knee effusion requiring drainage. The other patient had a liver biopsy showing mild disease who became anaemic with a haemoglobin value of 6 g/dl during treatment. There was no evidence of overt loss of blood on history or examination. On Upper GI endoscopy he was found to have varices. Re-examination of his liver biopsy by the original histopathologist was performed. The histopathologist felt that the liver biopsy was a poor fragmented specimen and showed cirrhosis.

It must be remembered that as the numbers for each arm were small due to problems with recruitment and treatment withdrawal we are unable to draw any significant results between the two arms.

Comparing the small numbers that completed the study subjectively it seems that the herbal preparation did not have an effect on viral load compared to placebo (Figure 8).

Figure 8: Change in viral load + = increase



For quality of life data- eight SF-36 scales (Figure 9).

For the fatigue symptom score herbs had higher scores than placebo (Figure 10)

ALT levels probably fell slightly during treatment with herbs rather than placebo (Figure 11 -13).

Both the herbs and placebo preparations did not alter the full blood count or renal function during the study (Figures 14 – 17). The herbal preparation did not affect glucose levels during the study; however the placebo seems to have increased the glucose levels possibly due to some sugar additive (Figure 18).

Figure 9: QOL Scores –SF36 (+ = feels better)

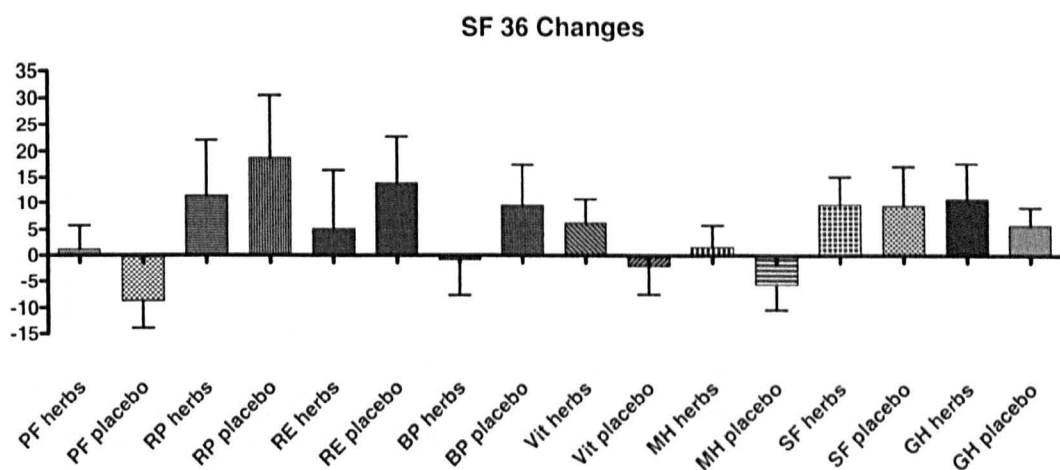


Figure 10: QOL Scores - FSS

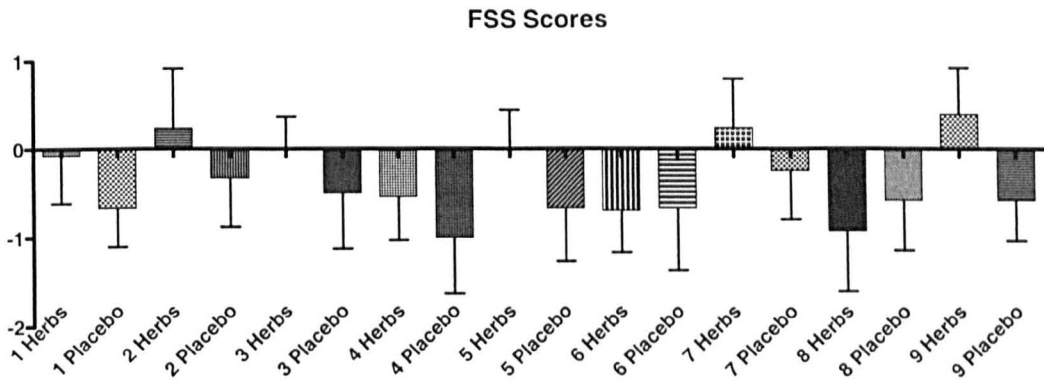


Figure 11: ALT values for herbs and placebo during the study (28 weeks).

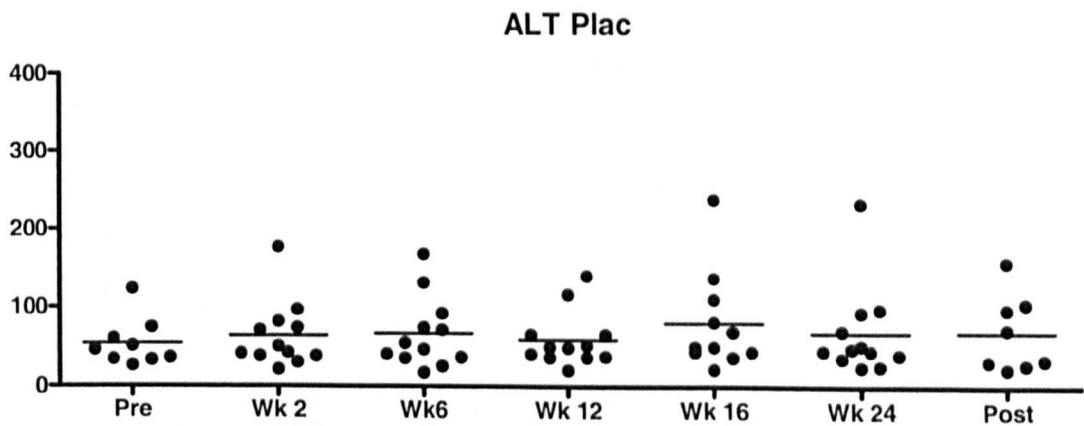
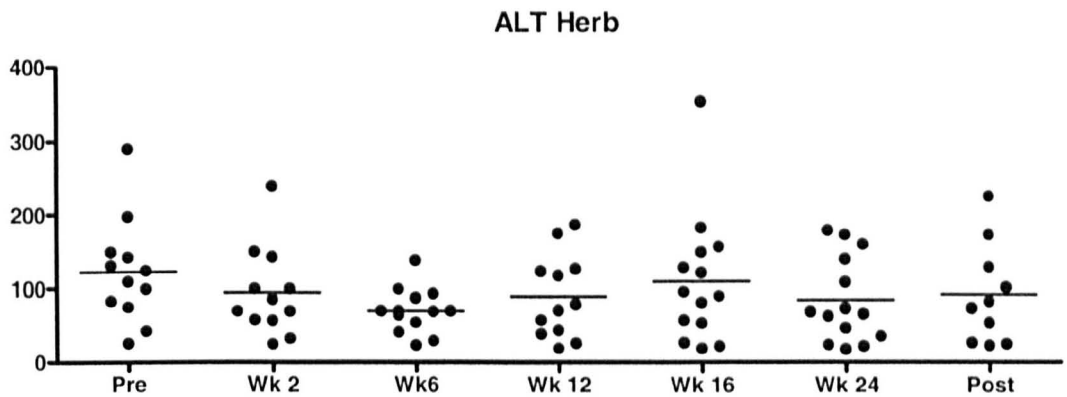


Figure 12: Herbs - ALT changes during the study.

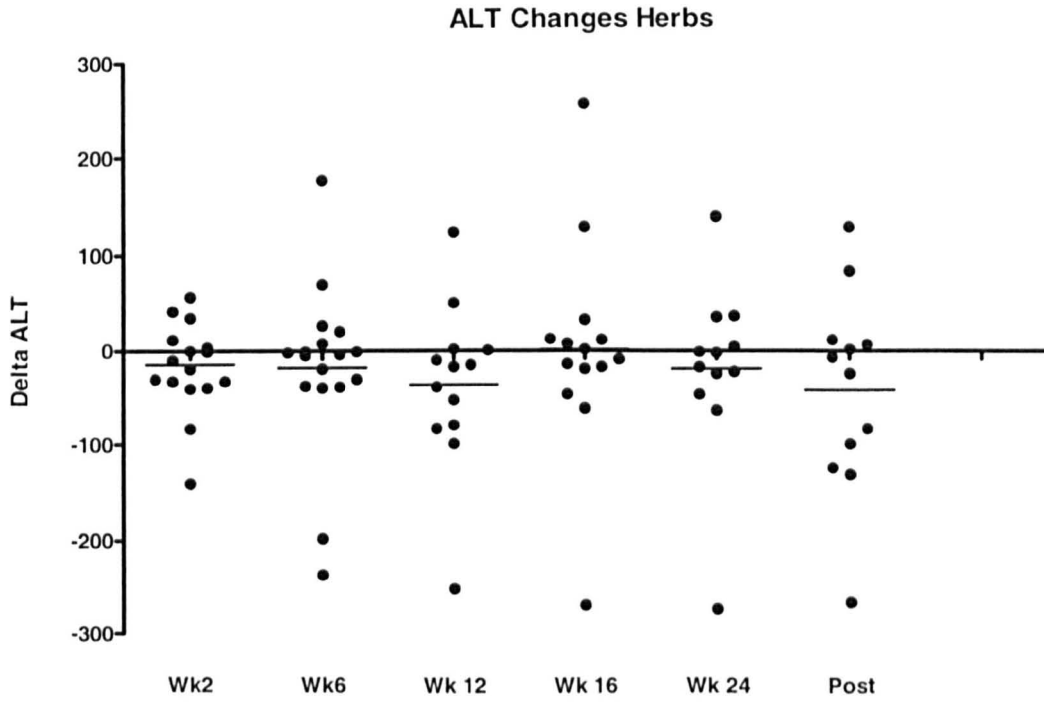


Figure 13: Placebo – ALT Changes during the study.

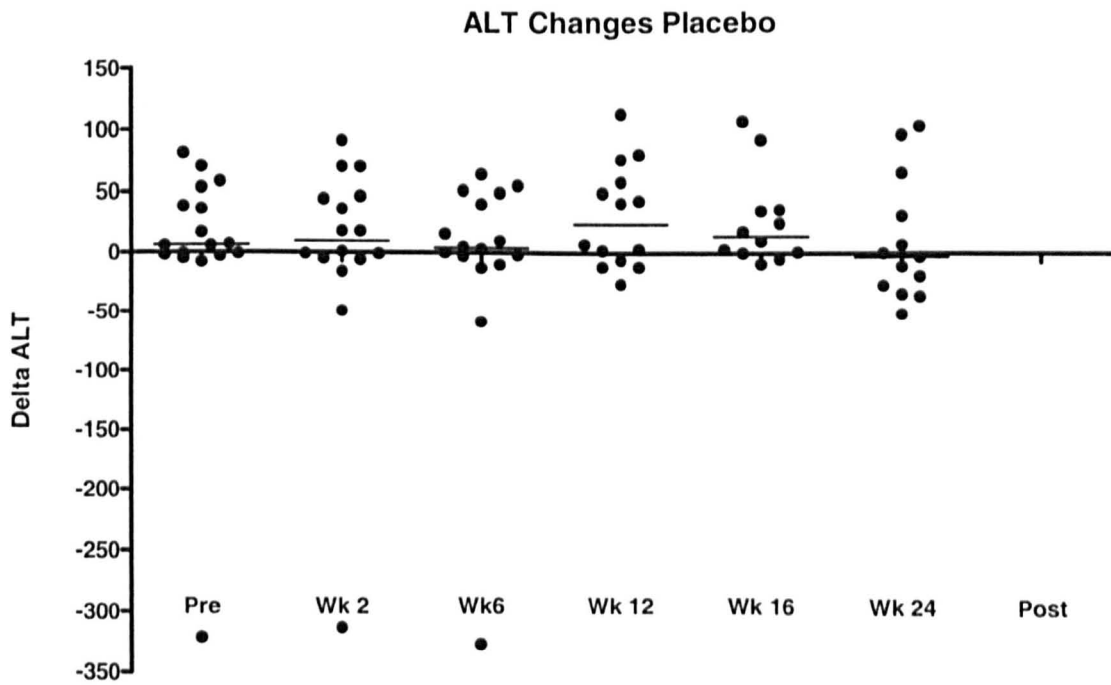


Figure 14: Safety – Haemoglobin levels during the study.

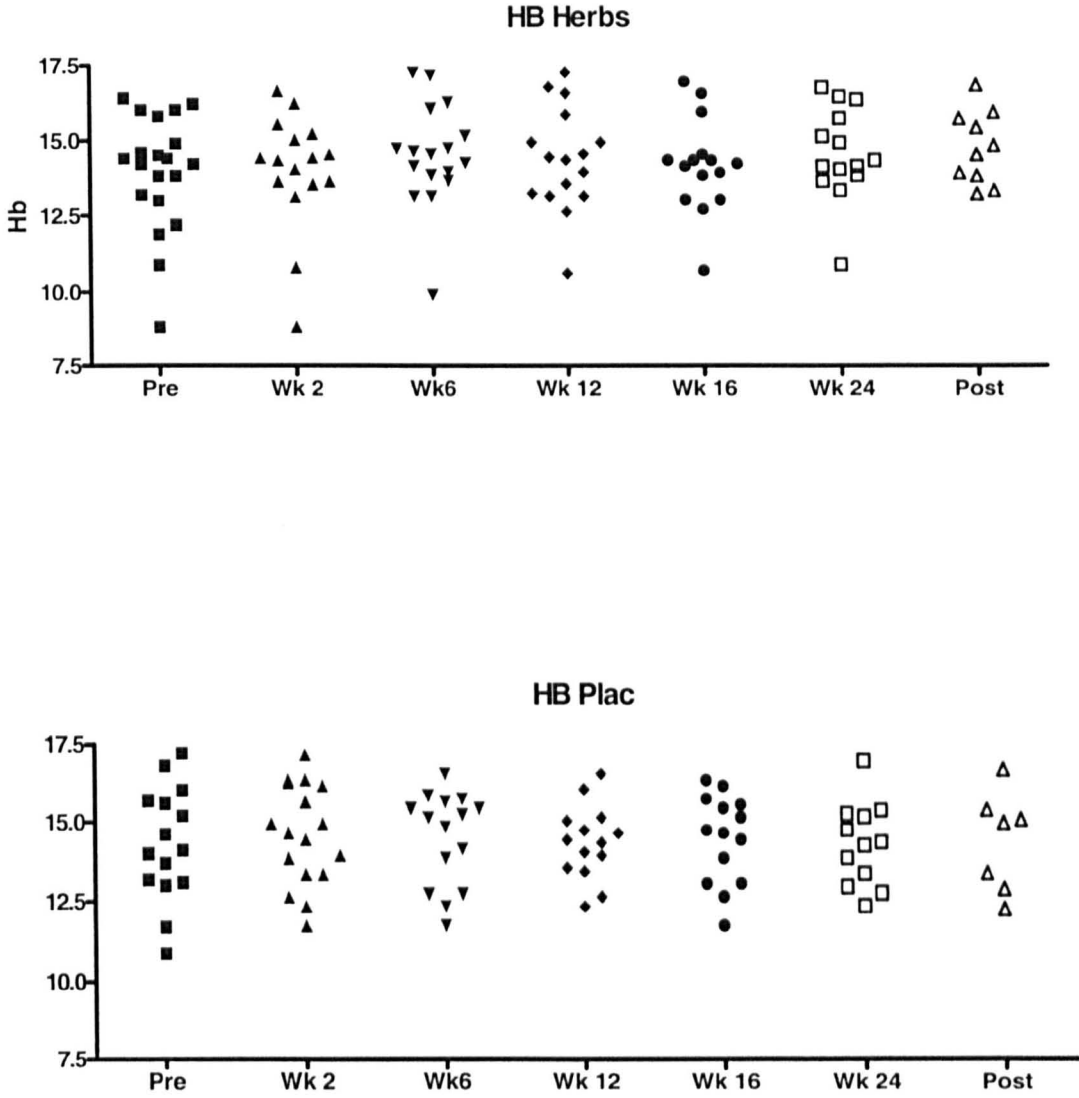


Figure 15: Safety - White blood cell count values during the study.

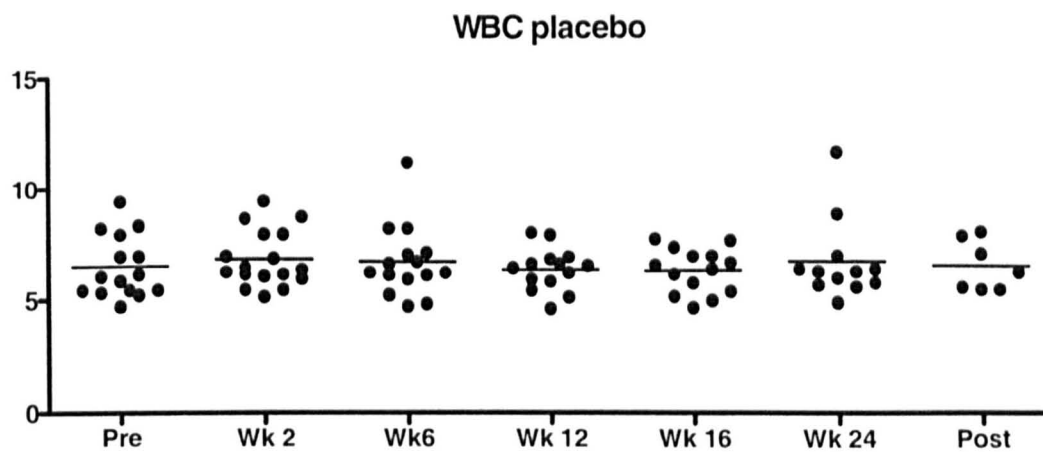
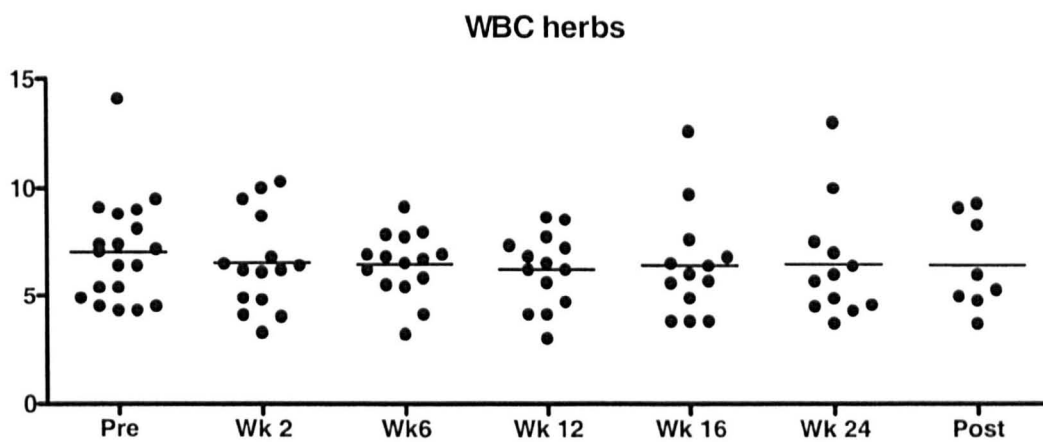


Figure 16: Safety - Platelet levels during the study.

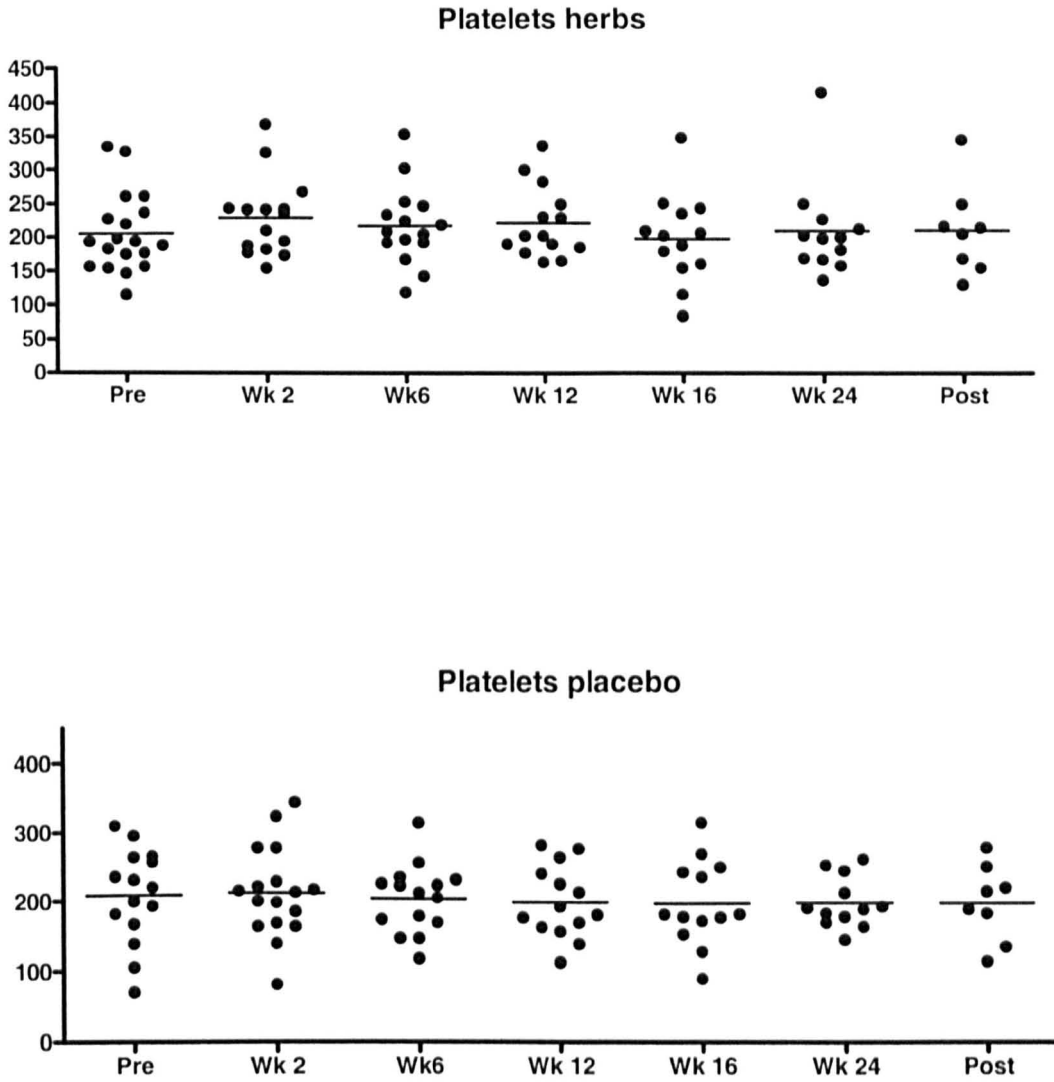


Figure 17: Safety - Creatinine levels during the study.

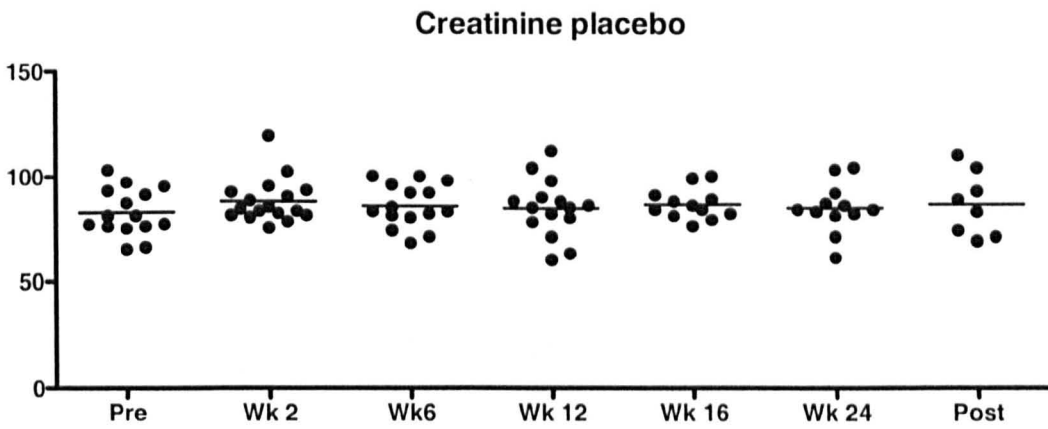
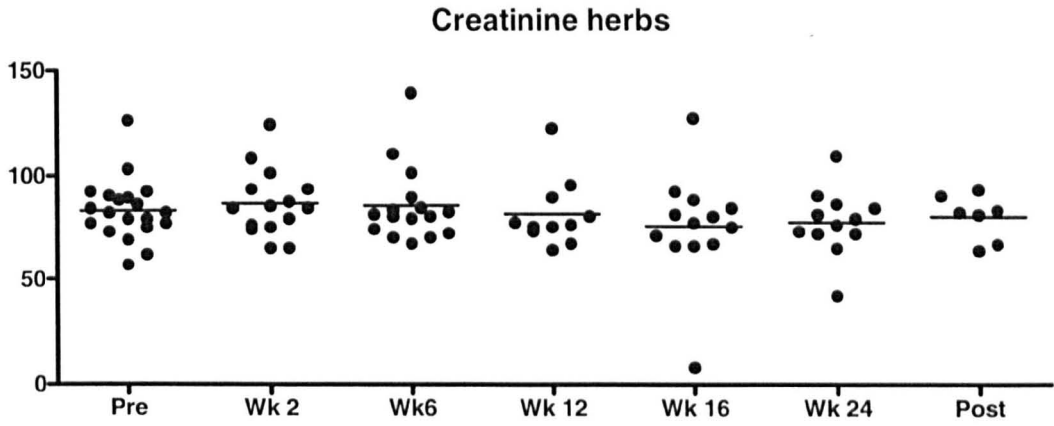
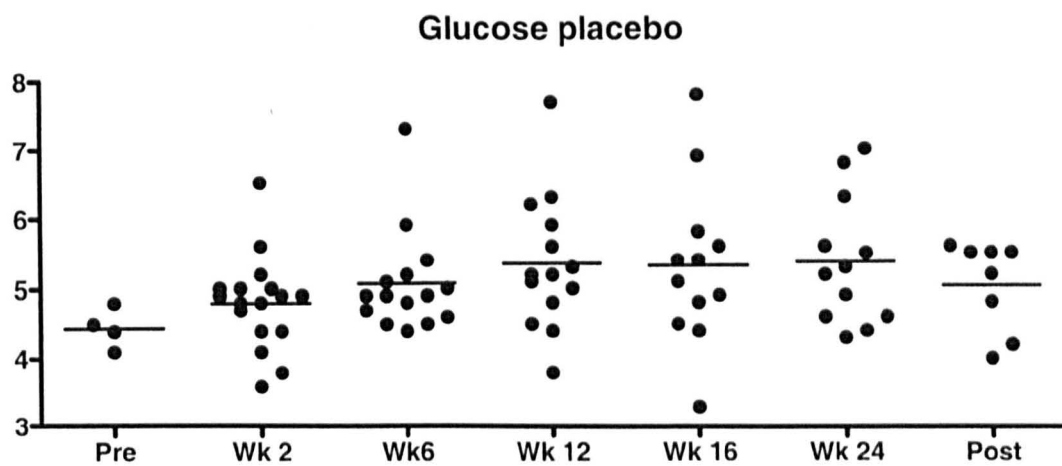
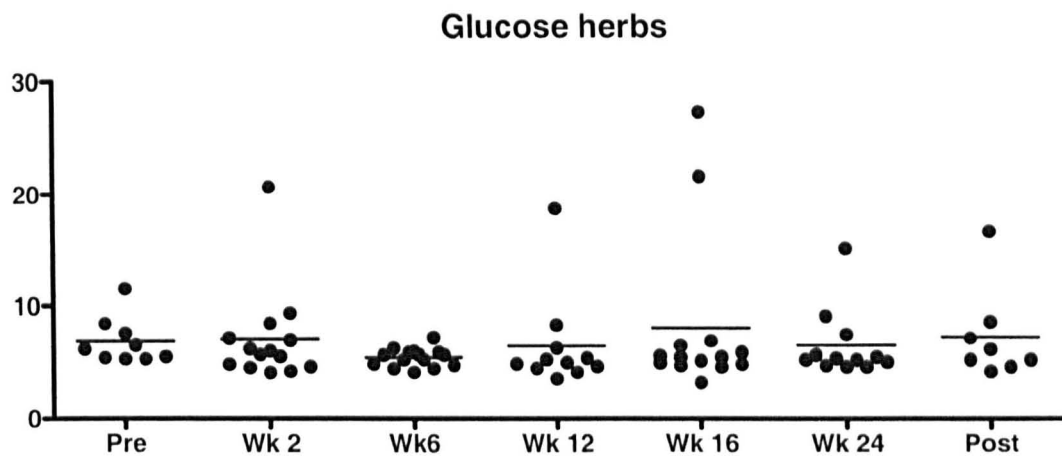


Figure 18: Safety – Glucose levels during the study.



CHAPTER 6: DISCUSSION

The natural history of infection with chronic hepatitis C over 2-3 decades has been determined by studying patients infected 20 to 30 years ago. The outcome is variable with around 15-20% of patients developing cirrhosis after 20 years (Alter et al.1992; Tong et al. 1995; Kenny-Walsh 1999). However prolonged (>30 years) follow up of chronically infected patients has not been completed. The large population of untreated elderly Asian patients who had acquired HCV in childhood gave us the unique opportunity to study the natural history of chronic hepatitis C in patients exposed to the infection for more than 40 years. We were also able to look at factors involved in fibrosis progression in chronic hepatitis C patients.

6.1 Natural History of Chronic Hepatitis C Infection: Life-Long Follow-up of an Unselected Population Infected in Early Childhood

This study addresses the outcome of chronic HCV infection up to 60 years after infection and represents the first very long-term study of the natural history of this infection. Our study involved elderly Asian patients and therefore the high rates of cirrhosis may be due to either Asian origin or advanced age. Ethnic groups have different response rates to antiviral treatment and small scale, studies in America suggest that fibrosis progression in African Americans and Latinos may differ from Caucasians (Wiley et al. 2002; Bonacini et al. 2001), perhaps indicating that our observations are only pertinent to Asian individuals. However in our study we found that fibrosis progression, measured both in single and paired biopsies was the same in Asian and Caucasian patients. This suggests that our findings may apply to other populations and that the high prevalence of cirrhosis, observed in Asian patients was due to prolonged infection and not to ethnicity.

During this study, patients were referred for a liver biopsy to one of three local hospitals (Barts and the Royal London, Newham District hospital and Homerton District Hospital), none of which operated as a tertiary referral center for hepatology. One of the possibilities was that the high prevalence of cirrhosis in elderly Asian patients may be due to referral bias rather than to a genuine increase in the prevalence of cirrhosis in this population. A number of different lines of evidence suggest that this was unlikely. The study was conducted at three local hospitals which at the time of the study did not offer advanced hepatological services (e.g. liver transplantation) and it therefore was unlikely that selective referrals of patients with end-stage liver disease would have biased our results. To reduce referral bias we actively excluded patients with decompensated liver disease who underwent a transjugular biopsy from the study and therefore referral bias of patients with severe liver disease does not explain our results. Some clinicians regard elderly patients as poor candidates for antiviral treatment and it is therefore possible that these patients did not have liver biopsies unless there was biochemical or other evidence of advanced fibrosis.

If this was the case it would have resulted in an increased proportion of elderly cirrhotic patients. This potential source of bias is unlikely to account for our results; as we show no fall in the frequency of liver biopsy in elderly patients and the increase in the prevalence of cirrhosis in Asian patients was seen in all age groups. Another potential source of bias is that Asian patients are less likely to present to their general practitioners than Caucasian patients unless they had symptoms of advanced disease. Since most patients with HCV are asymptomatic until the complications of cirrhosis have developed and as these patients with decompensated liver disease were actively excluded from the study, this possibility seems unlikely. Immigrant communities have lived in the East End of London for many decades and every effort has been made to ensure that all of the community has equal access to the free health care that is provided by the NHS in the UK. About 30% of the community in the East End are of Asian origin and as a similar percentage of Asian patients was seen in all of the age groups in our study there is no evidence that Asian patients only presented with advanced disease. Previous studies have shown that the prevalence of HCV induced cirrhosis is no more than 15-20% after 20 years

which compared favorably (15%) with the prevalence of cirrhosis in our Asian patients who had been infected for 20-40 years. Altogether this data suggests that the increase in prevalence of cirrhosis in elderly Asian patients was not due to selective referral of patients with advanced disease we cannot exclude the possibility that referral bias may have had a small impact upon our data.

Elderly Asian patients infected with HCV in early life who have a prolonged exposure to HCV (>60 years) have a very high prevalence of cirrhosis. Several lines of evidence indicate that this is so. Most of our Asian patients spent their childhood in Pakistan and Bangladesh where there is a high prevalence of HCV infection and, the majority probably acquired HCV infection in early life. Our data on disease progression also suggests that infection in childhood is likely. Disease progression rates are similar in Asians and Caucasians and if we speculate that infection in Asians occurred in adult life then we cannot explain the higher prevalence of cirrhosis in Asian patients. This evidence strongly suggests that infection in our Asian patients occurred in early life and the long duration of infection is responsible for the high prevalence of cirrhosis in

elderly Asians with HCV.

The possibility that another factor contributes to the high prevalence of HCV associated cirrhosis in Asian patients cannot be excluded. We have not been able to identify any additional factor that might predispose Asians to advanced disease. Multivariable analysis found that genotype 3 (commonest genotype in Asians) was associated with less fibrosis. Viral load measurements with a validated assay were not available for many of our cases and the effect of this variable on fibrosis progression could not be studied. However, a large French study have suggested that viral load and genotype did not influence disease progression (Poynard et al. 1997).

Alcohol accelerates disease progression in patients with chronic hepatitis C (Poynard et al. 1997) and our analysis confirms this effect. It must be remembered Asian populations have a high prevalence of NASH and it is possible that this is the cause for the high prevalence of cirrhosis found in elderly Asians. Whilst increased BMI was significantly associated with a higher fibrosis score in univariate analyses, adjusting for

BMI in multivariate analyses did not change the apparent relationships between fibrosis score, age and ethnicity. There was no increase in the prevalence of diabetes mellitus or steatosis in those patients with advanced fibrosis. This suggests that NASH is unlikely to account for the excess cirrhosis in elderly Asian patients. In a follow up study to this work, in a smaller cohort of patients who were due to receive therapy with pegylated interferon and ribavirin, we find that insulin resistance is associated with more advanced fibrosis in elderly Asian patients suggesting that insulin resistance may play a role in the development of advanced fibrosis. Further studies of larger cohorts studied prospectively will be required to determine the role of insulin resistance and glucose homeostasis in the development of hepatic fibrosis.

This study shows a very high prevalence of HCV induced cirrhosis in elderly Asians. This cannot be attributed to either by referral bias or more rapid disease progression and appears related to prolonged exposure to the virus. It is likely that long infection (>50 years) in other populations will also lead to advanced liver disease in the majority of patients. Some clinicians may be reassured to learn that infection for

30-40 years is benign in the majority but others will be alarmed to discover that infection for over 60 years is usually associated with advanced disease. All will agree that every effort must be made to identify and treat those infected in early life, as the lifetime risk of significant disease may be high.

6.2 Factors involved in Fibrosis Progression

i) Paired Biopsies: The role of steatosis in fibrosis progression

Hepatic steatosis is common in hepatitis C, with 81% of patients having some degree of steatosis. The small number of paired liver biopsies meant that it was not possible to run multivariable analysis to assess whether any of these factors were independently associated with fibrosis progression. We found a moderate positive correlation between the rate of fibrosis progression and the change in steatosis between the two biopsies and this is in agreement with other reports where hepatic steatosis is increasingly recognised as a cofactor influencing the progression of fibrosis in chronic hepatitis C (Hourigan et al.1999, Rubbia-Brandt et al. 2000, Adinolfi et al.

2001) . Higher grades of steatosis are associated with more advanced stages of fibrosis as suggested by our data (Adinolfi et al. 2001; Serfarty et al. 2002). However, recent data suggests that this association between fibrosis and steatosis is more complex. In one study the association was not found whilst other studies have only confirmed this association in certain patients' subgroups (Patton et al. 2004; Rubbia-Brandt et al. 2004). Patton et al. (2004) only found an association between steatosis and fibrosis between patients with genotype 1 infection; whilst Rubbia-Brandt et al. (2004) found the association in genotype 3 patients. However, after closer inspection these results are not necessarily conflicting. The study populations are quite different as are the histological and clinical variables (such as alcohol abuse) and even referral patterns differ at different locations. Patient populations from Australia are characterised by a higher average BMI and a lower prevalence of genotype 3 (Kumar et al. 2002), whereas European patients have a lower BMI (<25 kg/m² in all studies) and are more frequently infected with genotype 3 (Monto et al. 2002). Other factors such as genetic and dietary factors may also play a role. Several hypothesis have been put forward from the production of excess reactive

oxygen species and lipid peroxides to the insulin resistance syndrome (Okuda et al. 2002; Parola et al. 2001). If steatosis is associated with more rapid progression of fibrosis, then exercise and weight loss may improve hepatic injury due to HCV. Further research is eagerly awaited to confirm whether or not steatosis contributes to fibrosis progression and if so which patient groups are affected together with the pathogenesis.

ii) Relationship between Serum and Hepatic Markers of Iron, Diabetes Mellitus and Fibrosis Progression in Patients with Chronic Hepatitis C.

It has been proposed that iron overload in HCV-infected patients, like hereditary haemochromatosis may cause progression of HCV-associated hepatic fibrosis; resulting in cirrhosis with its associated complications of hepatocellular carcinoma and portal hypertension. So far, studies assessing the effect of elevated iron stores on the natural history of hepatitis C have been contradictory (Di Bisceglie et al. 1992;

Haque et al. 1996; Thorburn et al. 2002; Metwally et al. 2004). The role of both serum ferritin and hepatic iron staining on HCV fibrosis was examined in this study by comparing a relatively iron-deficient Asian population with the indigenous Caucasian population.

In our study 4.4 % of patients had raised serum ferritin levels. Elevated serum iron markers were more commonly found in males and Caucasian patients. Eleven percent of patients had elevated hepatic iron staining, which is similar to earlier studies where a prevalence of 10-36% has been recorded (Piperno et al. 1998; Tung et al. 1999; Kazemi-Shirazi et al. 1999). Serum ferritin from our study when elevated appears to be a good screening tool for abnormal iron stores. Nevertheless, it has to be realised that ferritin can often be elevated in the absence of increased hepatic iron stores, due to non-specific effects of hepatic inflammation that result in increased release of iron from damaged liver cells (Di Bisceglie et al. 1992, Prieto et al. 1975). Raised ferritin may also result from acute or chronic inflammation as part of an acute-phase reaction (Prieto et al. 1975). This is an unlikely explanation for our results as patients who had inflammatory

markers such as C reactive protein performed at the same time that ferritin levels were taken, had normal readings.

Racial differences in iron stores exist with some races responding to infection with HCV with an increase in iron stores. HCV-infected black patients were 5.4 times more likely to have increased iron stores (assessed by transferrin saturation and ferritin) than HCV-positive non-blacks (Ioannou et al. 2003). Other races were found to have only a small difference in the iron stores between HCV-infected and non-infected patients (Ioannou et al. 2003). Our Asian population had lower iron stores (assessed by serum ferritin and hepatic iron staining) than Caucasians and this is because the majority of Asians were vegetarian and most did not drink alcohol. Consumption of up to 2 alcoholic drinks/day is associated with a reduced risk of iron deficiency, whilst consumption of more than 2 alcoholic drinks/day is associated with a significant increase in the risk of iron overload (Ioannou et al. 2004).

The quantity of iron found both histologically and biochemically were unrelated to the stage of fibrosis, which suggests that iron does not play a significant role in the progression of HCV related liver injury. Even when controlling for alcohol intake Asians who had less iron than Caucasians had more fibrosis on liver biopsy.

Chronic hepatitis C infection compared to other liver conditions is associated with an increased risk for the development of type II diabetes mellitus (Allison et al. 1994; Mehta et al. 2000; Knobler et al. 2000; Alexander G. 2000). In cirrhotic patients being assessed for liver transplantation, HCV-infection was independently associated with type II diabetes (Allison et al. 1994). Furthermore, HCV infected patients aged 40 years or older had a three times higher rate of type II diabetes than similarly aged patients without HCV infection, even when cirrhotic patients are excluded (Knobler et al. 2000; Mehta et al. 2000; The Third National Health and Nutrition Examination Survey (N-HANES III) (1988-1994)).

However, a clear biological relationship between HCV infection and type II diabetes mellitus is not obvious

(Alexander G. 2000). One suggestion put forward is the association between hepatitis C and iron overload (Ford et al. 1978-1983; Paris R. 2001; Furantani et al. 2003). A significant association between elevated serum ferritin levels and newly diagnosed diabetes mellitus was noted on further analysis of the N-HAMES III data (Ford et al. 1978-1983; Paris R. 2001). We also found that serum ferritin levels were significantly higher in patients with diabetes or impaired glucose tolerance compared to those patients without diabetes. However, there was no association between diabetes or impaired glucose tolerance and the presence of iron deposition in the liver.

Other factors may also be involved. HCV infected patients can also have non-alcoholic fatty liver disease (NAFLD) which is strongly associated with features of the metabolic syndrome and this may explain the strong relationship between HCV infection and diabetes mellitus (Sanyal et al. 2003). Insulin resistance as previously discussed plays an important role in the development of type 2 diabetes mellitus and is the best predictor of diabetes mellitus. The mechanism for insulin resistance in chronic HCV infection are not yet known. Studies

from transgenic mice, that specifically express high levels of HCV core protein in hepatocytes, showed that hepatic insulin resistance could be induced solely by HCV core protein (Shintani et al. 2004). Increased levels of tumour necrosis factor alpha (TNFa) were produced which blocked phosphorylation of the insulin receptor-insulin substrate 1 pathway (Shintani et al. 2004). A defect in the Insulin receptor substrate- 1 tyrosine phosphorylation has been found in HCV patients' liver biopsies, but not in uninfected controls (Aytug et al. 2003) . This initial data suggests that TNF-a-mediated alterations in IRS-1 signalling are relevant to hepatitis C-induced insulin resistance, which may ultimately lead to the development of type II diabetes mellitus. Insulin resistance may cause development of liver steatosis in HCV-infected patients making them more susceptible to the development of diabetes mellitus (Monto et al. 2002).

Our study has several limitations. As it is a retrospective cross-sectional study it is unable to answer the question of causality. However, prospective studies of the natural history of HCV are difficult to perform due to the asymptomatic exceedingly prolonged course of the disease (Seeff L. 2002).

72 patients with an increased ferritin or hepatic iron staining had genetic studies performed for the haemochromatosis gene mutations. The numbers were too small to perform statistical analysis on and it is conceivable that we may have inadvertently included patients with heterozygosity for haemochromatosis in our study. However, a recent study showed that HCV patients who were heterozygote for HFE mutations did not develop iron accumulation or evidence of progression of liver fibrosis (Thorburn et al. 2002). We showed that serum ferritin levels were significantly higher in diabetic patients than in those patients without diabetes, although there was no difference between hepatic iron concentration between the two groups. However, we cannot eliminate conclusively the possibilities that the observed association reflected inflammation rather than excess body iron stores or that co-existing non-alcoholic fatty liver disease (NAFLD) was an important factor influencing alterations in serum iron markers

In summary , it must be remembered that raised serum ferritin can be a good indicator of abnormal hepatic iron deposition within the liver; however ferritin may be raised for other

reasons. Both serum ferritin and hepatic iron did not appear to be associated with advanced liver disease in HCV infected patients. These findings are not supportive for a role for iron depletion by venesection in patients with chronic HCV infection with elevated serum iron stores. The association between chronic HCV infection and type II diabetes mellitus exists, however the biological mechanism of this association still remains to be discovered. Prospective studies on this subject are needed.

iii) Clinical Significance of Insulin Resistance in Hepatitis C Virus Infection.

Our study suggests that insulin resistance plays an important role in hepatic fibrosis in HCV patients, irrespective of viral genotype. This association still remained when diabetic and cirrhotic patients were excluded. In multivariable analysis, both insulin resistance and older age were independently associated with a worse fibrosis stage. Asian HCV infected patients were found to have significantly higher insulin resistance than Caucasians.

HCV infection is more commonly associated with Type 2 Diabetes Mellitus (DM) than other liver conditions (Alexander G, Allison et al. 2000; Mehta et al. 2000; Knobler et al. 2000). Patients with cirrhosis have increased insulin resistance, and subsequently, a higher incidence of DM, however, when cirrhotic patients are excluded this association still exists (Mehta et al. 2000). Further studies now suggest that DM may be associated with increased fibrosis progression in patients with HCV infection (Monto et al. 2002; Ortiz et al. 2002).

Insulin resistance is a consistent finding in diabetic patients (Cahill et al. 1988; Reaven et al. 1976). It precedes the onset of DM by 10-20 years (Martin et al. 1992; Warram et al. 1990). Insulin resistance is increased in HCV infected non-diabetic patients when compared to healthy controls (Knobler et al. 2000). Our study confirms that insulin resistance is associated with fibrosis in HCV patients. This association remained when patients with cirrhosis, which is known to cause insulin resistance and impaired insulin clearance, and diabetic patients (according to the American Diabetes Association guidelines) were excluded from the study. This relationship was independent of other factors associated with

fibrosis, including older age, BMI and alcohol consumption. These findings are in concordance with other studies that suggest that viral-induced insulin resistance may cause fibrogenesis in chronic HCV (Hui et al. 2003, Monto et al. 2002; Ortiz et al. 2002). No genotype-specific effects on insulin resistance were found, which is in concordance with another study (Knobler et al. 2000).

The mechanisms for insulin resistance in chronic HCV infection are still not known. Studies from transgenic mice that express high levels of HCV core protein in hepatocytes suggest that hepatic insulin resistance can be induced solely by HCV core protein (Shintani et al. 2004). This resulted in increased levels of tumour necrosis factor alpha (TNF α) which block phosphorylation of the insulin receptor-insulin substrate 1 pathway (Shintani et al. 2004). This defect in the Insulin receptor substrate- 1 tyrosine phosphorylation has been found only in HCV patients' liver biopsies but not in non-infected controls (Aytug et al. 2003; Svegliati-Baroni et al. 1999; Knobler et al. 2003). These initial data suggest that TNF- α -mediated alterations in IRS-1 signalling are relevant to hepatitis C-induced insulin resistance. Some studies have

ascribed the increased insulin resistance to be secondary to the increase of iron deposits such as ferritin in HCV patients (Hichman et al. 2003; Lecube et al. 2004). Insulin resistance may cause fibrogenesis by directly stimulating hepatic stellate cells to proliferate (Svegliati-Baroni et al. 1999) or by up-regulation of connective growth factor, a cytokine involved in the pathogenesis of fibrosing liver diseases (Paradis et al. 1999).

Despite the small sample size, our study showed that increased insulin resistance is associated with the stage of fibrosis. However, worsening hepatic fibrosis can impair insulin degradation. This possibility has been excluded for two reasons. When cirrhotic patients are excluded from the analysis HOMA-IR is still an independent predictor of fibrosis score. Secondly, insulin resistance is higher in chronic hepatitis C than other chronic liver diseases (Knobler et al. 2000).

In this study, Asian HCV infected patients had significantly higher insulin resistance than Caucasians. BMI values for the diagnosis of obesity in the Asian population have been set lower ($>22 \text{ kg/m}^2$) than those for Caucasians ($>25 \text{ kg/m}^2$)

(WHO Expert Consultation Appropriate body-mass index for Asian populations). According to this new definition, all of the Asians in our study were classified as obese compared to only 62% of Caucasians. This may partly account for any ethnicity-related differences seen in insulin resistance. A recent study suggests that hyperinsulinaemia could be a factor that is responsible for the association between BMI and fibrosis in patients with HCV (Hickman et al. 2003).

Our study is only a small study and as such, the results from our study should be interpreted cautiously. However, despite the small sample size, our study showed that increased insulin resistance is associated with the stage of fibrosis.

In summary, insulin resistance in HCV patients is associated with the stage of fibrosis, even when cirrhotic patients are excluded. Asian patients have higher insulin resistance than Caucasians and the higher fibrosis progression in Asians may be related to this higher insulin resistance. However, numerous questions still need to be addressed, most notably those regarding the nature of the link between HCV infection and insulin resistance. Further studies on this subject are eagerly awaited.

6.3 Impact of Insulin Resistance on response to Therapy

The effects of insulin resistance on treatment response were assessed in the second part of the study. Patients infected with HCV genotype 3 were independently associated with an increased chance of a sustained virological response. Insulin resistance was significantly higher in non-responders to antiviral therapy than responders. In multivariable regression analysis Asian ethnicity, higher fasting insulin levels and higher HOMA-IR levels were all independently associated with a poorer sustained virological response to treatment.

Patients with obesity, steatosis and insulin resistance treated with antiviral therapy have reduced sustained virological response rates (Ortiz et al. 2002; Furanatani et al. 2003). Also, different ethnic groups have different sustained virological response rates to antiviral therapy (Hourigan et al. 1999). The Asians in our group develop the metabolic features of the insulin resistance syndrome at lower values of BMI than

Caucasians. Therefore, it can be expected that insulin resistance might mediate the differences in the response rate to antiviral therapy seen here.

Obesity, steatosis and insulin resistance have previously been found to be important in fibrosis progression (Ortiz et al. 2002), and measures aimed at weight reduction prior to antiviral therapy may improve insulin sensitivity and the likelihood of viral eradication. Our study is a small study and as such, the results from our study should be interpreted cautiously. In particular, it was not possible to tease out the independent effects of Asian ethnicity, fasting insulin or HOMA-IR levels when considering factors associated with response to therapy.

In conclusion, liver fibrosis is independently associated with insulin resistance in HCV patients confirming other studies and highlighting the fact that progression of liver disease in chronic hepatitis C infection may share the same mechanisms involved in the progression of non-alcoholic fatty liver disease (NAFLD) from fatty liver to cirrhosis, via non-alcoholic steatohepatitis (NASH). This paper shows that insulin resistance is also an important predictor of sustained

virological response to antiviral therapy. Further studies on this subject are needed.

6.4 Knowledge of Chronic Hepatitis C among East London Primary Care Physicians following the Department of Health's Educational Campaign

A significant number of patients who are chronically infected with the hepatitis C virus will develop advanced liver disease (Fried et al. 2002; Tong et al. 1995). Early intervention is therefore of paramount importance in order to reduce the costs and mortality associated with advanced liver disease (Poynard et al. 1997). The Department of Health recognises these problems associated with chronic hepatitis C infection and has begun to address the issue by writing to all general practitioners about this virus. Our study is the first to assess general practitioners knowledge of hepatitis C in the UK and reveals that knowledge about this virus is limited.

We assessed knowledge by two approaches:- (i) an open interview in a small group of general practitioners and (ii) a postal questionnaire of over 800 general practitioners. Both approaches led to very similar conclusions and, resulted in response rates over 55%. The consistent patterns of response suggest the results are truly representative. The response rate was higher than that obtained in an American study (33%) on primary care physician's knowledge and practice patterns concerning HCV and similar to the response rate of 54.4% in Australia obtained in a study assessing management of HCV in general practice.

Hepatitis C is a rapidly developing disease area, and it is not surprising to find that general practitioners are unaware of the latest therapy. However, we were concerned to find that a significant minority of general practitioners are unable to interpret hepatitis C antibody test results. As a result patients with hepatitis C are likely to be under-diagnosed and lost to follow-up. We were dismayed to receive feedback suggesting that some general practitioners appear to restrict access to health care to those who are actively using drugs. Further

urgent work will be required to assess whether there is discrimination against intravenous drug users.

Our primary care colleagues acknowledge their shortcomings and all wish to be better informed about management of hepatitis C so that they can improve their clinical practice of this constantly changing condition. In order to improve the recognition of patients at risk for hepatitis C and to ensure appropriate testing and referral specific educational initiatives and practice guidelines for GPs are required. Our findings are similar to studies in other countries, which have also demonstrated the poor knowledge base and practice patterns of their primary care physicians (Ouzan et al. 2003; Ouzan et al. 2003; Shehab et al. 2001). In Canada the delivery of CD-ROM based medical programmes to remote rural primary care physicians dramatically improved their knowledge and confidence in managing patients with hepatitis C (Stein et al. 2002). The hepatitis C detection rates have increased since the French Health Insurance Fund set up open access clinics for HCV screening (Zdanuk et al. 2001). Hence other health care services have been able to improve knowledge about HCV in the community (Pradat et al. 2001; Fischer et al. 2000)

and we hope that in UK the Department of Health will recognize that its current information campaign has not had the desired effect as yet and will go on to produce and audit further educational campaigns.

6.5 Improving General Practitioners

Knowledge of Chronic Hepatitis C Infection

Chronic hepatitis C is a significant health care problem in the UK and current figures indicate that the majority of infected individuals are unaware that they are infected (WHO 1999; Hepatitis C Strategy for England). To improve diagnosis and treatment rates among groups at high risk of infection it is essential that primary care physicians are well informed about this relatively new disease. Our previous survey showed that following the UK Department of Health information campaign in HCV the knowledge of East London GPs was still poor (D'Souza et al. 2004). To address this issue we launched an information campaign and we find that this has effectively increased GPs awareness and knowledge of chronic HCV, at least in the short term.

However we only assessed knowledge immediately after completion of the education session and, clearly, such a testing regime does not address knowledge retention (Fischer et al. 2000; Davis et al. 1995; Dillman et al. 1978) and it will be important to repeat our survey in the future to assess long term knowledge retention. It was clear from our survey that knowledge retention from both the lunch time educational sessions and the postal information leaflet were similar but the lunchtime sessions were relatively poorly attended. The lunchtime educational sessions were relatively labour intensive and therefore it may be more efficient to inform general practitioners using postal information leaflets. However in this study GPs were asked to complete a questionnaire after reading the leaflet and this may have contributed to the knowledge retention that was achieved.

Educational programmes and postal surveys can serve as a model to provide educational programmes to health professionals. In remote areas of Australia practitioners are able to receive information via the internet concerning HCV and discuss cases with hospital based clinicians (Zdanuk et al. 2001) and literature for non-English speaking patients is available (Dev et al. 2002) . Thus, educational initiatives and

guidelines specifically designed for GPs are needed to assist them in identifying patients at risk, conducting initial diagnostic evaluation, and initiating appropriate referrals, so patients with hepatitis C can be correctly diagnosed and treated while they are still in the early stages of disease. The best method of providing information should be provided and GPs may wish to become more directly involved in shared care arrangements with specialists that will broaden education and treatment opportunities for HCV-infected patients.

6.6 Assessment of the safety and efficacy of ONP-17 in the treatment of Hepatitis C virus related symptoms.

The use of Chinese herbal medicine is an exciting area of development in this field and the studies so far have been inconclusive. Before October 2002 there were seven randomised clinical studies of traditional Chinese medicine in the treatment of hepatitis C (Han et al. 1997; Pei et al. 1996; Batey et al. 1998; Chen et al. 1998; Jiang et al. 1999; Xiao et

al. 1999; Yu et al. 1995). Six of these studies were of poor methodological design. The best quality trial compared Chinese herbs with placebo and found a significant reduction in ALT levels during treatment, but no decreases in HCV RNA levels (Batey et al. 1998). However four patients on the active herbal preparation developed palpitations, diarrhoea and abdominal discomfort. Recently a well-designed trial by Jakkula et al. (2004) randomised 45 patients to receive a combination of Chinese herbal medications or placebo for 12 weeks. Participants randomised to active treatment received a combination of 10 traditional Chinese medicinal herbs (Radix astragal, Radix acanthopanax, Radix bupleur, Radix et tuber curcumae, Rhizoma polygonum, Radix glycyrrhiza, Radix isatis, Radix paeoniae rubra, Radix salviae, and Herba tarazaci). Outcome measures were changes in health-related quality of life using the validated Hepatitis Quality of Life Questionnaire and alanine aminotransferase, HCV viral load and adverse effects. There was no improvement in quality of life using the health questionnaire, liver chemistry results or viral load. No significant adverse effects were observed.

In our study we used two herbs namely Astragalus and Salvia root which have been investigated previously. In addition we

added Schisandra fruits and milk thistle. Unfortunately recruitment was poor despite extending the study to another site and advertising in a local newspaper. The study had to be terminated early as the herbal company were worried about the efficacy of the herbal preparation. There were a lot of fallouts during the study. As a result there was insufficient power in the study to see significant differences between the two arms. The results from this study show that recruitment and retention in trials of alternative therapies are problematic.

CHAPTER 7: CONCLUSIONS AND SUMMARY

In this thesis, I have explored the natural history of hepatitis C in the North-East of London. Studies on prolonged (>30 years) follow up of chronically infected patients have not been performed. Seeff L. (2002) has used extrapolations from the current natural history studies to estimate long-term natural history. If the progression is linear then approximately 50% of infected patients will develop cirrhosis after 50 years. If the rate of fibrosis progression decreases with increasing duration of infection then the proportion of patients who develop cirrhosis after 50 years will be reduced and, conversely, if the rate of disease progression increases with age then a greater proportion of infected individuals will go on to develop cirrhosis. Our groups' study on natural history addresses the outcome of chronic HCV infection up to 60 years after infection and reports on which of the outcomes described by Seeff L. (2002) are correct.

Our study included elderly Asian patients infected in childhood. Fibrosis progression, measured in several hundred single biopsies and in nearly 50 paired liver biopsies, was the

same in Asian and Caucasian patients. We found that the longer the exposure to hepatitis C virus the greater the degree of fibrosis progression. A methodological limitation with this study was the use of cross-sectional, observational data to estimate longitudinal parameters. There was an absence of multiple liver biopsies in the same patient. The other limitation is the presumed variability in the estimate for duration of exposure. In the absence of prospective follow-up of patients from the date of contamination of HCV until the date of the liver biopsy, any estimate of the duration of infection relies on patient history.

We confirm that factors such as age and alcohol excess as reported by Poynard et al. (1997) are associated with faster fibrosis progression. It must be remembered that alcohol consumption was a difficult factor to assess in this study and the risk of variability is great. However, all HCV-infected patients should strongly be advised to limit or cease alcohol consumption.

Our study did not demonstrate significant differences between fibrosis stage and sex, which may be due to the large majority of postmenopausal women in the cohort. Menopause appears to be associated with accelerated liver fibrosis progression in

HCV- infected women, an effect that may be prevented by hormone replacement treatment (Di Martino et al. 2004; Codes et al. 2006). Studies have suggested a protective effect of oestrogens on fibrogenesis by inhibition of stellate cell proliferation (Bissekk D. 1999; Ashcroft et al. 1997).

Despite the small sample of paired biopsies moderate to severe steatosis was associated with rapid fibrosis progression. However, no association was found between steatosis and fibrosis in single liver biopsies and the reasons for this may be the progressive nature of development from steatosis to fibrosis which would be missed on a single biopsy. Secondly, there are two types of steatosis – metabolic and genotype dependent and due to the small numbers these groups were not analysed separately.

Insulin resistance was associated with fibrosis progression, however serum ferritin and hepatic iron played no role.

As to the future, I see many potential areas of development of my research. Further longitudinal studies of untreated patients with chronic hepatitis C would be extremely useful. Studies are required to examine the pattern of natural history of HCV disease progression in persons infected for at least four

decades such as those infected as infants. However, as the course of chronic hepatitis C is markedly protracted and because treatment is now almost universal, these are extremely unlikely to be performed in the future. Further research activity should be directed toward a better understanding of the pathogenesis of disease progression. One example is the effect of age at the time of infection and of the process of ageing (its effects on the liver as well as the immune system) in the patient with established chronic hepatitis C infection. Ethnic minorities are often underrepresented in treatment studies and studies to determine the applicability of currently accepted treatment need to be performed.

The mechanisms by which insulin resistance promotes hepatic fibrogenesis should be explored. Strategies to improve insulin sensitivity should be developed as they will complement antiviral therapy in the management of chronic HCV infection, and also will prevent fibrosis progression in non-responders to interferon-based antiviral therapies.

Furthermore, research into the development of non-invasive dynamic measures of hepatic fibrosis is strongly encouraged.

The fibrosis probability index proposed by Sud et al. (2004) based on five parameters (insulin resistance, age, aspartate aminotransferase (AST), total cholesterol level, and past alcohol intake) should be further validated directly with the gold standard, fibrosis stage of the liver biopsy. The association between chronic HCV infection and type 2 diabetes mellitus is highly probable, but the biological mechanism still needs to be addressed. Prospective studies assessing whether HCV associated type 2 diabetes mellitus can be treated with antiviral therapy need to be performed.

In the second part of the thesis we assess the knowledge of General Practitioners (GPs) in the East End of London following the Department of Health campaign. The knowledge from the two regions in the East End of London were similar. Therefore it is likely to assume that this is a good reflection of the knowledge in the UK. Our survey clearly revealed that significant deficits in knowledge regarding hepatitis C among GPs continue to exist despite the provision of a 45 page booklet discussing all aspects of HCV . This booklet was sent to every GP in the United Kingdom by the Department of Health approximately 10 months prior to our questionnaire. Several factors may contribute to the knowledge deficits regarding hepatitis C among GPs: the rapid evolution of new information, the lack of established guidelines catered for GPs, the need for GPs to keep up with advances in many diverse areas of medicine, and the possible ineffectiveness of current educational resources.

We endeavored to improve GPs knowledge by inviting them to educational meetings on HCV or by improving their knowledge via a postal service. This strategy has met with success, although an immediate post-test does not address knowledge retention. GPs are the 'gate-keepers' to the NHS and will be

the first health care professionals to have the opportunity to screen high risk patients for HCV. Thus, educational initiatives and guidelines specifically designed for GPs are needed to assist them in identifying patients at risk, conducting initial diagnostic evaluation, and initiating appropriate referrals, so patients with hepatitis C can be correctly diagnosed and treated while they are still in the early stages of disease. GPs may wish to become more directly involved in shared care arrangements with specialists that will broaden education and treatment opportunities for HCV-infected patients. The Department of Health has launched a second campaign for tackling hepatitis C in the UK. This includes an information pack for general practitioners. This will assist professionals recognise the main risk factors, advise on how to avoid infection, discuss testing with patients, if appropriate, and follow-up if they discover patients are infected. This will be followed by education of the public about avoiding the risk of infection and considering testing where appropriate.

In the final part of the thesis we assess treatment of hepatitis C. Patients with raised insulin resistance should be advised about lifestyle modifications (lose weight if obese, regular

exercise) to achieve the best chance of success from combination therapy for HCV.

Studies assessing the effectiveness of Chinese herbal treatment for hepatitis C have been inconclusive and poorly designed. Our randomised double blind controlled study on Chinese herbal treatment had problems with recruitment and a lot of patients withdrew during the study from both arms. As a result there was insufficient power in the study to see significant differences between the two arms. Subjectively there was some improvement in ALT levels and quality of life data. However, this study highlights the fact that recruitment and retention in trials of alternative therapies are very difficult.

As to the future, I see many potential areas of development of my research. Further longitudinal studies of untreated patients with chronic hepatitis C would be extremely useful. However, as the course of chronic hepatitis C is markedly protracted and because treatment is now almost universal, these are extremely unlikely to be performed in the future. Further research activity should be directed toward a better understanding of the pathogenesis of disease progression. One example is the effect of age at the time of infection and of

the process of aging (its effects on the liver as well as the immune system) in the patient with established chronic hepatitis C infection.

The mechanisms by which insulin resistance promotes hepatic fibrogenesis should be explored. Strategies to improve insulin sensitivity should be developed as they will complement anti-viral therapy in the management of chronic HCV infection, and also will prevent fibrosis progression in non-responders to interferon-based antiviral therapies.

Furthermore, the fibrosis probability index proposed by Sud et al. (2004) based on five parameters (insulin resistance, age, aspartate aminotransferase (AST), total cholesterol level, and past alcohol intake) should be further validated directly with the gold standard, fibrosis stage of the liver biopsy. The association between chronic HCV infection and type 2 diabetes mellitus is highly probable, but the biological mechanism still needs to be addressed. Prospective studies assessing whether HCV associated type 2 diabetes mellitus can be treated with antiviral therapy need to be performed.

General practitioners awareness of hepatitis C is clearly low and we await the outcome of the governments' second campaign launched on the 29th June 2004 whose objectives

are to improve the knowledge amongst general practitioners and the general public.

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APPENDIX

Table 3 – The Short Form 36 Survey Questionnaire.

Fatigue Symptom Scale (FSS)

Please read each statement below and think about how well it has applied to you over the past week. Then circle the corresponding number to the left: a low value indicates that the statement is not very appropriate, whereas a high value indicates agreement with the statement.

1. My motivation is lower when I am fatigued.	1	2	3	4	5	6	7
2. Exercise brings on my fatigue.	1	2	3	4	5	6	7
3. I am easily fatigued.	1	2	3	4	5	6	7
4. Fatigue interferes with my physical functioning.	1	2	3	4	5	6	7
5. Fatigue causes frequent problems for me.	1	2	3	4	5	6	7
6. My fatigue prevents sustained physical functioning	1	2	3	4	5	6	7
7. Fatigue intereferes with carrying out certain duties and responsibilities.	1	2	3	4	5	6	7
8. Fatigue is among my three most disabling symptoms.	1	2	3	4	5	6	7
9. Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7

SF-36 Health Survey

INSTRUCTIONS: This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is:

- Excellent1
- Very good.....2
- Good3
- Fair.....4
- Poor.....5

2. Compared to one year ago, how would you rate your health in general now?

- Excellent1
- Very good.....2
- Good3
- Fair.....4
- Poor.....5

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

<u>ACTIVITIES</u>	Yes, Limited a Lot	Yes, Limited a Little	No, Not Limited At All
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf.	1	2	3
c. Lifting or carrying groceries	1	2	3
d. Climbing several flights of stairs	1	2	3
e. Climbing one flight of stairs	1	2	3
f. Bending, kneeling or stooping	1	2	3
g. Walking more than a mile	1	2	3
h. Walking half a mile	1	2	3
i. Walking one hundred yards	1	2	3
j. Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	Yes	No
a. Cut down on the amount of time you spent on work or other activities	1	2
b. Accomplished less than you would like	1	2
c. Were limited in the kind of work or other activities	1	2
d. Had difficulty performing the work or other activities (for example, it took extra effort)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	Yes	No
a. Cut down on the amount of time you spent on work or other activities	1	2
b. Accomplished less than you would like	1	2
c. Did not do work or other activities as carefully as usual	1	2

6. During the past 4 weeks to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

Not at all.....1
 Slightly.....2
 Moderately.....3
 Quite a bit.....4
 Extremely.....5

7. How much bodily pain have you had during the past 4 weeks?

Not at all.....1
 Slightly.....2
 Moderately.....3
 Quite a bit.....4
 Extremely.....5

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework) ?

Not at all.....1
 Slightly.....2
 Moderately.....3
 Quite a bit.....4
 Extremely.....5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks-

	All of the Time	Most of the Time	A Good Bit of The Time	Some Of the Time	A Little of the Time	None of the Time
a. Did you feel full of life?	1	2	3	4	5	6
b. Have you been a very nervous person?	1	2	3	4	5	6
c. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
d. Have you felt calm and peaceful?	1	2	3	4	5	6
e. Did you have a lot of energy?	1	2	3	4	5	6
f. Have you felt downhearted and low?	1	2	3	4	5	6
g. Did you feel worn out?	1	2	3	4	5	6
h. Have you been a happy person?	1	2	3	4	5	6
i. Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problem interfered with your social activities (like visiting with friends, relatives, etc) ?

- All of the time.....1
- Most of the time.....2
- Some of the time.....3
- A little of the time.....4
- None of the time.....5

11. How TRUE or FALSE is each of the following statements for you?

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
a. I seem to get ill more easily than other people	1	2	3	4	5
b. I am as healthy as anybody I know	1	2	3	4	5
c. I expect my health to get worse	1	2	3	4	5
d. My health is excellent	1	2	3	4	5

Sickness Impact Profile

Name of Questionnaire

Sickness Impact Profile (SIP)

Description

Generic measure used to evaluate the impact of disease on both physical and emotional functioning. Patients are asked to respond to the items as they are on that day. The measure has also been used in patients with COPD and asthma.

Developer

B Gilson & M Bergner, 1976 (revised 1981)

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Cost & availability

Fee. Contact the Medical Outcomes Trust for information and permission to use.

Administration

Self, interview

Time to complete

20-30 minutes

Number of items

136

Domains & categories

2 overall domains (physical and psychosocial)

12 categories (sleep and rest, eating, work, home management, recreation and pastimes, ambulation, mobility, body care and movement, social interaction, alertness behavior, emotional behavior, communication)

Name of categories/domains

Physical: ambulation, mobility, body care and movement

Psychosocial: social interaction, communication, alertness behavior, emotional behavior; sleep and rest, eating, home management, recreation and pastimes, employment

An overall score can also be computed.

Scaling of items

Respondents check the items that apply to them (yes/no).

Scoring

Overall score, 2 domain scores, and 12 category scores; items are weighted according to a standardized weighting scheme.

Reliability

a. Test-retest/reproducibility Reported

b. Internal consistency Reported

Validity

Compared to clinical indices of asthma and disease-specific quality of life measures (AQLQ-Juniper, SGRQ, AQLQ-Marks, symptoms & clinical efficacy)

Responsiveness

Reported

Minimally important difference

Not determined.

Research use

Reported, including clinical trial.

Clinical use

Not reported.

Language

Original: English

Translations: None currently authorized by the copyright holder.

Guidelines for translation are available through the Medical Outcomes Trust or through Johns Hopkins University.

Nottingham Health Profile

VARIABLES MEASURED:

Subjective health status in a number of areas: physical mobility, pain, sleep, emotional reactions, social isolation and energy

PURPOSE:

To provide direct access to perceived health status, which can give basic information required for determining goals in health care and assessing how far they have been met.

DESCRIPTION/DEVELOPMENT:

There are six sections: physical mobility, pain, sleep, emotional reactions, social isolation, and energy. Each contains a number of statements to which the respondent answers yes or no. Statements in each section are weighted –a score of 100 indicates the presence of all possible perceived problems in that section, while a score of zero on a section indicates the respondent does not believe he/she to have any of the problems in that section.

ADMINISTRATION: Self-administered.

Dear Colleague,

Re: Hepatitis C Questionnaire

Chronic hepatitis C is an increasing health problem. The Department of Health has recently addressed this issue with the publication of its 'Hepatitis C strategy for England' in August 2002 and information about this virus has been sent to general practitioners. The strategy document sets out proposals to improve the effectiveness of prevention, diagnosis and treatment services for hepatitis C.

We are currently conducting a survey amongst general practitioners about hepatitis C. We want to assess your views of this condition and the services available for patients in your area. The survey may help to improve local services for hepatitis C. We would be grateful if you would take part and respond to the following questions. We thank you for your assistance in this study.

With kind regards

Please circle the right answer or write down a single answer to the following questions.

- | | | | |
|----|--|-----|----|
| 1. | Patients who are hepatitis C antibody positive no longer have active disease | Yes | No |
| 2. | Hepatitis C is common (> 40 %) in those who have ever used intravenous drugs | Yes | No |
| 3. | The prevalence of HIV is higher than hepatitis C in the East End | Yes | No |
| 5. | More than 50 % of pregnant women infect their children | Yes | No |
| 6. | Blood transfusion in the 1990s carries a high risk of transmitting hepatitis C | Yes | No |
| 6. | In patients using intravenous drugs, transmission of hepatitis C can be prevented by meticulous hygiene and non- | Yes | No |

sharing of all paraphenalia

7. Therapy for hepatitis C is effective in more than 50% of patients treated Yes No

8. Out of 100 patients with chronic hepatitis C which proportion of patients will develop cirrhosis in 30 years

5 10 20 50 70

9. Where do you refer your patients with hepatitis C

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10. What changes do you feel would improve the services offered to your patients with hepatitis C in your area

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Thank you for your assistance in this pilot study, your time is much appreciated. Please return to the following address in the enclosed prepaid envelope please or return this by fax to 020 7882 7241.

Dear Colleague

We received an excellent response following our questionnaire in hepatitis C and we would like to thank all our colleagues in general practice for their wholehearted support in this project. The majority of GPs called for a one page summary of the Department of Health booklet titled 'Hepatitis C strategy for England'. A one page summary has therefore been enclosed and we would be grateful if after reading this you could fill in the enclosed questionnaire and fax back to **020 7882 7241**. Hopefully if this proves to be useful we will send the information sheet out to the rest of our colleagues in the East End. The survey may help improve local services for hepatitis C.

Thank you for your assistance.

With kind regards

Hepatitis C Information Sheet

In the UK the number of people chronically infected with hepatitis C is estimated to be around 200 000, the majority of whom are asymptomatic and unaware of their infection. HCV is a common infection and more common than HIV. Our aims together with our general practitioner colleagues is to prevent new cases of HCV infection and to reduce the risks of those infected progressing to severe liver disease

Transmission:- In the UK HCV is mainly transmitted by injecting drug users sharing blood contaminated equipment. Other less efficient routes of transmission include sexual intercourse (<5%) and from mother to baby (<5%). Blood transfusion prior to 1991 carried a high risk of transmitting hepatitis C.

Natural History:- 20% of patients who have been infected with HCV in 30 years will develop cirrhosis with its inherent complications.

High risk groups:- There should be increased testing of at risk groups by GPs. These include the following groups:- injecting drug users (past or present), history of receiving blood or blood products prior to 1991, organ transplant prior to 1991, needlestick injury, regular sexual partners of patients with HCV and patients being investigated for abnormal liver function tests.

Testing :- patients suspected of having HCV should be screened with a hepatitis C antibody test. Those who are positive should be referred to the hospital for further testing (PCR HCV).

Treatment:- Chronic infection with HCV can lead to severe liver disease and liver cancer, but moderate to severe disease can be treated successfully in at least 50% of people. Patients are treated with interferon and ribavirin.

Prevention:- There should be increased public awareness, needle exchange services and teaching drug users about meticulous hygiene and nonsharing of paraphenelia.

Information on HCV should also be freely available, including prisons.

After reading this information sheet please fill in the questionnaire and return this by fax to 020 7882 7241.