The Long Term Outcomes of Community Acquired Hepatitis C Infection in a Cohort with Sera Stored from 1971 – 1975

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By

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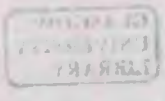

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ABSTRACT

Aim: To examine the long term outcomes of hepatitis C virus (HCV) infection in a cohort of patients admitted with acute viral hepatitis between 1971 and 1975. The availability of stored sera enabled testing to identify anti-HCV positive subjects.

Methods: A retrospective cohort study design was chosen. The exposure of interest was the presence of anti-HCV in stored sera. Systematic approaches were used to locate the cohort and outcomes assessed with the SF-36 questionnaire, a study specific questionnaire and by clinical, serological, virological and biochemical assessment.

Results: Sixteen percent (n=238) of the cohort tested anti-HCV positive and formed the exposed group. The unexposed group (n=476) was randomly selected from those who were anti-HCV negative. Complete follow up was achieved on 98 anti- HCV positive individuals and 202 negatives. At 25 years follow-up, 54% of the anti-HCV positive group had evidence of chronic HCV infection (both anti-HCV and HCV RNA positive). Of those chronically infected 69% had elevated serum ALT levels, but only 8% had progressed to overt cirrhosis and no cases of HCC were identified. Anti-HCV positive subjects were 4 times more likely to have died from suicide or drug overdose than from HCV related disease. Quality of life measures were significantly reduced in the exposed group and significantly worse for anti-HCV positive individuals aware of their serostatus, compared to those unaware.

Discussion: The reduced quality of life in those aware of their HCV diagnosis may be partially an effect of 'labelling'. Anti-HCV positive study subjects were at increased risk of liver related pathology after 25 years follow up, but few had progressed to overt cirrhotic liver disease. Excess mortality in the anti-HCV positive group was not due to liver disease. This suggests that the natural history of community acquired HCV may be more benign than previously thought.

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2. Rodger AJ, Thomson JA, Thompson SC, Jolley D, Mijch AM, Lanigan A, et al. Assessment of long-term outcomes of hepatitis C virus infection in a cohort of patients with acute hepatitis in 1971-1975: results of a pilot study. *J Gastroenterol Hepatol* 1999; 14 (3): 269-73.

3. Rodger AJ, Jolley D, Thompson SC, Lanigan A, Crofts N. The impact of diagnosis of hepatitis C virus on quality of life. *Hepatology* 1999; 30 (5): 1299-301.

4. Rodger AJ, Roberts S, Lanigan A, Bowden S, Brown T, Crofts N. Assessment of long-term outcomes of community-acquired hepatitis C infection in a cohort with sera stored from 1971 to 1975. *Hepatology* 2000; 32 (3): 582-7.

5. Rodger AJ, Lanigan A, Hocking J, Crofts N. Methodological Approaches to Tracing a Cohort of Individuals Admitted with Hepatitis from 1971 to 1975. [In press] *ANJPH*

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LIST OF ABBREVIATIONS

αFP	Alpha-fetoprotein
AIHW	Australian Institute of Health and Welfare
Anti-HAV	Hepatitis A Virus Antibody
Anti-HCV	Hepatitis C Virus Antibody
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
BBV	Blood Borne Viruses
CAI	Chronic Active Hepatitis
CI	Confidence Interval
DNA	Deoxyribonucleic Acid.
ELISA	Enzyme Linked Immunosorbent Assay
FHF	Fulminant Hepatic Failure
FIDH	Fairfield Infectious Diseases Hospital
GGT	Gamma Glutamyl Transpeptidase
GP	General Practitioner
HA	Hyaluronate
HAI	Histological Activity Index
HAIgM	Hepatitis A IgM
HAV	Hepatitis A Virus
HBcAb	Hepatitis B Core Antibody
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Lymphocyte Antigen

ICD	International Classification of Disease
IDU	Injecting Drug Use/r
IgG	Human Immunoglobulin G
IM	Intramuscular
IQ	Interquartile
IV	Intravenous
LFTs	Liver Function Tests
NANBH	Non-A, Non-B Hepatitis
NHIC	National Health Insurance Commission
NHLBI	National Heart and Lung Blood Institute
NR	Normal Range
OD	Optical Density
PCR	Polymerase Chain Reaction
PY	Person Years
QC	Quality Control
QOL	Quality of Life
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
SD	Standard Deviation
SE	Standard Error
SF-36	Short Form 36
SMR	Standardised Mortality Ratio
STD	Sexually Transmissible Disease
TA	Transfusion Associated
VIDRL	Victorian Infectious Diseases Reference Laboratory

THE ROLE OF THE AUTHOR

I was integrally involved in all aspects of the study except for retrieval and testing of the stored sera which occurred in 1996. From 1997 to study completion in 2000 I was, as the sole study investigator, responsible for all aspects of the project.

My role involved study design, development of study instruments, tracing subjects for the study (in association with Anna Lanigan) and assessment of outcomes. I undertook the clinical assessment of all subjects seen in the study centre (the Alfred Hospital), undertook all data analysis and wrote all papers relating to the study. From 1997 to date I have written all grant applications to secure funding for the study.

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Lastly, I would like to thank my husband and son, Craig and Alexander, and it is to them that this thesis is dedicated

CHAPTER 1: INTRODUCTION

1.1 Background

The natural history of Hepatitis C Virus (HCV) remains unclear. Review of available literature demonstrates that most data published are either based on studies reporting on transfusion acquired cases or cross-sectional studies, mainly from tertiary referral liver centres with their inevitable referral and selection bias in estimating proportions and rates of disease progression. This data is currently used to project HCV disease burden on a population and individual basis despite the fact that in most industrialised nations the principal ongoing route of infection with HCV is community acquired, principally through injecting drug use (IDU).

There is a pressing need to establish the natural history of community acquired - as opposed to transfusion acquired - HCV infection, and to provide reasonable estimates of disease progression as well as long term morbidity and mortality associated with chronic community acquired HCV infection. However there are methodological difficulties associated with conducting these studies. Firstly, detection of newly acquired infection is problematic as a small number of cases appear to display clinical illness and it is not yet possible to identify acute HCV by an IgM antibody response. A further issue impeding natural history studies has been the difficulty in gaining access to and following for long periods groups at high risk of infection, such as injecting drug users (IDUs). In addition chronic HCV runs a protracted and highly variable course before the development of adverse outcomes and it may be that four or five decades of follow up are required to ascertain fully the impact of HCV infection.

The estimated risk of progression to serious sequelae of HCV infection differs depending upon the populations studied, the methods used and the period of follow up. Transfusion related studies report rates of progression to cirrhosis of over 20% at 20 years. Liver clinic studies

estimate similar or higher rates of progression to cirrhosis, although it is likely that such series suffer from considerable referral bias. Studies in anti-D recipients indicate that in certain populations the progression of HCV to cirrhosis may be significantly less than the estimates from transfusion associated non-A, non-B hepatitis (TA-NANBH) or liver clinic studies.

There have been very few population-based studies in IDUs and none with follow up of any significant length. Natural history estimates from such studies are essential as they represent more closely current HCV infected populations.

Our planned study was able to overcome many of the problems traditionally associated with HCV natural history studies. The population involved has community acquired disease - predominantly through IDU, the length of follow up will be sufficient for many of the long-term consequences of HCV to arise and the duration of disease is documented clearly through testing of stored sera from the early 1970s.

1.2 Aim and Objectives

Aim

The aim of this study is to clarify the burden of disease, disability and death associated with community acquired HCV 25 years after initial infection.

Our hypothesis was that the natural history of community acquired HCV is more benign than that associated with other modes of transmission, principally transfusion acquired infection. We estimated that the rate of progression to cirrhotic liver disease in subjects with community acquired infection, would be at least half that observed in those infected through transfusion of contaminated blood products.

Objectives

Study objectives were to:

1. Test stored sera from 1971 to 1975 at FIDH for serological hepatitis markers.

2. Identify exposed (anti-HCV positive) subjects and unexposed (anti-HCV negative) cohort subjects.

3. Obtain contact information recorded in the original FIDH medical record and systematically apply tracing methods to trace and recruit cohort members to the study 25 years after exposure to HCV.

4. Assess the health status of study members with regard to mid and long term sequelae of chronic HCV.

5. Document progression to adverse outcomes - including death, liver related morbidity (chronic hepatitis, cirrhosis and hepatocellular carcinoma [HCC]) and reduced quality of life (QOL).

6. Explore associations with adverse outcomes - virus related, host related and other.

7. Measure QOL in study subjects and explore the impact of knowledge of HCV serostatus on QOL.

CHAPTER 2: LITERATURE REVIEW

2.1 Genetic Variation of Hepatitis C Virus

HCV is a single-stranded ribonucleic acid (RNA) virus, which is a member of the flaviviridae family. Viral replication occurs through an RNA dependent polymerase which results in the rapid development of diverse, but related quasispecies within an infected person and presents a major challenge to immune-mediated control of HCV.[1]

HCV isolates demonstrate considerable global genetic variation with the genomic sequences of the most distantly related isolates varying by as much as 35%. It is generally accepted that, based on differences of its non-structural and core proteins, HCV has evolved into six major genotypes with a further 50 subdivisions or subtypes.[2]

Genotypes are identifiable within particular geographic distributions, with the distribution of HCV genotypes in Australia similar to Europe and North America.[3] Isolates of genotype 1, 2 and 3 are widely distributed, but significant variation is seen in the subtypes. Within genotype 1, subtype 1a is common in western Europe and North America whereas subtype 1b is more common in Japan and southern Europe. Genotype 3a is seen commonly in younger western populations, especially IDUs, whereas subtypes 3c-3f are found in Nepal and 3b in Japan, Thailand and Indonesia. Genotype 4 is found in Africa, except for South Africa, where genotype 5 predominates and genotype 6 is seen in Asia.[4] [5] [2] In Australia, genotypes 1a and 3a are the most frequent isolates.[6] Genotypes 4 to 6 are uncommon in Australia, and are mainly represented by immigrant groups from areas where such genotypes predominate.[7]

Genotypes also vary by mode of acquisition, with those contracting HCV via transfusion more likely to be infected with subtype 1b, whereas those infected via IDU tend to be infected with

3a. There is a trend therefore that younger people are more likely to be infected with subtype 3a, and older people with subtype 1b.[4 8 2]

Table 2.1 Distribution of HCV genotypes in Australia [6]

Genotype	%
1	
1a	23
1b	16
Undefined	16
2	
2a	3
2b	1
Undefined	3
3	
3a	38
3b	<1
4	
4a	<1
Undefined	<1

The influence of genotype on outcome of treatment with interferon is well established,[9 10] although its influence on transmission or natural history remains controversial.

2.2 Diagnostic Tests for Hepatitis C Virus Infection

Diagnostic tests for HCV are divided into serological assays for antibodies and molecular tests for viral particles.

2.2.1 Antibody Tests

Following the development of HCV antibody (anti-HCV) assays in 1991,[11] there have been three generations of modifications made to improve the sensitivity and specificity of these assays. HCV assays are based on recombinant or synthetic antigens, but the first generation assays only contained recombinant antigens from the NS4 region of the HCV genome. The sensitivity of these early generation assays was significantly lower for individuals with genotypes 2b and 3a than for those with genotypes 1a or 1b.[12] The second generation assays reduced the number of false-reactive results by incorporating recombinant antigens derived from the core, NS3 and NS4 regions.[13] [14] The addition of such antigens also increased the sensitivity of the assays, as antibodies to NS3 are generally the first to appear during seroconversion.[14] Third generation assays have included NS5 antigens, with some improvement in sensitivity and specificity.[15] [16] Some individuals demonstrate false-reactivity to NS5 which can decrease the specificity of certain assays.[17]

False-negative results occur in infected individuals who may be in the 'window period' prior to seroconversion and have also been documented in subjects with Human Immunodeficiency Virus (HIV) infection.[18] False-positive reactions have been recorded in subjects with syphilis, rheumatoid arthritis and systemic lupus erythematosis.[19-21]

2.2.2 Direct Measurement of Viral RNA

The only method to directly measure HCV infection is by the detection of viral RNA, typically by reverse transcriptase polymerase chain reaction (PCR), although other methods include in situ hybridisation and branched deoxyribonucleic acid (DNA) amplification assays. The DNA amplification assay is easy to perform and reproducible, but is significantly less sensitive than PCR assays.[22]

Assays can be qualitative or quantitative. Qualitative tests are based on PCR and have a lower limit of detection of 100 copies of RNA per ml.[23] Genotype and viral load results are used to predict the outcome of interferon or combination therapy.[9 10] PCR tests also aid identification of infection in individuals who are in the acute stage of infection, but with no detectable antibodies.[24]

The absence of PCR detectable viraemia appears to indicate an extremely low risk of HCV transmission by any route. A systematic review by Dore *et al*[25] identified 29 published studies of HCV transmission. They found that in 1148 subjects exposed to anti-HCV and PCR positive sources, 148 cases of transmission occurred, compared with no definite case of transmission among 874 subjects exposed to anti-HCV positive, but PCR negative sources.

2.3 Routes of Transmission and Risk Factors for Hepatitis C Virus Infection

2.3.1 Overview

The primary route of transmission of HCV is through percutaneous exposures to blood and blood products. The most common exposures associated with transmission are transfusion of contaminated blood products and IDU. The relative importance of these two routes of transmission has changed over the past two decades. Blood transfusion, which accounted for a substantial proportion of HCV infections acquired over 15 years ago, accounts rarely for recently acquired infections due to widespread screening of blood donors and products. The prevalence of IDU increased dramatically from the early 1980s and is currently the most common route of transmission of HCV, due principally to the high prevalence of HCV in IDU communities and unsafe injecting practices.

There is a small risk that HCV can be transmitted through sexual contact and also through non-sexual household contact. HCV infection can also occur through vertical transmission, as well as via therapeutic injections, acupuncture, tattooing, and body piercing if poor infection control practices exist.

2.3.2 Injecting Drug Use

Injecting drug users are at risk of a number of infectious diseases, mainly due to the unsterile injecting techniques employed as a consequence of the illegal nature of this activity in most populations.

The prevalence of HCV in IDUs in consistently very high across a wide range of populations studied (*Table 2.2*).[26-37]

Table 2.2 Prevalence of anti-HCV in injecting drug users

Country	Year	Sample size	Study setting	Anti-HCV positive (%)
India [37]	1996	191	Community	98%
China [34]	1994	507	Drug rehabilitation	95%
Australia [32]	1995	1428	Prison entry	64%
New Zealand [31]	1995	116	Treatment centre	84%
Italy [33]	1994	255	Treatment centres	81%
UK, North England [30]	1998	773	Treatment centre	67%
USA, Baltimore [38]	1996	312	Community	77%
USA, San Francisco [26]	1999	308	Young IDUs	45%
Brazil [28]	1999	102	Prison	56%

In other populations, such as sexually transmissible disease (STD) clinic attenders, who are at risk of both blood-borne viruses and STDs, a concomitant history of IDU is a strong risk factor for infection with HCV.[39] Similarly in blood donors found to be anti-HCV positive, IDU is again the commonest association.[40-42]

The strongest association with HCV seroprevalence in IDUs is duration of injecting, and by association, age.[30 35 36 43-51 37 52] However, in certain situations new injectors are at very high risk of infection.[38 45 53] In one study in the USA, injectors who had been injecting for between five to 8 months had an anti-HCV prevalence of 75%.[38] Associations with HCV seroprevalence in these new injectors were; injecting daily, reusing syringes at least once in the past 6 months, injecting the first time with someone > or =5 years older and injecting cocaine or speedball exclusively.

HCV seropositivity is also associated with increased frequency of injecting.[52 43 38] Injecting daily has a strong association with HCV, particularly in new or young IDUs.[38 45] In populations of IDUs with low frequencies of injecting the prevalence of HCV is relatively low. [54]

Several studies have found associations with HCV seropositivity and the injection of certain drugs. [37 52 55 56 45 51 57] HCV is more common in those injecting heroin or other opiates than amphetamines, and where cocaine is commonly injected, there is a very strong association with HCV seropositivity. The explanation for these findings is likely to be the frequency (and cumulative frequency) with which the drugs are injected - cocaine users often inject multiples times per day - or the risk behaviours of certain injecting networks, rather than any characteristics of a particular drug.

The sharing of needles and syringes has also been associated with HCV seropositivity.[37 55 39 31 43 45] However, there is also evidence of HCV exposure in IDUs who do not admit to a history of sharing. A study of young IDUs in a treatment setting in Sydney reported a HCV incidence of 11.9% per year among those who reported never sharing needles and syringes, compared to 30% per year among those who shared.[58] A cohort study of IDUs in the Australian State of Victoria reported an incidence of HCV seroconversion of 16.3 cases per 100 person years (PY) among those who shared, compared to an incidence of 4.3 per 100 PY in those who denied sharing.[59] This raises the concern that HCV can be transmitted amongst IDUs through other injecting behaviours or environmental contamination, not just though the re-use of contaminated needles and syringes. Consequentially the control of HCV becomes more difficult in IDUs. This may partly explain why the prevalence of Human Immunodeficiency Virus (HIV) has remained low in some communities of IDUs who have access to needle exchange programmes, while HCV has spread rapidly.[27]

Hepatitis C has higher average transmission efficiency than HIV. The rate of HCV virus antibody seroconversion among health care workers exposed to needlestick injuries ranges from 1.8 to 9%,[60 110] compared to seroconversion for HIV after needlestick of 0.3%.[61] It may be that HCV can be transmitted between injecting drug users on pieces of injecting equipment other than needles and syringes i.e. swabs, spoons, water or tourniquets. One study in Melbourne studied injecting equipment from 10 different injecting settings for the presence of HCV RNA by PCR and found evidence of HCV RNA on 70% of spoons tested, 67% of swabs and 33% of spoons implying that HCV could be transmitted through this equipment to IDUs who do not share needles.[62]

2.3.3 Contaminated Blood Products

2.3.3.1 Blood Transfusion Recipients

After the development of serological tests for Hepatitis B Virus (HBV) and Hepatitis A Virus (HAV) in the late 1960s and early 1970s, it became apparent that the majority of cases of post transfusion hepatitis were associated with neither.[63] The term non-A non-B hepatitis (NANBH) was developed to describe this type of hepatitis and studies in the mid 1970s reported an incidence of about 20% NANBH post transfusion.[64] Screening for surrogate markers of NANBH (elevated alanine aminotransferase [ALT] and Hepatitis B Core Antibody [HBcAb]) from the mid 1980s reduced the risk of transfusion associated (TA) NANBH by 42%.[65]

The causative agent for post transfusion NANBH was identified after isolation of the HCV virus in 1989 and the subsequent development of an antibody test. Screening using first

generation assays further reduced post-transfusion HCV risk by 85%, and using second-generation assays by 95%.[65 66]

Currently, HCV is rarely transmitted by blood transfusion. In addition to screening tests and the availability of commercial PCR kits, the use of donor interviewing for risk factors and donor deferral has led to a reduction in the risk of acquiring HCV infection from blood transfusion in Australia to 4.27 per million donations, compared with 0.79 per million donations for HIV.[67] This data is comparable to figures observed in other industrialised countries. [67]

2.3.3.2 People with Haemophilia

Receipt of clotting factor concentrates prepared from plasma posed a high risk for HCV infection until effective procedures to inactivate viruses, including HCV, were introduced during 1985-7. Persons with haemophilia who were exposed to non-heat treated clotting factor concentrates before this time have prevalence rates of HCV infection as high as 96%.[68 69] Treatment of blood products from the mid 1980s, and the implementation of HCV screening in 1992, essentially eliminated the risk of contracting HCV through heat-treated blood products although sporadic cases do still occur.[70 71 72] Those who are placed on recombinant clotting factors have no risk of contracting any blood-borne infections. However, this treatment is predominantly given to children and many adults still receive heat-treated blood products.[73]

2.3.3.3 Intravenous Anti-D Immunoglobulin.

Human immunoglobulin G (IgG) preparations are used for a variety of clinical indications. They are usually administered either by the intramuscular route (IM) or by the intravenous route (IV).

Anti-D is manufactured from donations that have high titres of specific antibodies against the Rhesus D antigen and used for the prevention of haemolytic disease of the newborn. Almost all anti-D is manufactured as IM preparations, rather than as IV preparations.

Two known episodes of transmission of HCV by contaminated anti-D occurred in East Germany in 1978 and in Ireland in 1977.[74 75] Both were associated with IV preparations that had been prepared using a procedure that did not include cold ethanol fractionation, which contributes towards the removal and inactivation of viruses. This manufacturing process was used only in Ireland, Germany and Canada.[76]

2.3.4 Sexual Transmission

Inconsistencies exist among studies in this area, but current evidence suggests that sexual practices are not an efficient means of spreading HCV, in comparison to that of HIV and HBV.[77-96]

Wyld *et al.*[96] reported that over an eleven year study period, 40% of heterosexual partners of HCV/HIV co-infected index cases contracted HIV, whereas no one was infected with HCV when sexual contact was the only risk factor. However, when heterosexual contact and IDU were both present, 52% of partners contracted HIV, and 80% HCV.

Thomas *et al*[83] evaluated potential transmission of HCV between STD clinic patients, who denied other risk factors, and their steady partners. Prevalence of HCV infection among male patients with an anti-HCV-positive female partner (7%) was no different than that among males with a negative female partner (8%). However, female patients with an anti-HCV-positive partner were three times more likely to have HCV infection than females with a negative male partner (10% versus 3%,) indicating that similar to other STDs, sexual transmission of HCV from males to females might be more efficient than from females to males. There is generally no significant difference in HCV transmission with different types of sexual behaviour [78]

Risk factors found to be associated with HCV in heterosexual populations include greater number of partners, not using a condom, history of other STDs and having high-risk partners. In homosexual men the risk factors include a history of syphilis, rectal gonorrhoea, anal insertive intercourse with ejaculation, and douche or enema use before anal receptive intercourse.[87]

Long-term sexual partners of haemophiliacs or recipients of HCV-contaminated immunoglobulin preparations rarely become infected.[77 79 85] These discrepancies are not fully understood. Other sexual behaviours or confounding non-sexual transmission routes could play a part. In addition, the stage at infection (and the resultant level of viraemia) may impact on the ability to transmit HCV sexually.

2.3.5 Other Routes

Nosocomial transmission of HCV is possible where poor infection control practices exist. Among hemodialysis patients, anti-HCV prevalence averages 30%, but there are significant

variations by geographic region; from 0% in a centre in Chile,[97] to 28% in an Italian centre,[98] 76% in Indonesia,[99] and 74-100% in Eastern European centres.[100] In the USA, an overall HCV prevalence of about 10% was reported in a 1995 survey of 2,647 dialysis centres,[101] and similar figures have been reported from the UK.[102]

It is likely that factors other than dialysis alone are responsible for a proportion of HCV exposures in these patients. A number of studies have demonstrated an association between anti-HCV status and the total number of transfusions and duration of dialysis.[103-105] It may be that in older subjects much of the HCV exposure is secondary to transfusion of contaminated blood products, although HCV prevalence is higher in non-transfused dialysis patients than the general population suggesting that nosocomial transmission may also occur. Several reports provide evidence of patient-to-patient HCV transmission with environmental blood contamination the most significant factor in transmission. There is no evidence that HCV has been transmitted by re-use of dialysis machines, but being dialysed next to an HCV positive patient is associated with a significant risk of HCV acquisition.[106]

Unsafe therapeutic injections are associated with transmission of HCV and other blood borne pathogens in settings with inadequate infection control practices and can represent a significant source of infection. [107-109]

Transmission of HCV from patients to health care staff also occurs, although in industrialised countries the risk of transmission is low and is related primarily to needle stick injuries. The risk of seroconversion after needlestick is documented at 1.8 to 9%.[60 110] Transmission rates of HCV from health care staff to patient, or patient to patient are very low.

Perinatal exposures account for a small proportion of HCV infections and appear to occur solely in mothers who are HCV RNA positive at the time of delivery. There appears to be no increased risk for vaginal delivery or breast-feeding although data is limited.[25]

Tattooing is also a recognised risk factor for transmission of HCV, [111] although the efficiency with which transmission occurs is unknown. One study tested stored sera obtained from tattooists in Victoria in the mid eighties for anti-HCV and HBV markers in 1995.[112] No sera were HIV positive or Hepatitis B Surface Antigen (HBsAg) positive. Of 35 specimens tested for anti-HCV, only two (5.6%) were positive despite markers of HBV in 48.6% of the same sera.

Despite frequent needlestick injuries reported by tattooists at the time, the low seroprevalence of HCV in this group suggests that HCV may not be transmitted efficiently by intra-dermal inoculation using solid-bore tattooing needles.

2.4 Natural History of Hepatitis C

2.4.1 Acute HCV Infection

Persons with acute HCV infection typically are either asymptomatic or have a mild clinical illness. The pattern of acute illness is documented most thoroughly in transfusion associated cases where 70-80% of cases are anicteric. In a series of 86 consecutive cases of post transfusion hepatitis only 30% were jaundiced with a bilirubin concentration greater than 25mg/dl. Over two thirds had no discernible symptoms and none had protracted severe illness.[113] It is more difficult to ascertain the clinical picture of acute HCV in community acquired infection as individuals are generally identified only if clinically unwell. In one study in community acquired infection 70% of people were icteric, but as the cases were ill and had sought treatment this is not representative of the usual clinical path of acute HCV.[114] Extrapolation from transfusion studies indicates that clinically apparent illness occurs in no more than 25% of HCV infections.[113]

In a study of community acquired HCV infection, antibody was detected between one week and 32 weeks after the onset of dark urine and more than half the patients (54%) had seroconverted by four weeks and a third (34%) developed antibodies within two weeks.[115] In transfusion acquired infection, the average time period from exposure to symptom onset is 6-7 weeks and from exposure to seroconversion 8-9 weeks. Anti-HCV can be detected in 80% of patients within 15 weeks after exposure.[113]

The course of acute hepatitis C is variable, although elevations in serum ALT levels, often in a fluctuating pattern, are its most characteristic feature. Fulminant hepatic failure following acute hepatitis C is rare.[116] Prevalence of HCV RNA in cases of fulminant hepatitis of unknown cause have been reported as being between 0-12% in the USA and Europe, but significantly

higher (45-59%) in Asia.[117] It may be that differences in patient populations or other risk factors account for the differences observed.

It is also possible that HCV acts as a cofactor with other viral agents particularly HBV in acute liver failure. One study from Taiwan reports an incidence of fulminant hepatitis in subjects with underlying HBV infection and superimposed acute HCV of 23%, compared to 2.9% in those with HCV infection alone.[118] An association between acute HAV infection and fulminant hepatitis in subjects with chronic HCV infection was reported by Vento *et al*,[119] although these findings have not been reproduced elsewhere.

2.4.2 Chronic HCV Infection

2.4.2.1 Long Term Outcomes

The significance of HCV lies in its ability to persist in the host and progress – often asymptomatically - over many decades to severe liver disease including chronic hepatitis, cirrhosis and HCC. What is not clear is what proportion of individuals infected will progress and over what time scale. It is likely that chronic hepatitis C does not have one typical course, but can follow a rapidly progressive, slowly progressive or non-progressive route. The determinants of severity are not fully understood and are likely to be multifactorial. Viral, host and environmental factors are known to influence the course of disease. It is important to identify subjects who are likely to progress to cirrhosis – as they would require early treatment and prolonged intensive follow up – as compared to those likely to follow an indolent course.

A wide range of rates of progression to chronicity following acute infection have been reported and there is continuing debate about whether an individual can be a healthy carrier of HCV and

have persistently detectable HCV RNA, but no symptoms or signs of chronic liver disease.[120-123] At the other end of the spectrum are those with persistent viraemia that corresponds to mild hepatitis with some inflammatory or necrotic changes, which can progress over time to cirrhosis.

Liver biopsy samples are graded according to Histological Activity Index (HAI) which grades inflammation on a cumulative 18 point scale based on piecemeal necrosis, confluent necrosis, lobular inflammation and portal inflammation. Fibrosis is classified on the following increasing severity scale – no fibrosis, periportal or portal fibrosis, portal-portal bridging, portal central bridging and probable or definite cirrhosis.[124] In subjects with chronic HCV the extent of fibrosis on biopsy is the most important prognostic factor as it is predictive of progression to cirrhosis.[125] Once an individual develops cirrhosis the symptoms of end stage liver disease can appear including marked fatigue, fluid retention, haemorrhage, jaundice, pruritis and muscle wasting. At this stage the only effective treatment option is liver transplantation.

Hepatitis C is also associated with a range of non-hepatic manifestations including arthritis, keratoconjunctivitis sicca, cryoglobulinaemia and glomerulonephritis.[126 127]

There is also strong evidence to support an association with chronic HCV infection and HCC. The pathogenesis of HCV related HCC is unknown, although virtually all reported subjects with HCV related HCC have established cirrhosis or advanced fibrosis, which is known *per se* to be a precursor of malignancy.[128] It may be that HCV induces malignant transformation indirectly by causing necroinflammatory hepatic disease and continued hepatocyte regeneration, which eventually leads to tumour formation.

The prevalence of anti-HCV in patients with HCC varies in different countries from 72% in Italy,[129] to 34% in the USA,[130] and 13% in Taiwan.[131] In regions where HBV is endemic, a well

documented risk factor for HCC, anti-HCV is present in a smaller proportion of subjects with HCC.

The rates of progression to HCC in cohorts with HCV related cirrhosis in the USA and Europe varies from 1-3%/year.[132-135] The rate of progression in studies from Japan is greater at 4-7%,[136 137 138] for reasons that are not currently clear.

It is evident that, many years after initial infection, HCV can progress to chronic hepatitis, cirrhosis and HCC. However, the estimated risk of progression to long term outcomes of HCV infection differs depending upon the populations studied, the methods used and the period of follow up. Accordingly natural history studies can be grouped broadly into four main types; post transfusion, liver clinic series, population based and newly diagnosed blood donors.

2.4.2.2 Post Transfusion Longitudinal Studies

Several studies have followed subjects prospectively from the onset time of transfusion acquired acute NANBH (*Table 2.3*).[139-143]

Koretz *et al*[140] reported on follow up of subjects 16 years after TA hepatitis in the 1970s and found that the probability of developing clinical evidence of cirrhosis in subjects with HCV (n=64) was 20% after a mean interval of 16 years.

Table 2.3 Progression to cirrhosis in subjects with transfusion associated HCV infection

Country	Year	Sample size	Mean age (years)	Mean duration of infection (years)	Cirrhosis prevalence
USA [140]	1993	64	65	16	17%
USA [139]	1991	39	62	10	20.5%
USA [142]	2001	222	61	25	17%
Italy [143]	1992	135	54	8	15.6%
Sweden [144]	1993	39	55	13	8%

Di-Besceglie *et al* [139] reported on 39 subjects with NANHB post cardiac surgery with morbidity and mortality results for 33 subjects. The mean age at transfusion was 52 years and after a mean follow up time of 9.7 years (range 1 to 24), 20% had developed cirrhosis and 12% end stage live failure. No cases of HCC were reported and no liver related deaths occurred, although one third of subjects died during follow up from other causes (the majority from progressive heart disease).

Tremolada *et al* [143] reported on 135 Italian patients who developed NANBH after cardiac surgery and were followed for a mean of 7.5 years (range 1.1 to 15). Of the 65 subjects who underwent liver biopsy at the end of follow up, 21 (32%) of those biopsied had developed cirrhosis and one (0.7%) HCC. Advanced age at the time of transfusion was significantly associated with progression to cirrhosis.

In Sweden, Mattson *et al* reported on thirty-nine subjects followed prospectively for 13 years after TA-NANBH. After 13 years follow-up, 1.6% of the patients had died of end-stage liver disease, 8% had cirrhosis and three quarters remained chronically infected with HCV.[144]

The American National Heart, Lung and Blood Institute (NHLBI) study of TA-NANBH began in 1987 and followed subjects from five major studies of TA hepatitis in the early 1970s,

to compare outcomes in 222 subjects who developed TA-NANBH (attributable to HCV) to matched transfused subjects from the same studies who had not developed hepatitis. The study reported outcomes at 18 years and 25 years.

At 18 years follow-up, all-cause mortality was 51% for those with HCV, compared with 52% for controls. Mortality related to liver disease in the cases was 3% and 2% in the controls. Thirty percent of those with NANBH/HCV had evidence of chronic hepatitis compared to 1% of the control group, and of those biopsed, two thirds had evidence of chronic active hepatitis (CAH) or cirrhosis.[141]

At 25 years follow up, the all-cause mortality was high in both groups, but no different between cases and controls (67% vs 65%). Liver-related mortality was low (<3%), but significantly higher among the cases than the controls. Among HCV infected patients, 23% had spontaneously lost HCV RNA and the estimate for progression to cirrhosis was 17%.[142]

The advantages of such longitudinal studies is that they began at disease onset and have reasonable follow up periods (25 years in the NHLBI study).[142] In addition, as they include a range of subjects - not just those presenting clinically with liver disease - they include those with milder disease which gives a more complete picture of the spectrum of HCV related disease than studies from tertiary liver centres which only includes those who present with established liver disease. However, due to the advanced age of subjects at the time of HCV infection, and because so many deaths are attributable to non HCV related disease, they are not helpful in determining the outcome of chronic HCV in younger populations or for determining the likely outcome of HCV in the third and fourth decades following infection. In addition, as the majority of those currently infected with HCV are IDUs, and because the natural history of HCV may differ by route of acquisition - due to the variation in the initial infecting dose - the natural history in transfusion recipients may be becoming less relevant.

2.4.2.3 Cross Sectional Studies from Liver Clinics

There have been a number of studies from liver clinics that have estimated progression of liver disease at rates similar or higher to those from longitudinal studies of TA-NANBH (*Table 2.4*).[4] [120 128 132 134 136 137 145-165]

Table 2.4 Progression to cirrhosis in HCV infected subjects referred to liver clinics

Country	Year	Sample size	Mean age (years)	Mean duration infection	Cirrhosis prevalence
Italy [145]	1997	429	50	Unknown	25.4%
Germany [146]	1997	187	43	Unknown	15%
Italy [147]	1998	96	47	Unknown	16.7%
USA [120]	1997	50	51	Unknown	14%
UK [148]	1995	42	37	13 years	4.7%
Europe [5]	1998	292	49	11 years	24%
Spain [149]	1998	253	43	Unknown	6.4%
Japan [166]	1990	205	54	Unknown	35%
Germany [152]	1998	838	49	10 years	16.8%
France (OBSVIRC Group) [154]	1997	1138	44	11 years	12.5%
France (DOSVIRC Group) [154]	1997	607	46	14 years	17%
France (METAVIR Group) [154]	1997	490	49	Unknown	31%
Australia[159]	1995	152	36	Unknown	32%
Japan[136]	1993	333	49	19	18%
USA[161]	1995	131	57	22	51%
UK[165]	1997	140	36	Unknown	7%

Tong *et al*[161] reported on 131 patients with chronic post transfusion HCV referred to a tertiary liver centre with chronic liver disease. The mean age at transfusion was 35 years and 57 years at the time of assessment. The mean period of follow up after referral was 3.9 years (range 1 to 15). On assessment, 46% of the subjects had cirrhosis and 10.6% had HCC. The mean interval

between the time of transfusion to diagnosis of chronic hepatitis was estimated at 13.7 years (range 1 to 42), to cirrhosis of 20.6 years (range 3 to 42) and to HCC of 28.3 years (range 8 to 42). Kiyosawa *et al*[66] studied outcomes in 231 subjects, and reported similar intervals for progression of disease i.e. a mean interval between the date of transfusion to chronic hepatitis, cirrhosis and HCC of 10, 21 and 29 years, respectively. In a subset that underwent successive liver biopsies, sequential progression through chronic hepatitis and cirrhosis to HCC was observed.

Poynard *et al*[54] assessed the natural history of liver fibrosis in three sets of subjects (n=2,235). They identified an estimated median duration of infection to cirrhosis of 30 years, ranging from 13 years in men infected after the age of 40, to 42 years in women who did not drink alcohol and were infected before the age of 40 years. They estimated than in 20 years, over one third of subjects would progress to cirrhosis, but that a further third would not progress for 50 years or never progress. The independent factors that were associated with progression, were age at infection of over 40 years, alcohol consumption greater than 50g per day and male sex.

Takahashi *et al*[36] studied 333 Japanese subjects with chronic hepatitis and observed a mean time interval of 12 years from blood transfusion to histological diagnosis of chronic persistent hepatitis, and of 24 years to liver cirrhosis.

An Australian study studied 342 patients referred to a liver clinic of which 53% were IDUs. The median age of this group was 33 years (range 14 to 47). This group estimated a median time from first exposure to a risk factor for HCV infection to cirrhosis of 18 years (range 5-48), and to chronic hepatitis of 13 years (range 1-26).[159]

The principal problem with such studies is that as they report on those with established liver disease they focus on the more severe end of the disease spectrum and may therefore over-

estimate rates of progression and frequency of liver pathology. Accurate data on the duration of infection is lacking and the majority of studies estimate disease duration by extrapolating backwards to the time of first risk exposure. As they do not include those with asymptomatic HCV or those with established dates of acquisition of infection they cannot give estimates of rates of progression to sequelae. What such studies do provide are estimates of disease progression in those with established liver disease, although as they report on the shortest time period to development of severe outcomes such estimates are also biased.

Fattovitch *et al*[132] reported on the outcome after development of cirrhosis in 384 patients with compensated cirrhosis. During a period of 50 months follow up, 9% of patients died from liver related causes. Survival probability was 96% at 3 years and 91% at 10 years unless decompensation occurred in which case survival dropped to 50% at five years. Two hundred and five patients (53%) were treated with interferon and after adjustment the 5-year estimated survival probability was 96% and 95% for treated and untreated patients, respectively.

2.4.2.4 Population Based Longitudinal Studies

Population based or community studies involve subjects, followed up prospectively, who were not known to have underlying disease at the time they were identified being anti-HCV positive. In such studies the date of exposure is clearly established and in only a minority of cases is the exposure route transfusion of contaminated blood (*Table 2.5*).[74 75 144 167-171]

Table 2.5 Progression to cirrhosis in population based studies of community acquired HCV infection

Country	Dominant transmission route	Sample size	Mean age (years)	Mean duration infection	Cirrhosis prevalence
Sweden[167]	IDU (75%)	10	54	29	20%
Germany[168]	Anti-D IG (100%)	74	34	10	0%
Denmark[169]	Unknown	178	51	23	9%
Ireland [75]	Anti-D IG (100%)	390	45	17	1.9%
Sweden [144]	Unknown	24	41	13	8.3%
Germany [74]	Anti-D IG (100%)	917	44	20	0.4%
USA [171]	Unknown	17	65	45	5.8%
Austria [172]	Plasmapheresis (100%)	20	42	18	20%

In 1999 the Irish Hepatology Research Group reported on 17 year follow up of women who had been infected with HCV through contaminated anti-D immunoglobulin in 1977/78.[75] Of a total of 62,667 screened, 704 were anti-HCV positive, and of these 390 (55%) were also positive for HCV RNA (all genotype 1), indicating a high overall level of clearance of infection. Assessment of those chronically infected revealed normal ALT levels in 45%. Liver biopsy in this group indicated that about half had evidence of hepatic inflammation, but of these only 15 % had bridging fibrosis and only 2% had probable or definite cirrhosis. Two of the seven subjects with cirrhosis reported excessive alcohol consumption. This contrasted considerably

with estimates from liver clinic and transfusion studies of 20% progression to cirrhosis at 20 years.

Similar data were obtained from an East German study that reported on 1,018 women followed for 20 years after receiving contaminated anti-D.[74]After 20 years, 85% were still anti-HCV positive, although only 55% were HCV RNA positive. Only 4 (0.4%) had overt cirrhosis of whom 2 had died, one of fulminant hepatitis B and the other of cirrhosis secondary to alcohol dependence. Histology obtained in 44% of the viraemic women demonstrated hepatitis of minimal to moderate grade in 96%, and septal fibrosis in 3% of the cases.

Both these studies have low rates of progression to cirrhosis and appear to support the opinion that women infected with HCV at a young age have a low rate of progression to cirrhosis. However, although these studies are informative, the natural history of HCV in women infected through contaminated anti-D may not reflect the natural history of HCV acquired through other routes, in particular IDU. In the former case all women were infected with one genotype (1b) on one occasion only, which does not reflect the pattern in IDUs of multiple small exposures to different HCV genotypes.

Three further population based studies that involved small numbers of subjects have reported varying outcomes after infection with HCV. However the relatively low power of such studies limits the usefulness of study findings.

A study from Austria reports outcomes in 20 of 30 subjects infected with HCV (all genotype 1a) at a plasmapheresis centre in 1977/78.[172] The mean age at time of infection was 24 years and 90% were male. Assessment after 18 years in this group indicated that 20% had cirrhosis. A further study with similarly small numbers reports on 29 subjects identified as anti-HCV positive through testing of stored sera from 1969 to 1972.[167] At 25 years follow up, 4 had died

(only one from liver related causes) and 15 were untraceable. Of the remaining 10 cases none had overt cirrhosis.

Seeff *et al* report on the outcome of HCV infection in a group of male airforce recruits who had blood stored between 1948 and 1954.[171] Stored samples were obtained for 8568 subjects and 17 (0.2%) tested anti-HCV positive – of whom 11 were also HCV RNA positive. Follow up revealed that 7 subjects had died (only one of liver disease) and among the 10 remaining alive, liver disease had been identified in only 1. The numbers involved are extremely small, but indicate that mortality and morbidity from liver disease in these groups are probably low.

2.4.2.5 Studies in Blood Donors

Several studies in blood donors found to be anti-HCV positive during pre-donation screening support the picture of a more benign course of HCV. Alter reports on 248 asymptomatic anti-HCV positive blood donors followed prospectively. Among 81 patients who underwent liver biopsy, only 13% had evidence of severe hepatitis (8%) or cirrhosis (5%), despite a duration of infection that generally exceeded 15 years.[173] Shakil *et al* enrolled 60 anti-HCV-positive blood donors into a prospective study.[174] Mean duration of infection was 19 years and only 2% had evidence of cirrhosis. Rates of progression to cirrhosis are low in both studies, although both estimated duration of disease as the length of time from first exposure to an identified risk factor and may therefore have overestimated the duration of disease.

2.4.3 Factors Influencing Progression of Disease

It is emerging that end-stage liver disease is not an inevitable consequence of HCV infection and that only a subset will develop progressive disease. A number of factors appear to influence progression of disease although their precise role remains unclear. These factors include viral factors (genotype, viral load), host factors (age at infection, duration of infection, gender, genetic susceptibility, co-infection with other viruses in particular HBV and HIV, co-morbid medical conditions such as iron overload) and external factors (excess alcohol, diet, smoking, medicines, hepatotoxins or undefined environmental contaminants).[175]

2.4.3.1 Genotype

The association of viral genotype with outcome is unclear. Many studies have found that genotyope 1b is associated with more severe disease than other genotypes although such studies may be confounded by length of infection. In many countries the distribution of genotypes varies with the age of the subjects, possibly reflecting the introduction of genotypes through different routes of infection such as IDU. In general those who contracted HCV via transfusion were more likely to be infected with subtype 1b, whereas IDUs tend to be infected with 3a.[5 146] Consequently there is a trend that younger people are more likely to be infected with subtype 3a, and older people with subtype 1b.[48] Cirrhosis has been documented in subjects with all genotypes and there does not appear to be a non pathogenic genotype.

Pozzato *et al*[176] were among the first to report a link between genotype and cirrhosis when they compared genotypes from Italian and Japanese patients with HCV. They found genotype 1b was more commonly associated with cirrhosis, but as most subjects (74%) were genotype 1b it made comparison with other genotypes difficult. Kobayashi *et al*[177] followed 100 patients with

genotype 1, and 36 with genotype 2 over 8 years, and found that those with genotype 1 were more likely to have higher HCV-RNA titres, greater deterioration of liver histology and development of HCC. Zein *et al*[178] examined explanted livers for the presence of HCC and found that 5 of 18 patients infected with genotype 1b (28%) had HCC, but only one of 30 patients (3%) infected with all other genotypes (1a, 2a, 2b, 3a, and 4a) had HCC. Gordon *et al*[179] found that Genotype 1b appeared to be associated with a higher degree of post liver transplant fibrosis and cirrhosis than non-1b genotypes.

However other studies have found no association between genotype and adverse outcomes[5 180] and indeed it is difficult to interpret the influence of HCV genotype on the progression of disease from cross sectional clinic based studies with only approximate data on duration of disease. One study found that while genotype 1 was significantly more common in patients with cirrhosis, in multivariate analysis the effect disappeared and the only factors associated independently with cirrhosis were older age at exposure and longer duration of infection.[181]

However, a large prospective study of 163 patients with cirrhosis identified that those with genotype 1b were significantly more likely to develop HCC and similar findings were reported in two case control studies,[133 182] although two other prospective studies found no association.[134 145]

It remains controversial whether genotype does influence outcome of disease and it is likely that confounding factors make interpretation of studies difficult. Such confounding factors include age, duration of infection and lifetime alcohol consumption.

2.4.3.2 Age

Age at infection is strongly associated with progression to adverse outcomes such as cirrhosis and HCC. Age has also been associated with reduced ability to clear the virus following infection.

Vogt *et al*[183] reported on 67 German children infected with HCV by contaminated blood during cardiac surgery before 1991. Their mean age at first operation was 2.8 years. At follow up a mean of 19.8 years later, 37 (55%) of the 67 who were positive for anti-HCV had detectable HCV RNA in their blood. Only 1 of the 37 had elevated levels of liver enzymes and that patient had severe right-sided congestive heart failure. Of the 17 patients who underwent liver biopsies, only 3 had histological signs of progressive liver damage and these 3 had additional risk factors: two had congestive heart failure, and the other active HBV infection. The authors concluded that the clinical course of those infected as children seems more benign than would be expected in people infected as adults. However the impact of HCV on such children as they age is as yet unknown. Similar findings are reported by Locasciulli *et al*[184] who report on 114 subjects cured of childhood leukaemia followed prospectively for at least 10 years since chemotherapy withdrawal. Forty subjects (35%) remained HCV-RNA positive and none developed signs or symptoms of decompensated liver disease.

In adults the mildest outcomes have been observed in those infected under the age of 40 and age has been reported in many studies to be a predictor of poor outcome.[132 137 154 159 185 186]

Poynard *et al*[154] reported that age at infection of over 40 years old was independently associated with progression of HCV related liver fibrosis (as was male gender and alcohol consumption). Khan *et al* followed a cohort of 455 subjects with clinically compensated HCV for a mean

period of 4.7 years and identified age among the independent factors associated with poor outcomes.[186]

The more benign course of disease in those infected as young adults was demonstrated by the anti-D immunoglobulin studies,[74 75] and the adverse outcomes reported in studies of TA-NANBH could be due to the relatively advanced age of subjects at infection.[142]

The reasons for the apparent impact of age are unclear; it could represent infection with a possibly more virulent genotype, as there is a trend that younger people are more likely to be infected with subtype 3a, and older people with subtype 1b,[8] although the impact of genotype on outcome remains unclear. The observed association could also be due simply to a longer duration of infection. It may also represent age related immune-based changes that lead to acceleration of disease in affected individuals. If this is the case, it could be that those infected at younger age would also demonstrate acceleration of the disease process as they age.

2.4.3.3 Gender

Male sex is associated with poorer outcomes from HCV, both from initial clearance of infection[187 188] and long-term outcomes.[125 186 189].

Poynard *et al*[154] described risk factors for fibrosis in 2235 patients and found that histological staging of fibrosis in men was higher than in women irrespective of age, duration of disease or alcohol consumption. Poorer outcomes in men have been reported in a number of other studies.[186 190]

2.4.3.4 Alcohol

The relationship between alcohol use and poor outcomes of HCV infection has been demonstrated in numerous studies.[153 153 154 164 185 190-193]

One retrospective study found a threefold increased risk of liver cirrhosis and decompensated liver disease in those with high alcohol intake (>50gms per day), and that the rate that subjects developed cirrhosis was faster in the high alcohol group compared to the non alcohol group – by the second decade 58% were cirrhotic, as opposed to 10% in the non-alcohol group.[192]

It is now widely accepted that HCV and alcohol are not only independent risk factors for chronic liver disease, but also act in combination to increase rates of progression to poor outcomes. The NHLBI study reported on the relationship between development of cirrhosis and alcohol abuse in 1030 subjects with TA-NANBH.[193] The authors found that the risk for cirrhosis was 17% among subjects with HCV, and 2.8% among HCV negative controls. A history of heavy alcohol use (>125gms alcohol per day) was associated with a fourfold-increased risk of cirrhosis, but in those with a history of heavy alcohol abuse and HCV the risk of cirrhosis increased thirty-fold.

In another study of 6,917 subjects in a north Italian town, of those who were HCV infected and did not drink excessively 11.5% had cirrhosis, compared to 31% of HCV infected subjects who drank excessively.[194]

There is also a strong association between IDU and alcohol use. In one study over half of anti-HCV positive alcohol patients gave a history of IDU compared to 2% of the anti-HCV negative subjects.[191]In this group the combination of alcohol excess and HCV led to higher levels of chronic liver disease.

2.4.3.5 Race

The impact of ethnicity on adverse outcomes of HCV is unclear. The majority of studies have been conducted in white populations, although one Australian study identified birth in Asia, the Mediterranean regions or Egypt as a risk factor for progression to adverse liver outcomes.[186].

Black subjects have been observed to have reduced response rates to interferon monotherapy although the reasons for this are unclear.[195] Thomas *et al* assessed HCV viral clearance in a subset of 919 patients infected through IDU over a median period of 8.8 years. Viral clearance was observed in 90 (9%), 722 (78%) had persistent viraemia and in 107 (12%) viraemia was 'not resolved'. Viral clearance occurred five times more often in non-blacks although the reasons for this were not clear.

2.4.3.6 Co-infections

Co-infection with HIV

There is clear evidence of an increase in the incidence of chronic liver disease in HCV/HIV co-infected individuals compared to those with HCV alone. In one study the relative risk of HCV mono-infected haemophiliacs developing hepatic decompensation after 20 years was 10.8%, rising 21-fold in HCV/HIV co-infected subjects.[196] The authors concluded that HIV infection in those with HCV accelerates progression to cirrhosis and speculate this may be due to enhanced HCV replication in the presence of immune deficiency.

Eyster *et al*[197] reported on a prospective cohort study of subjects with haemophilia. In those co-infected, 9% had liver failure after 10 years of HCV infection compared to 0% of HCV mono-

infected individuals. In this cohort, co-infected subjects who survived for longer than 10 years had a risk of liver failure twice that of progression to Acquired Immune Deficiency Syndrome (AIDS), and mortality due to chronic HCV in HIV negative subjects was uncommon.

The main hypothesis for the increased risk of liver disease in co-infected individuals is the association with significantly higher levels of HCV viraemia,[198] which in turn may correlate with increased liver damage. In one study, HCV-RNA levels increased 3-fold in those who were HIV-negative, whereas in co-infected individuals a 58-fold increase was observed during the same time period.[198]

Co-infection with HBV

There is a complex relationship between HBV and HCV. While HBV and HCV are independently associated with the development of HCC, there is also evidence of a synergistic interaction between HBV and HCV and development of HCC.

Two studies from Taiwan, one a matched case-control study[199] and the other a cohort study[200] examined the association between HBV and HCV in patients with HCC. Tsai *et al*[200] reported that HCV/HBV co-infection, anti-HCV alone, and HBsAg alone were independent risk factors of HCC and that the increased risk of HCC in patients with combined HBV and HCV demonstrated a linear trend compared to those without. Chuang *et al*[199] reported that both HBsAg and anti-HCV were important risk factors for HCC, but the risk for HCC was elevated significantly with combined HBV and HCV infection indicating an additive and independent effect modification of HCV and HBV infection on HCC development. An Italian prospective study of 290 patients with cirrhosis reported that age, positivity for HBsAg and HCV antibodies, male sex and previous alcohol abuse were independently related to development of HCC.[201] These studies suggest that there is an additive and independent effect modification of HCV and HBV infection on HCC development.

There has been renewed interest in the impact of occult HBV infection in subjects with chronic HCV infection. Cacciola *et al*[202] demonstrated HBV DNA in 33% (66/200) patients with chronic hepatitis C liver disease. Among the 66 subjects, all were HBsAg negative, 46 were HBcAb positive and 20 were negative for all HBV markers. Thirty three percent (22/66) had cirrhosis, compared with 19% (26/134) of the patients with hepatitis C infection, but no HBV sequences. The authors concluded that occult hepatitis B infection occurs frequently in patients with chronic hepatitis C liver disease and may have clinical significance. The prognostic importance of low level HBV infection remains unclear however and other studies have failed to find an association between the prevalence of markers for HBV in those infected with HCV and the development of liver disease.[186]

2.4.3.7 Other Factors

The dose of HCV at initial exposure varies by different routes of transmission. IDUs are exposed to multiple small doses of often varying genotypes, while transfusion recipients are usually exposed to a single large inoculum. Routes of HCV transmission have been shown to influence disease progression.

Gordon *et al*[203] reported on blinded examination of liver histology of 124 consecutive subjects referred to a liver clinic of whom 43 were blood transfusion cases and 40 intravenous drug users. All had similar durations of infection. The authors found that those with transfusion acquired disease had more aggressive histological inflammatory activities, even after allowing for the impact of age and gender. The implication from this paper is that the initial volume of viral inoculum may affect the severity of the initial pathological lesion, which in turn affects outcome although they may have underestimated duration of infection in subjects with IDU.

Similar findings have been reported in other studies,[156 204] although the results may be biased by factors including referral bias and unknown disease duration.

Genetic factors may also influence outcomes from HCV infection and studies have demonstrated an association between human lymphocyte antigen (HLA) alleles and outcomes. Several studies report that the class II DR and DQ alleles were found significantly more frequently in patients with spontaneous viral clearance compared to those with chronic infection.[205-208]

The association with HLA alleles and long term outcomes is less clear, but Kuzushita *et al*[209] reported on 130 HCV infected patients and demonstrated that subjects with haplotype class 1B54 were 13 times more likely to have liver injury than those without B54. However, liver injury in this study was based on serum ALT levels, which do not correlate well with histological activity.

Hypogammaglobulinamia has also been associated with poor outcomes from HCV.[210 211] One study reported rates of progression to cirrhosis of 35% after 10 years.[211] The immuno-suppression associated with HIV also influences outcomes and although there is a correlation with low CD4 counts[212 213] the exact mechanism by which HIV adversely affects outcome is not known.

2.5 Treatment

Interferon monotherapy has been used since the early 1990s to treat chronic HCV infection, but response rates were limited with high relapse rates. The introduction of combination therapy with ribivirin improved treatment outcomes, with approximately half of treated subjects achieving sustained viral clearance as measured by negative PCR test for HCV RNA at least 6 months after treatment. [10] [9] A sustained virological response is associated with a prolonged biochemical and histological response.[214] Factors that predict a good response to treatment include young age, female sex, minimal fibrosis on liver biopsy, low viral load and infection with genotype 2 or 3.[215] [216]

Interferon therapy can be associated with severe side effects[217]and is not indicated in all patients, the difficulty lies in deciding who are appropriate candidates for treatment. Those with persistently normal liver function tests (LFT) and absent or only minimal changes on biopsy have an excellent prognosis without treatment. Therapy should be recommended for those at risk of progression – i.e. persistently elevated LFTs and fibrosis on biopsy. Patients who fall in-between the two extremes can be offered treatment or monitored with repeat LFTs and serial liver biopsies.[216] [218]

The benefit of treatment in cirrhotics is less clear, although several studies have suggested that treatment with interferon in subjects with cirrhosis will reduce the risk of progression to decompensated liver disease and HCC.[134] [219] [138]

2.6 Quality of Life

Individuals with HCV complain of a number of non specific symptoms such as fatigue, irritability, headache, nausea and right upper quadrant pain which are not always related to objective evidence of liver or other disease. [121] There have been a number of studies which report broadly impaired health related quality of life (QOL) in subjects with chronic HCV which improves with successful interferon treatment, although the mechanisms underlying this impairment remain far from clear.

Foster *et al.* [220] reported Short Form 36 (SF-36) scores in 72 non-cirrhotic patients with chronic HCV and found them reduced across all modalities. Scores were also significantly less when compared to subjects with chronic HBV. The reduction in QOL in the HCV group could not be attributed to the degree of liver inflammation or to the route of infection.

Hunt *et al.* [221] used the SF-36, the Beck Depression Inventory and the Hospital Anxiety and Depression Scale in a small study of 38 patients with chronic HCV and found reduced QOL scores pre-treatment with interferon. They also report that the presence of cirrhosis did not influence scores on any of these scales and that there were no differences in QOL after interferon treatment completion between responders and non-responders.

Bonkovsky *et al* [222] measured QOL at baseline and at the end of interferon treatment in 642 subject with chronic HCV. Baseline data indicated that compared to normal controls, subjects scored poorly on all eight scales of the SF-36. Scores significantly improved in those who responded successfully to interferon therapy.

The reasons for reduced QOL in those with HCV are unclear. It is possible that biological mechanisms related to HCV account for low QOL, and this hypothesis is supported by studies that found that QOL improved in those with sustained biochemical or virological response to

interferon compared to non responders.[222-224] While it is likely that diminished QOL in chronic HCV is, at least in part, a consequence of the disease, the positive psychological impact of being 'cured' of HCV was not accounted for in these studies.

A recent study investigated whether HCV could cause a cerebral effect and found elevations in basal ganglia and white matter choline/creatine ratios in patients with histologically mild HCV, compared with healthy volunteers and patients with HBV. This elevation was unrelated to hepatic encephalopathy or a history of IDU. [225] The clinical relevance of such a finding remains unclear.

Most QOL studies are from tertiary liver centres, whose patients encompass the more severe end of the HCV disease spectrum. Such studies do not include those with milder or asymptomatic disease.

The effect of co-morbidities such as alcohol abuse or IDU could also contribute to the observed reduction in QOL in subjects with HCV, as both are independently associated with reduced QOL and psychiatric co-morbidities. [226] [227] [228] [229] However, Foster *et al* [220] found that although IDUs had the lowest QOL scores, scores were also reduced in those with no history of IDU. In addition, Fontana *et al* [230] reported on 107 subjects with chronic HCV – approximately half of whom had a history of IDU -and found that a history of alcohol abuse or IDU did not correlate with reduced QOL scores, although having one or more active medical co-morbidities did.

It is likely however that psychological factors play a role in the reduced QOL of HCV patients. One study [231] investigated fatigue and psychological disturbance in subjects with chronic HCV compared to other chronic disease groups. They report that fatigue experienced by subjects with HCV is more severe and responds poorly to relieving factors, and that subjects with HCV

harboured greater feelings of anger and hostility compared with those with non-liver chronic diseases.

In all QOL studies, subjects had been diagnosed and were aware that they had HCV. It may be that subjective health perceptions had been influenced by diagnosis of HCV alone which caused individuals to view their health related QOL in a less positive light. In order to investigate the impact of diagnosis it is necessary to measure QOL prior to imparting the diagnosis of HCV. We were able to undertake this in our study due to the availability of stored sera.

CHAPTER 3: METHODS

3.1 Study Design

A historical cohort study design was chosen. The exposure of interest was anti-HCV in archived stored sera from individuals admitted with clinical hepatitis to the Fairfield Infectious Diseases Hospital (FIDH) in Melbourne during the years 1971 to 1975. Individuals whose sera tested anti-HCV positive formed the exposed cohort, and those testing anti-HCV negative the unexposed.

Relevant study outcomes were mortality, liver-related morbidity (chronic hepatitis, cirrhosis and HCC) and QOL.

3.2 Identification of Cohort Sampling Frame

In Melbourne, in the 1960s and 1970s, all cases of clinical hepatitis were admitted to the Infectious Diseases Hospital at Fairfield. From early 1970 it became standard practice to systematically store sera from all cases of hepatitis admitted to the hospital. The aim of this practice was to create a sera archive for future research purposes. The sera was frozen at <40 degrees centigrade and retained on site at the hospital. This practice continued until the early 1980s.

We decided to retrieve and test sera from the period 1971 to 1975 as it was the earliest point that systematic storage of sera began and also a previous study had identified anti-HCV in samples of the stored sera from that time.[232] In addition coding using the International Classification of Disease - 9th version (ICD-9) was introduced to FIDH in 1970. This

standardised the diagnostic coding of patients allowing for accurate identification of those admitted with hepatitis.

Discharge records for FIDH for the years 1971 to 1975 were obtained. All persons admitted to FIDH with a clinical or biochemical diagnosis of acute viral hepatitis from 1/1/71 to 31/12/75 were included in the cohort sampling frame. Exclusion criteria included those without an original serum sample for testing and those for whom no medical record could be located. Those aged under 16 years at the time of admission were also excluded as their admission was generally precipitated by HAV, which was endemic at this time. [233]

The original hospital records were obtained and details of the admission transcribed. Information obtained (*Table 3.1*) included clinical information, diagnosis on discharge, results of any serological testing undertaken and risk factors for blood borne viruses, if available.

Table 3.1 Data transcribed (if available) from the original FIDH medical records

General
FIDH unit record number, date of admission, date of discharge and sera number
Sociodemographic
Surname, first given name and second name (if any) on admission
Date of birth, age at admission, gender, occupation and country of birth
Full address (including postcode) and telephone number
Next of kin details including name, full address and telephone number
Risk factors for blood borne viruses in the six months prior to admission
Transfusion of blood or blood products, renal dialysis, tattooing, injecting drug use, occupational exposure, recent surgery, contact with a case of hepatitis, overseas travel
Diagnostic data (ICD-9)
Original discharge diagnosis, other diagnoses
Test results
Hepatitis Australia antigen, hepatitis A IgM, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis D (delta) antibody

3.3 Ethics

3.3.1 Ethical Approval

Ethical approval for the study was sought and obtained from Ethics Committees at all sites involved in the study - FIDH, the Alfred Hospital and the Australian Institute of Health and Welfare (AIHW).

The Ethics Committees required resolution of a number of issues prior to granting full ethical approval. The ethics of testing sera for HCV without the consent of the individuals from whom the sera was originally taken was considered, but all Committees deemed it to be in the individual and public interest that such testing was undertaken and the individuals traced and assessed if possible. Guidelines about the manner of making initial contact with subjects and how information was released to them at that contact were prepared and approved.

The ethics of undertaking a liver biopsy for study purposes alone, when it was not clinically necessary was also discussed. It was decided, under the guidance of the Ethics Committees, not to request consent for a liver biopsy for histological assessment of the degree of liver involvement if it was not clinically relevant to the ongoing care of the individual.

3.3.2 Informing Study Subjects of Anti-HCV Status

As routine testing for anti-HCV only became available in the early 1990s, it was likely that many of the identified anti-HCV positive subjects were unaware of their serostatus. This was particularly likely in those who had remained asymptomatic.

In conjunction with the Ethics Committees it was decided that at the time of recruitment, subjects should only be told that the study was looking at outcomes in persons who had been admitted to hospital with 'hepatitis' 25 years ago. The results of testing of the archived sera were not disclosed at this time. This was principally because tracing and recruiting subjects to the study was performed via telephone and letter – mediums deemed inappropriate to discuss sensitive and potentially very distressing material.

Subjects were sent and then completed the study specific questionnaire and the QOL questionnaire. Once the study centre received both, clinical review was arranged. It was at this point in the study that the examing physician informed subjects that they had tested anti-HCV positive on stored sera (to those affected that were unaware of their HCV serostatus) and that they could be chronically infected. Blood results and other investigations were also undertaken at this time.

This approach was taken to obtain current anti-HCV status, HCV RNA by PCR and LFT results to allow appropriate counselling as to current health status, degree of infectivity and their likely prognosis. Facilities were also available for subjects who wished to undertake more formal or prolonged counselling.

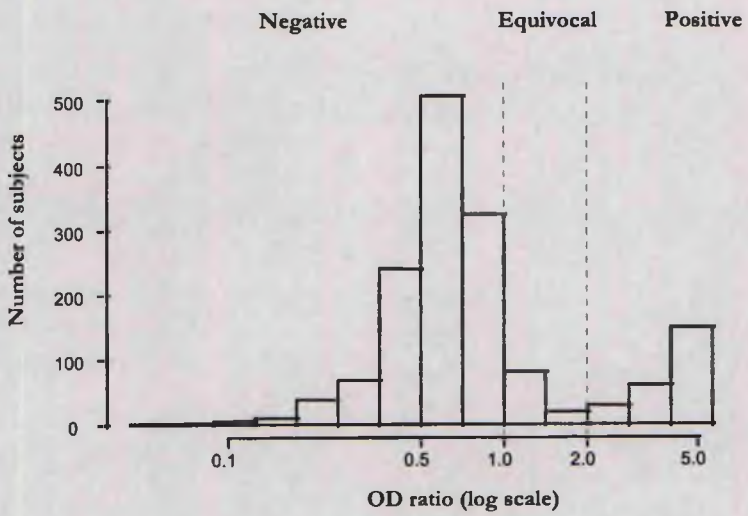
3.4 Measurement of Exposure

3.4.1 Definition of Exposure

The exposure was defined as an anti-HCV enzyme linked immunosorbent assay (ELISA) Optical Density (OD) to cut off ratio of greater than 2 in archived sera from 1971 to 1975. Individuals who had OD ratios of less than one were classed as unexposed.

Previous studies of frozen sera stored for long periods suggested a more stringent classification was required to avoid overestimation of the prevalence of anti-HCV. [234] This was a problem particularly with first generation immunoassays and although second generation assays (as used in this study) are more specific we used twice the manufacturers recommended cut off, as recommended in the literature, [234] to further increase specificity of a positive anti-HCV test result. This point was also chosen because there was a bimodal distribution of results in our study (*Figure 3.1*).

Figure 3.1 Log scale of ratio of sample anti-HCV ELISA optical density (OD) to recommended cut off OD in stored sera samples (n=1511)



Individuals with weakly positive results, i.e. sample ELISA to cut off OD ratio of 1-2 (n=99), were recorded as equivocal and excluded to minimise misclassification bias.

3.4.2 Testing of Stored Sera from 1971 to 1975

Multiple serum samples were stored for admitted patients between 1971 and 1975. Each sample had the subject's name and hospital number attached and samples were stored in chronological order. The last available serum sample from each patient associated with their final hepatitis admission and which was adequate for testing was selected for each individual and obtained from storage. This was to allow maximum time for seroconversion to occur.

Serology was performed on the stored sera for all hepatitis markers using commercially available immunoassays: hepatitis A IgM (HAIgM) (IMX Assay and EIA Assay-Abbott Diagnostics, Abbott Park, Illinois, USA), hepatitis B core antibody (HBcAb) (RIA - CORAB), hepatitis B surface antigen (HBsAg) (RIA - AUSRIA II, Abbott Diagnostics) and hepatitis C antibody (anti-HCV) (EIA second generation assay - Abbott Diagnostics). Testing was conducted by the Victorian Infectious Diseases Reference Laboratory (VIDRL) and the National Serology Reference Laboratory (NRL), Australia. The laboratories designated results as reactive or non-reactive. All results were entered into an ACCESS database.

VIDRL also undertook PCR testing on anti-HCV positive stored sera, but only one sample was HCV RNA positive. HCV RNA is unstable, so correct handling and subsequent storage of the samples at -80 degrees C is essential to avoid false negative results. [235] The most likely explanation for non-detection of HCV RNA in our stored study samples is deterioration of HCV RNA due to the age of the samples, inadequate storage and recurrent freeze thaw cycles.

3.4.3 Quality Control of Sera Testing

The NRL was established in 1985 to develop a comprehensive quality assurance programme for laboratories undertaking HIV, HTLV and subsequently, HCV serology. Components of the program include performance evaluation, specificity monitoring, quality control and quality assessment programmes. The NRL has gained certification/accreditation for its Quality Management System and is accredited to ISO Guide 25 and certified to the ISO9001 standards. The Victorian Infectious Diseases Reference Laboratory (VIDRL) uses the quality control mechanisms developed by the NRL.

Quality control (QC) samples are produced by the NRL for use with anti-HCV and HBsAg serological assays as well as with HCV nucleic acid testing and PCR assays. The QC samples are used in every assay run to confirm that test results are accurate and reliable. The NRL provides a mean and standard deviation for each QC batch which laboratories use as a guide for setting their validation limits.

In addition, quality assessment programmes are used to assess the accuracy of the entire laboratory testing process from receipt of the specimen to delivery of the result. A panel of unknown specimens (including anti-HCV positives, negatives, duplicates and replicates) are supplied from the NRL to a laboratory such as VIDRL, with instructions that the samples are handled and assayed according to normal procedures, the results are then audited.

In addition, the results of testing of the study stored serum samples were compared to original recorded results for HBsAg using Kappa to measure agreement. Ten subjects who had equivocal anti-HCV results were randomly selected, traced and current anti-HCV status determined to assess the impact of misclassification in these subjects.

3.5 Construction of the Cohort

Stored serum of 238 patients was strongly reactive - greater than twice the standard cut-off - for anti-HCV, these formed the exposed group and all were selected for follow up. A random sample (n= 476) of the anti-HCV seronegative individuals (the unexposed group) was selected using standard computer randomisation soft ware (Excel) to give a 2:1 ratio of unexposed to exposed subjects.

The subjects contact information from the time of admission was transcribed from the original medical records from 1971 to 1975 and used for tracing purposes. This included hospital number, surname and given names as recorded, title, date of birth, address at time of admission, telephone number at time of admission, next of kin contact details (name, address, telephone, relationship), general practitioner (GP) at time of admission and date of admission. The individual's occupation and any risk factors for blood borne viruses (BBV) - e.g. IDU, tattooing or blood transfusion - recorded in the medical history were also documented (*Table 3.1*).

The public access electoral roll was obtained from the electoral office on microfiche and gave alphabetical listings of the complete electoral roll by surname with full name and address. Voting is compulsory by law in Australia and as a result the voting register is more comprehensive and up to date than that of other countries such as the UK. The electoral role does not however give date of birth. An electronic telephone directory ('Oz on Disc') was purchased and gave the ability to search on any field (given name, surname, address, and state).[236] Similar search tools are available on the World Wide Web, but do not have the flexibility of commercially available products and their use is limited in studies such as this.

Records were entered onto an ACCESS database and the tracing methods used on each participant and the number of searches required to determine the current address or contact telephone number documented.

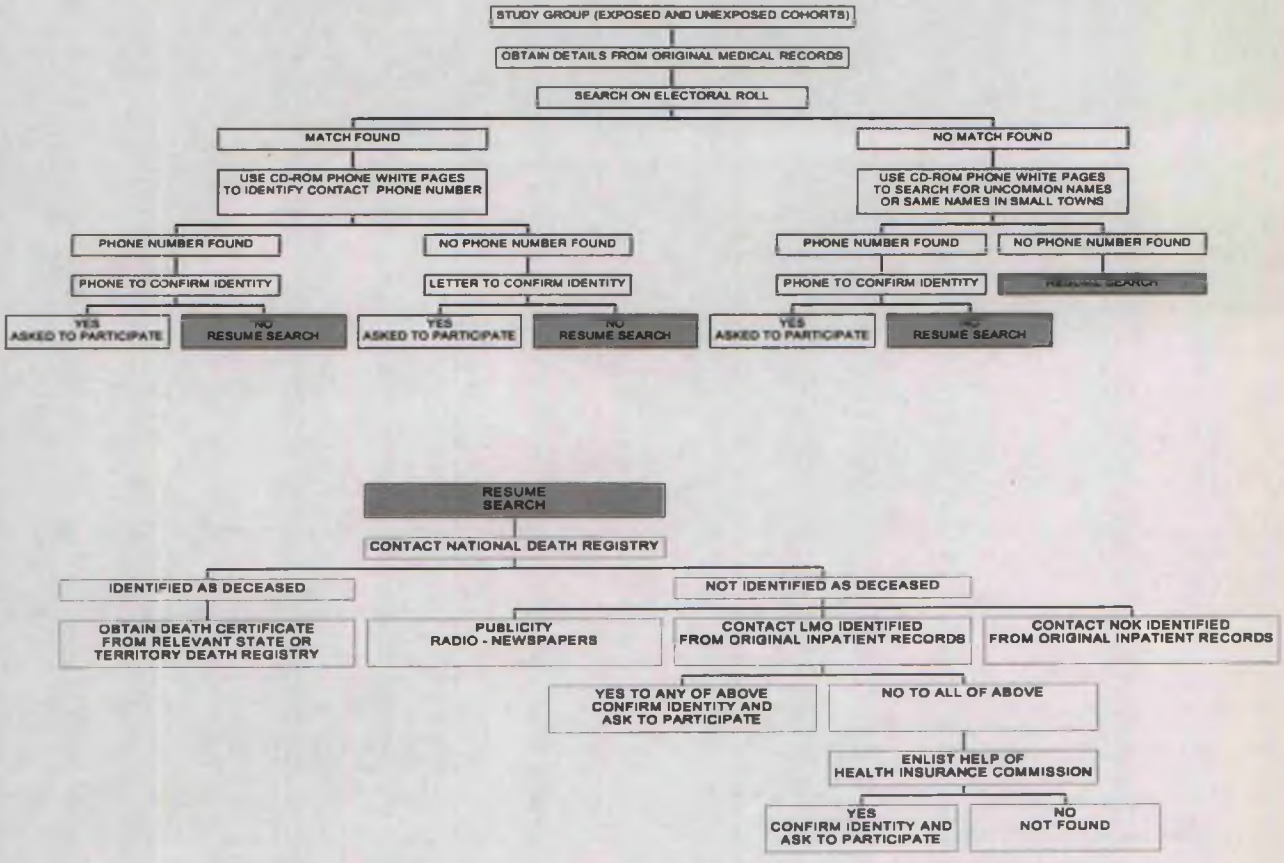
Tracing was undertaken initially by matching full names on the electoral roll, followed by searching for a telephone number to match the electoral roll address on electronic telephone directory. If a matching telephone number was located, the individual was contacted by phone and asked if they were the person and asked to verify their date of birth. Protocols were prepared to deal with telephone contacts. To protect confidentiality, no details about the study were released until the subject had confirmed their name and date of birth. At this stage very brief details of the study were released – that is the study was a follow up review on subjects admitted with hepatitis between 1971 to 1975. Subjects were asked if they would consent to take part and an information pack - including a consent form - sent to subjects (*Appendix 1*). It did not reveal the results of testing of the stored sera or that serum had been tested. This information was only released at the time of clinical review. *Figure 3.2* outlines the tracing algorithm used.

Study subjects located on the electoral roll, but for whom no contact telephone number could be located, were sent a letter with a request to contact the study centre if they were the named individual. No further information was given and 'hepatitis' was not mentioned in the letter. Subjects who contacted the centre received the same study pack as those identified by telephone contact.

Persons not found on the electoral roll were searched for on the aforementioned electronic telephone directory, particularly if they had uncommon surnames. The search then widened to location of next of kin recorded in the original medical records. This was performed in the same manner with initial searches on the electoral roll and use of electronic telephone

directory. If a telephone number was found, the next of kin were contacted and asked if they could provide contact details for the named individual or pass on the study centre details with a request to call. If only an address could be matched, a letter was sent asking them to contact the study centre

Figure 3.2 Study tracing algorithm



Approaches to local doctors listed in the original medical admission records were also made to assess whether subjects were still attending the same practice. Other sources used included advertising in the national daily newspapers in the Public Notices Section on Saturdays for two weeks. Features were also placed in the Hepatitis C Support Foundation newsletter and study co-ordinators appeared on local state radio.

Information was also obtained from the National Death Registry maintained by the AIHW. Names and dates of birth were supplied to the registry who searched their database for a fee. This registry was computerised in 1982 and could not search before that date. We also purchased on microfiche the Victorian death index to 1985 and manually checked the years from 1970 to 1982.

When all other methods had been exhausted an application was made to the National Health Insurance Commission (NHIC). The Commission – which assisted with the study only as it was deemed to be in the individual and public health interest - searched their database to determine if study subjects had any contact with health services in the period from computerisation of records in 1982 to the present day. If subjects were located, the last recorded addresses on the database were supplied to the study for the exposed group, and a letter forwarded to the rest asking them to contact the study centre.

All study subjects located were invited to take part in the study by completing two postal questionnaires and, following completion of the questionnaires, to participate in follow up clinical examination.

No financial remuneration was offered to study participants at any point in the study.

3.6 Assessment of Outcomes

3.6.1 Definition of Outcomes

The main outcomes measured were mortality, liver-related morbidity (chronic hepatitis, cirrhosis and HCC) and QOL.

Chronic hepatitis was defined as

- histological evidence on liver biopsy of chronic hepatitis (based on HAI[124]), or
- a serum hyaluronate level greater than 37μg/l,[237] or
- ALT level > 1.5 times upper limit of normal (NR 25-50u/l).

Cirrhosis was defined as

- histological evidence on liver biopsy of cirrhosis (based on HAI[124]) or,
- a serum hyaluronate level greater than >200μg/l,[237] or
- clinical or radiological evidence of hepatic cirrhosis.

Hepatocellular Carcinoma was defined as

- a hepatic space occupying lesion with a vascular appearance consistent with HCC on MRI, or
- cytological or histological evidence of HCC on examination of liver tissue.

3.6.2 Mortality Assessment

All causes of death including liver specific mortality data was obtained from searches of the Victorian and New South Wales State Death Registries and the National Death Registry maintained by the AIHW. For each subject in the cohort who was known to have died, a copy of the death certificate was obtained from the Victorian and New South Wales Death Registries. Coding for the underlying cause of death was by the ICD revision in use at the time of death. For deaths between 1971-1977, ICD-8 was employed by the death registries and from 1977 onwards, ICD-9 was used. For analysis, bridge coding of ICD revisions was conducted to give the corresponding ICD-9 categories in all cases

3.6.3 Morbidity Assessment

Morbidity data was collected through a self-administered study specific questionnaire (*Appendix 2*) and a clinical proforma (*Appendix 3*). The process of assessment was identical for both exposed and unexposed subjects.

Self-Administered Questionnaire: The questionnaire was developed specifically for the study and piloted at the local liver clinic. Study subjects were sent the questionnaire by post at the same time as the quality of life questionnaire. Both were self completed and returned to the study centre again by post.

The questionnaire collected information on;

- Risk factors for blood borne viruses both before admission in the early 1970s - to ascertain the possible route of infection - and also after admission to determine risk behaviours which could have led to transmission of HCV to others.

- Sociodemographic and lifestyle information (including detailed information on IDU history)
- Lifetime alcohol consumption – regular drinking habits and binge episodes –was obtained. The questionnaire collected information on the duration and amount of alcohol consumed in addition to the type of alcohol (wine, beer, spirits, other)
- Other factors which could influence the natural history of the disease such as smoking, diet, the use of prescription medications such as the oral contraceptive pill and non prescription medications such as anabolic steroids.
- General health and other medical history including liver related and non-liver related conditions
- Testing history for hepatitis A, B and C, the results of testing and carriage status if know.
- The management of HCV including contact with health services (whether GP or specialist) and treatment offered and accepted (including interferon +/- ribivirin and naturopathic medications)

Once the questionnaire had been returned to the study centre the participants were invited to undergo clinical examination. Clinical examination was carried out either by a doctor of choice (for those who lived interstate) or by the study co-ordinator (myself) through the cohort study centre at the Alfred Hospital.

Clinical Assessment: A standardised clinical form was developed for the examining doctor to record the clinical history, examination and investigation findings. The form was piloted through liver clinics.

The form included questions determining the subjects full medical history, risk factor assessment, treatment history and the results of relevant investigations (including serological, biochemical, histological and radiological). A sample of sera was also taken under controlled

conditions. This was taken by study staff at the study centre and by enrolled laboratories for those assessed interstate and subsequently sent by courier to the study centre. This sample was used for virological assessment and for measurement of serum hyaluronate.

The following information was collected by the clinical assessment:

- Clinical signs of chronic liver disease and portal hypertension (enlarged liver, spider naevi, gynaecomastia, testicular atrophy, bleeding disorders, oedema/ascites, caput medusa, varices and splenomegaly). The Child Pugh score was calculated from the relevant indices (*Table 3.2*). Child Class A was classed as a score of 5-6, Child Class B a score of 7 – 9, and Child Class C a score of > 9.

Table 3.2 Grading system for cirrhosis: the Child-Pugh score[124]

Score	Bilirubin (g/dl)	Albumin (g/dl)	Prothrombin time (Sec)	Hepatic encephalopathy	Ascites (grade)
1	<20	>35	1-4	None	Mild
2	20-30	28-35	4-6	1-2	Mod
3	>30	<28	>6	3-4	Severe

- Hepatitis serology including hepatitis A IgM (HAIgM), hepatitis B core antibody (HBcAb), hepatitis B surface antigen (HBsAg) and hepatitis C antibody (anti-HCV).
- Laboratory indicators including aspartate aminotransferase (AST)/ALT ratio, prothrombin index, albumin, bilirubin, platelets, alpha-fetoprotein (αFP)
- Serum hyaluronate (HA): Serum HA has been shown to be a sensitive and specific non-invasive marker of fibrosis.[238] HA levels were measured by the HA laboratory at Monash University in anti-HCV positive participants at follow-up through analysis in triplicate by a radiometric competitive assay (HA test, Upjohn, Pharmacia, Uppsala, Sweden) based on the use of specific HA binding proteins (HABP) isolated from bovine cartilage. Intra- and

inter-assay coefficient of variance was <4% and <5% respectively. Results greater than 37μg/l were considered elevated. [237]

- Ultrasound (U/S) was performed if clinically indicated to assess development of collateral circulations, portal vein diameter, ascites, liver echotexture for homogeneity, hepatic length at right kidney and splenic length.
- Liver histology: Liver biopsy was carried out only if clinically indicated and the patient gave full consent. The relevance to the study was explained to each study subject and if any participant underwent biopsy at any time histological staging and HAI was obtained and recorded.
- Virology: Both qualitative and quantitative virological assessment were conducted by VIDRL. Qualitative PCR was carried out using the Roche AMPLICOR kit (Roche Molecular Systems, Branchburg, NJ). Subjects with HCV RNA positive samples were genotyped by the Innogenetic line probe assay (LIPA; Innogenetics, Ghent, Belgium) [239] or by direct sequencing and viral load quantified.

3.6.4 Quality of Life Assessment

All subjects completed the SF-36 scale (*Appendix 4*) prior to clinical review at the same time as the study specific questionnaire.

The SF-36 was chosen as a generic and widely used quality of life instrument, which has been adapted for use in Australia. [240-243] It is a standardised medical outcome questionnaire consisting of thirty-six questions in eight subscales and two summary scales and is constructed for self-administration (in about 10 minutes) or for administration by a trained interviewer in person or by telephone. The SF-36 measures the following eight health concepts, which are relevant across age, disease and treatment groups:

1. limitations in physical activities because of health problems;
2. limitations in usual role activities because of physical health problems;
3. bodily pain;
4. general health perceptions;
5. vitality (energy and fatigue);
6. limitations in social activities because of physical or emotional problems;
7. limitations in usual role activities because of emotional problems;
8. and mental health (psychological distress and well being).

The SF-36 survey has a standardised scoring system which yields a profile of eight health scores, a self-evaluated change in health status and two summary scales - a physical component summary and a mental component summary. Subscale scores range from 1 to 100, so each individual then receives a score that can be converted to a centile score with reference to 'standardised' scores.

In this study, subjects were told at the time of recruitment that the study was looking at outcomes in persons who had been admitted to hospital with 'hepatitis' 25 years ago. Subjects completed the QOL questionnaire at the same time as the study questionnaire. Once the study centre received both, clinical review was arranged. It was at this point in the study that the process of imparting the diagnosis of chronic HCV infection (to those affected who were unaware of their HCV serostatus) and the results of sera testing was commenced. In this way subjects were unaware of their anti-HCV status at the time of recruitment and hence completed all parts of the study, including the SF-36 questionnaire, without this knowledge. This approach was taken partly to assess the impact of knowledge of HCV diagnosis on QOL and also to allow appropriate counselling as to current health status, degree of infectivity and likely outcome.

We compared the SF-36 scores of the exposed and unexposed cohorts to each other, and also to population norms for Australia.[244]

In order to ascertain the impact of a diagnosis of HCV on quality of life an additional analysis was undertaken in those who were chronically infected with HCV (i.e. HCV PCR positive), but who had no clinical liver disease or other conditions that could affect quality of life. Subjects were therefore excluded from this analysis if found to be currently anti-HCV or HCV RNA by PCR negative, have cirrhosis or clinically detectable liver disease, if symptomatic at the time of their recent anti-HCV testing or with other medical or psychiatric conditions which could affect quality of life.

We then examined the QOL scores of this subgroup of the subjects with long standing chronic HCV infection, only approximately half of whom were aware that they were HCV seropositive at the time the QOL scores were measured. To investigate the impact of HCV diagnosis on quality of life, we compared the scores between those aware of their serostatus and those not aware.

3.7 Data Management and Quality Control

Self completed questionnaires that were returned incomplete or with ambiguous entries were completed by trained study staff via telephone interview with subjects. If possible all questions were answered

Collection of clinical data was performed by a number of clinicians. All subjects attending for review in the study centre were seen by the study co-ordinator (myself) to minimise measurement bias. Where possible, interstate subjects were reviewed by specialist

gastroenterology staff. The clinical proformas were reviewed and ambiguous or unclear items were rechecked with the examining physician.

All forms were identifiable only by the subjects study number and were stored in locked cabinets at the study centre. The forms were coded prior to data entry onto the database package ACCESS. A number of forms were created in ACCESS to facilitate data entry. These forms contained consistency and range checks to alert staff to data entry errors. A data dictionary was complied and contained a list of all variables, coding instructions, values allowed and the names of relevant computer files. Training was given to the two study staff who entered data.

Data entry errors were detected by double entry verification and by range and consistency checks once all data had been entered. Back up copies of the database were made weekly and one copy stored of site.

3.8 Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS).[245] For each cohort member, person-years at risk were calculated from the date of the stored sera to the date of death or study follow up assessment. Cohort mortality data was compared to that of the general population. Expected deaths by cause in the cohort were calculated by multiplying the age, sex and year specific person years at risk within the cohort, by the corresponding national cause specific mortality rates. Standardised mortality ratios (SMRs) were then calculated by dividing the number of deaths in the cohort with the expected number and multiplying the result by 100. Confidence intervals (CI) and 2-sided p-values were calculated based on the Poisson distribution.

Morbidity outcomes were measured as binary responses or as continuous measures where appropriate. Exposures were measured as either binary or cumulative continuous during the entire cohort interval since the 1970s. Logistic regression analysis was used for binary outcomes and linear regression for continuous outcomes.

The impact of confounders (alcohol use, age, gender, co-infections and smoking) was controlled for through the use of multivariate regression analysis – either linear for continuous outcomes or logistic for binary variables.

Study subjects QOL scores were compared to population norms for Australia.[244] Multiple linear regression analysis was used to compare SF-36 scores between groups based on knowledge of HCV sero-status after adjusting for recognised QOL confounding variables such as age, sex, marital status and ethnic group.

3.9 Power Calculations

Study power was limited by the ability to trace and recruit subjects through to completion of the study. The expected outcome rates and numbers of clinical end-points were based on the results of the pilot study.[246] For chronic hepatitis we expected approximately 50% of subjects to be affected and for cirrhosis approximately 10%. The precision of absolute estimates was estimated for 100 subjects completing follow up, based on 95% confidence interval for rates. We estimated that on this number of subjects we could estimate the true value for chronic hepatitis (based on 50 events) to within plus or minus 30% and for cirrhosis (based on 10 events) to within plus or minus 60%.

CHAPTER 4: RESULTS

4.1 Identification of Cohort Sampling Frame

A total of 1,798 patients (aged greater than 16) were identified from FIDH discharge records as having been admitted from 1971 to 1975 with a diagnosis of viral hepatitis (ICD-9 Classification 070.0 to 070.9). Medical records were found for 1,737 (97%). Five percent had been admitted with hepatitis more than once over the study period so the first admission was used as the index admission for study purposes. Serum specimens were found for 1559 (90%) of identified subjects and testing for all the measured hepatitis (HAIgM, HBcAb, HBsAg, anti-HCV) markers completed for 1,511 (87%).

4.2 Measurement of Exposure

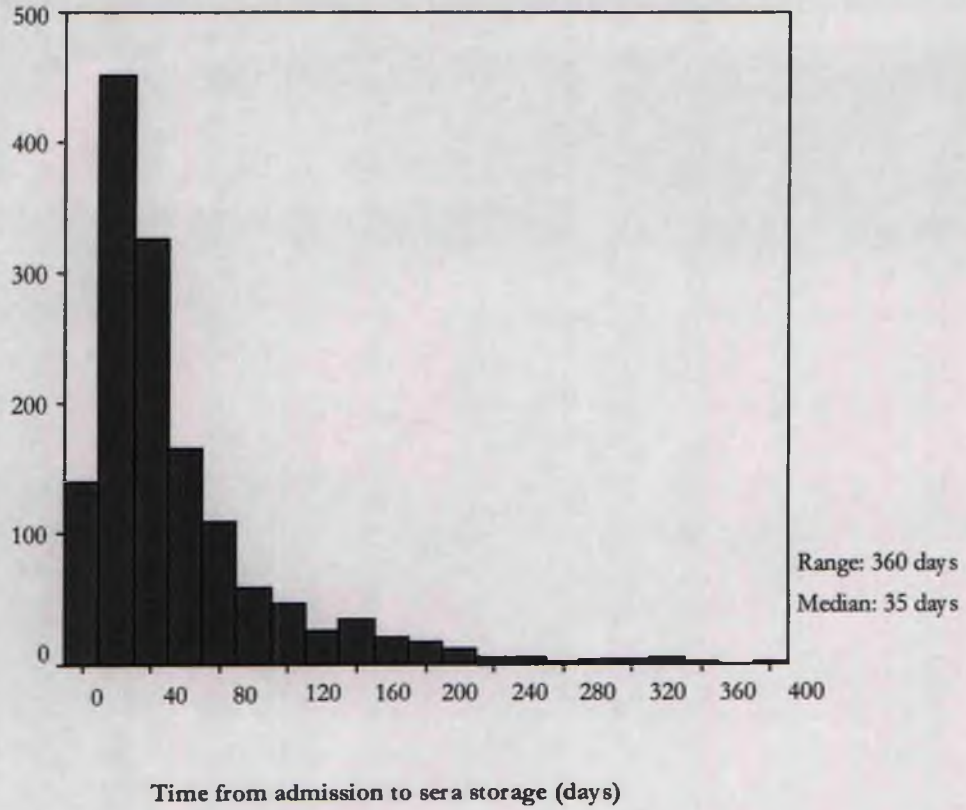
4.2.1 Serological Analysis of Stored Sera (1971 to 1975)

Ninety-nine (7%) of the 1,511 cohort members had equivocal anti-HCV results (OD ratio 1-2) and were excluded from the study to minimise misclassification bias. A random sample of 12 of these individuals were traced and found to be currently anti-HCV negative supporting our hypothesis that their equivocal anti-HCV results in the stored sera were borderline false positives.

Of the remaining 1,412 subjects, 238 patients had strongly reactive sera - greater than twice the standard cut-off - for anti- HCV. These formed the exposed group and all were selected for follow up. A random sample (n= 476) of the anti-HCV seronegative individuals (the unexposed group) was selected to give a 2:1 ratio of unexposed to exposed.

The mean time from admission to FIDH to storage of the sera sample was significantly shorter for the anti-HCV positive group (mean 48 days, SD 54) compared to the anti-HCV negative group (mean 57 days, SD 58), (mean difference -8.6, 95% CI: -16 to -1, p=0.027).

Figure 4.1 Histogram demonstrating distribution of the interval (in days) between admission to storage of sera sample for all cohort subjects with sera available for testing (n=1511)



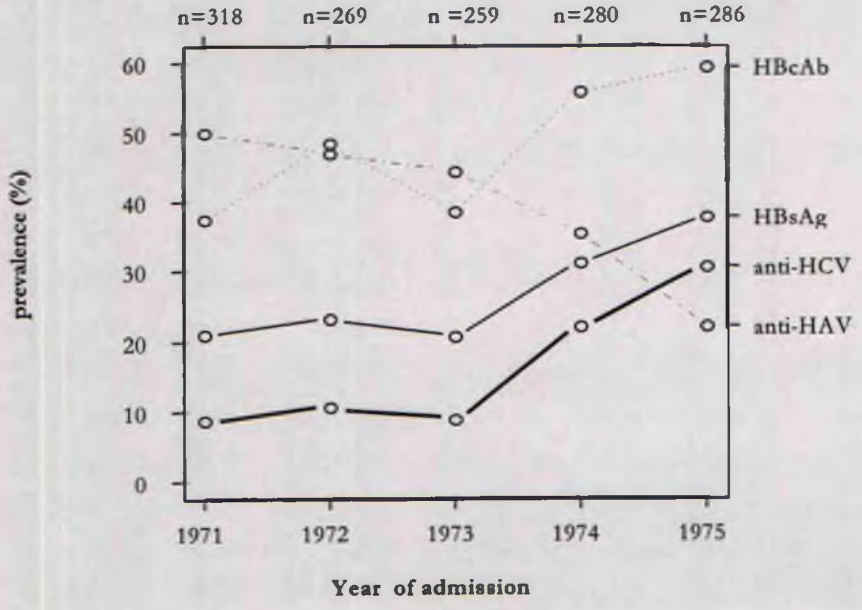
Only 15% (n=37) of those who were anti-HCV positive had no evidence of other hepatitis markers. Furthermore those who were anti-HCV positive were significantly more likely to have markers for HBV (*Table 4.1*).

Table 4.1 Prevalence of markers of viral hepatitis infection in anti-HCV positive compared to anti-HCV negative patients admitted to FIDH from 1971 to 1975

	Anti-HCV positive (n=238)	Anti-HCV negative (n=1182)	RR (95%CI)	p value
Anti-HAV IgM	14.3%	45.1%	0.2 (0.1 to 0.3)	<0.001
Anti-HBc	75.2%	42.6%	4.0 (3.2 to 5.6)	<0.001
HBsAg	45.7%	23.3%	2.7 (2.1 to 3.7)	<0.001

The trends in prevalence for each of the measured hepatitis markers were significant, the prevalence of anti-HCV increasing four fold from 1971 to 1975, with the biggest increase between 1974 and 1975. Increases were also seen in the prevalence of HBcAb and HBsAg. The prevalence of anti-HAV IgM declined over the period studied (*Figure 4.2*). In those who were HCV seropositive the prevalence of markers of hepatitis A and B did not change significantly over the study period.

Figure 4.2 Prevalence of markers of viral hepatitis (anti-HAV IgM, HBcAb HBsAg, anti-HCV) in stored sera from patients admitted to FIDH from 1971 through 1975



4.2.2 Validation of Sera Testing Results

A comparison of study testing of stored sera for HBcAb and anti-HCV with the results of follow up (1997) serology in a traced subset of the original cohort (n=161) demonstrated that the overall agreement was good, with complete agreement for the former of 91% (Kappa 0.79 [95% CI 0.67 to 0.91]) and for the latter of 88% (Kappa 0.74 [95% CI: 0.66 to 0.82]).

There was moderate agreement when results for HBsAg recorded at the time of original admission were compared with our results of testing of the stored sera, with complete agreement for 65% (Kappa 0.58 [95%CI: 0.43 to 0.72]).

4.3 Characteristics of Cohort at Baseline

4.3.1 Sociodemographic Data

Sociodemographic data were available from medical case records for all subjects. The 238 anti-HCV positive individuals were significantly younger than anti-HCV negative individuals, (geometric mean age at time of original admission: 22 years [SD 5.3 yrs] and 29.5 years [SD 12 yrs] respectively, p< 0.001). Those who were anti-HCV positive were also significantly more likely to have been born in Australia than those who were negative (80% and 69% respectively, (RR 1.5, 95% CI: 1.4 to 1.8. p<0.001) and to be male (62% and 52% respectively, (RR 1.4, 95% CI: 1.1 to 1.9. p<0.001).

4.3.2 Risk Behaviours for Viral Hepatitis

Information on risk factors was incompletely recorded in medical records. In total only 40% had recorded information for IDU, 69% for contact with a case of hepatitis, 36% for transfusion, 15% for tattooing and 11% for travel.

A history of IDU and of contact with a hepatitis case were the only risk factors significantly associated with HCV sero-positivity (*Table 4.2*). There was good agreement between original recording of IDU in case records on admission and follow up information obtained from a subset of the cohort of IDU during that time (Kappa 0.83 [% CI 0.74 to 0.92]).

Table 4.2 Proportion of anti-HCV positive and anti-HCV negative admissions to FIDH (1972 through 1975) who have a positive history of a risk factor for blood borne viruses recorded in medical case records

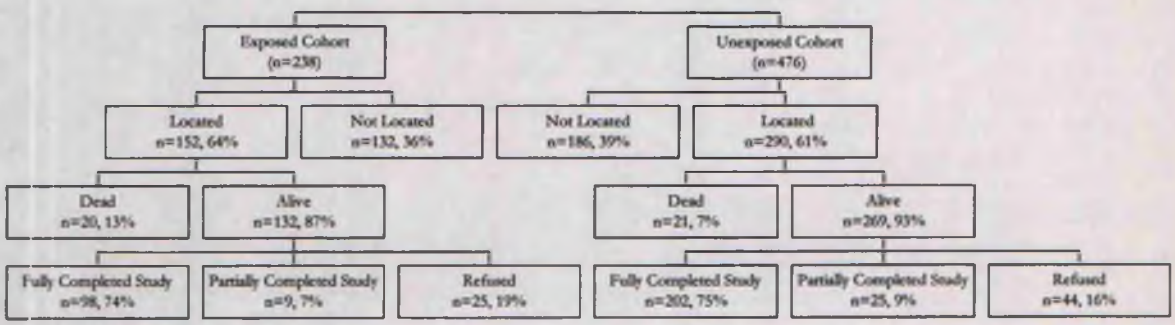
	Anti-HCV positive	Anti-HCV negative	RR (95%CI)	p value
IDU	90.4%	22.3%	32.9 (19 to 56)	<0.001
Tattoo	46.7%	40.6%	1.3 (0.7 to 2.5)	0.49
Travel overseas	44.4%	73.5%	0.2 (0.1 to 1.1)	0.08
Blood transfusion	17.9%	11.2%	1.7 (0.8 to 3.6)	0.11
Contact with a case	63.8%	35.2%	3.2 (2.3 to 4.6)	<0.001

4.4 Construction of the Study Cohort

4.4.1 Tracing and Recruiting the Cohort

One person undertook the tracing over the three-year study period. This individual, a female, has enormous experience in tracing and recruiting for longitudinal studies. Tracing was undertaken on all members of the group and 64% of the exposed group (152/238) and 61% of the unexposed group (290/476) were successfully located (*Figure 4.3*).

Figure 4.3: Cohort subjects located and recruited to the study



The ease and success of tracing methods varied considerably. The most successful methods employed were search of the electoral roll/CD-ROM telephone directory and of the NHIC database, which located 36% and 33% of subjects respectively. Other less successful and time consuming methods were letter to last known address (15%), contact of next of kin (10%), radio publicity (3%), newspaper advertisements (1%) and HCV support group newsletters, contact with last known GP or 'word of mouth' (less than 1% each).

The proportion traced by any particular method was similar between the exposed and non-exposed cohorts. Factors associated with successful tracing included being male, not admitting

to a history of IDU at the time of admission in the 1970s and not having tattoos (*Table 4.3*). We found no difference in response rates by method of contact, in particular between those contacted by phone and those contacted by letter. A phone protocol had been developed, but was rapidly abandoned as each phone call was individual in nature.

The participation rate of those approached was 81% for the anti-HCV positive group and 84% for the anti-HCV negative group. Participation rate did not vary by method of contact, socio-demographic features or risk behaviours (*Table 4.4*). Of those who did participate, 90% fully completed all aspects of the study, including clinical follow up.

Table 4.3 Socio-demographic and serological characteristics (at the time of original admission to FIDH in 1971 to 1975) associated with successful tracing

	Proportion traced (n=442)	**p value**
Age (median=23)		
>23	66%	
≤23	60%	0.194
Sex		
male	70%	
female	55%	<0.01
Country of birth		
Australia	65%	
other	58%	0.158
History of IDU		
no	80%	
yes	65%	<0.01
HBcAb status		
negative	65%	
positive	61%	0.221
Anti-HCV status		
negative	63%	
positive	64%	0.192

Table 4.4 Socio-demographic and serological characteristics (at the time of original admission to FIDH in 1971 to 1975) associated with participation in the study

	Proportion participated (n=332)	p value
Age (median=23)		
>23	82%	
≤23	83%	0.267
Sex		
male	81%	
female	85%	0.237
Country of birth		
Australia	84%	
other	78%	0.139
Tattooed		
no	98%	
yes	92%	0.713
History of IDU		
no	95%	
yes	81%	0.207
HBsAg status		
negative	82%	
positive	83%	0.785
HBcAb status		
negative	87%	
positive	78%	0.18
Anti-HCV status		
negative	84%	
positive	80%	0.482

4.4.2 Completeness of Cohort Follow-Up

Almost two thirds of the cohort was located, 66% of the exposed group (152/238) and 61% of the unexposed group (290/476). Of those located, 13% (20/152) of the former group and 7% (21/290) of the latter were deceased.

Of those who were located alive, 81% (n=107) of the anti-HCV positive group and 78% (n=225) of the anti-HCV negative group agreed at initial contact to participate in the study (*Table 4.5*). Follow-up was fully completed by 98 anti-HCV positive (exposed) individuals and 202 anti-HCV negatives (unexposed).

Table 4.5 Proportion of exposed and unexposed subjects who were located alive who participated in the study

	Anti-HCV positive traced subjects (n=132)	**Anti-HCV negative traced subjects (n=269)**
Full participation in all aspects of the study	98 (74%)	202 (75%)
Complete refusal to take part in any aspect of the study	25 (19%)	44 (16%)
Partially completed the study	9 (7%)	23 (9%)

Seventeen unexposed subjects, whose 1971-75 sera were anti-HCV negative, were found to be anti-HCV positive at follow-up and excluded from further analysis to avoid misclassification bias. All results presented for the unexposed group from this point are based on the 185 who remained anti-HCV negative at follow up.

Those completing follow-up did not differ sociodemiographically or serologically from the main cohort *(Table 4.6).*

Table 4.6 Comparison of the sociodemographic and serological characteristics at the time of admission to Fairfield Infectious Diseases Hospital from 1971 through 1975 of the study group with the full cohort by anti-HCV status

	Anti-HCV positive		**Anti-HCV negative**	
	Full cohort (n=238)	**Completing follow up (n=98)**	**Full cohort (n=476)**	**Completing follow up (n=185)**
Median age (admission)	21 yrs	19yrs	30yrs	29 yrs
Proportion male	62%	65%	52%	54%
Born in Australia	80%	81%	69%	77%
Hepatitis markers				
Anti-HAV IgM	14%	15%	45%	58%
HBcAb	75%	75%	43%	33%
HBsAg	46%	49%	23%	17%
Anti-HCV only	16%	17%	n/a	n/a

4.5 Characteristics of the Cohort at Follow Up

4.5.1 Sociodemographic Data

Anti-HCV positive individuals (n=98) were significantly younger than those anti-HCV negative (n=185) (median age 44yrs *cf* 52yrs, p=0.001), *Table 4.7.* Both seropositive and seronegative groups were predominantly male (65% and 54%) and born in Australia (81% and 77%). The highest level of education attained and current income level were similar between the two groups. However, anti-HCV positive individuals were significantly more likely to be divorced ($p<0.0001$) and to receive sickness benefit as their main source of income ($p=0.04$) than those who were anti-HCV negative.

Table 4.7 Sociodemographic data at follow up by anti-HCV status

	Anti-HCV positive (n=98)	Anti-HCV negative (n=185)	p value
Male (%)	64 (65%)	99 (54%)	0.56
Median age in years	44	52	<0.001
Education			
Secondary	47 (48%)	76 (41%)	
Tertiary	28 (29%)	63 (34%)	
Trade	17 (17%)	32 (17%)	
Other	6 (6%)	14 (7%)	0.0428
Employment status (%)			
Full time	46 (47%)	30 (43%)	
Part time	10 (10%)	18 (10%)	
Unemployed	6 (6%)	10 (5%)	
Sickness benefit	27 (27%)	23 (12%)	
Other	9 (9%)	69 (34%)	0.04
Income level per year (%)			
< $12,000	30 (31%)	57 (31%)	
$12,001-$25,000	28 (29%)	41 (22%)	
$25,001-$50,000	27 (28%)	48 (26%)	
> $50,001	11 (12%)	37 (20%)	0.25
Marital status			
Never married	17 (17%)	20 (11%)	
Married/defacto	47 (48%)	138 (75%)	
Divorced	29 (30%)	19 (10%)	
Other	5 (5%)	7 (4%)	<0.001
Country of birth			
Australia	79 (81%)	143 (77%)	
Other	21 (19%)	42 (23%)	0.57

4.5.2 Alcohol Intake

The exposed group was less likely to drink alcohol currently than was the unexposed group (RR 0.6, 95% CI: 0.4 to 0.8; p=0.03); however, this relative abstinence was only present in seropositive individuals aware of their HCV antibody status (n=52). Both groups had similar durations of alcohol intake, but the HCV positive group had consumed significantly greater average amounts of alcohol (mean 281gms per wk vs 123 gms per wk, p<0.001) over that period and were twice as likely to have had periods of 'binge' or dipsomaniac drinking (95% CI: 1.5 to 2.2; p=0.02).

The overall consumption of alcohol was significantly higher in the exposed subjects who had progressed to clinically apparent cirrhosis (mean 844 gms per week [SD 252]) than those who had not progressed (mean 312 gms per wk [SD 321], p=0.02).

4.5.3 Risk Factors for HCV Infection

Prior to admission to FIDH in the early 1970s, having a history of IDU conferred an increased risk of being HCV (RR 14.4, 95% CI: 8.6 to 24.5, p<0.001) *Table 4.8*. Eighty six percent of the anti-HCV positive group had injected drugs - two thirds doing so more than three times per week - for a mean period of 2 years prior to admission. Almost all (92%) shared needles.

A history of being tattooed also conferred an increased risk of being anti-HCV positive on admission (RR 2, 95% CI: 1.6 to 2.9; p<0.001) as did a history of having unprotected sex. Receiving transfusions of blood or blood products, or being born overseas were not associated with HCV serostatus.

Table 4.8 Risk behaviours for the transmission of blood borne viruses before admission to Fairfield Infectious Diseases Hospital in the early 1970s

	Anti-HCV positive (n=98)	Anti-HCV negative (n=185)	RR/Mean diff (95% CI)	p value	Adj. RR*	p value
Blood transfusion (%)	5 (5%)	13 (7%)	0.71 (0.3 – 1.7)	0.049		
Tattooed (%)	22 (22%)	12 (7%)	2.1 (1.6 – 2.9)	<0.001	0.99	0.95
IDU (%)	85 (86%)	3 (2%)	14.4 (8.6 – 24.5)	<0.001	14.22	<0.000
Mean duration yrs (SD)	2 (2.5)	0.7 (0.7)	1.4 (-1.5 – 4.4)	0.043		1
% injecting >3/wk	70%	0%				
% sharing needles	92%	0%				
Unprotected sex (%)	91 (93%)	138 (77%)	2.7 (1.4 – 5.6)	0.004	0.98	0.083
% one partner	14%					
% > six partners	65%					

*Adjusted for IDU, Tattoo, and Sex – two models used

In the period from admission to FIDH in the early 1970s to study follow up, anti-HCV positive subjects continued to be more likely to inject drugs (RR 8.4, 95% CI: 5.7 to 12.3, p<0.001) than the anti-HCV negative group (*Table 4.9*). Seventy six percent of the exposed group injected drugs over a mean period of 9.5 years. Sixty three percent injected more than 3 times per week and 73% shared needles.

Table 4.9 Risk behaviours for the transmission of blood borne viruses after admission to Fairfield Infectious Diseases Hospital in the early 1970s

	Anti-HCV positive (n=98)	Anti-HCV negative (n=185)	RR/mean diff (95% CI)	p value	Adj. RR*	p value
Tattooed (%)	25 (25%)	6 (3%)	2.8 (5.7 – 12.2)	<0.001	0.92	0.91
IDU (%)	74 (76%)	2 (1%)	8.4 (5.7 – 12.3)	<0.001	8.6	<0.0001
Mean duration yrs (SD)	9.5 (7.9)	1.5 (0.7)	1.42 (-1.5 – 4.36)	0.37		
% injecting >3/wk	63%	0%				
% sharing needles	73%	0%				
Unprotected sex (%)	98 (100%)	157 (85%)	3.8 (1.3 – 11.2)	0.016	1.12	0.65
% one partner	14%					
% > six partners	65%					

*Adjusted for IDU, Tattoo, and Sex – two models used

4.5.4 Hepatitis Testing History

In the period between discharge from FIDH to study follow up, the exposed group were significantly more likely to have undergone testing for the serological markers of hepatitis A, B and C (*Table 4.10*). In total, 56% (n=55) of anti-HCV positive subjects had been tested for anti-HCV prior to taking part in the study. The remainder (n=43) had not been tested and were therefore unaware that they were anti-HCV positive at the time of enrolment in the study. The 8% of the unexposed subjects who had tested for HCV had all tested negative.

Of those in the exposed group who had previously and knowingly tested for hepatitis C, 94% (n=52) had been told they were anti-HCV positive. The 6% (n=3) that tested 'negative' were all tested in 1991 and probably reflected a proportion of the false negative results that were obtained with first generation anti-HCV assays. All three subjects tested anti-HCV positive at the time of study follow up – using second or third generation assays.

Three percent of the exposed group and 1% of the unexposed had been vaccinated against HAV. The proportions vaccinated against HBV were 5% in the exposed group and 7% in the unexposed.

Table 4.10 Proportion of the cohort who had undergone testing for serological markers of current or previous hepatitis A, B or C from the time of admission to FIDH to study follow up

Serological marker	Anti-HCV positive % tested	Anti-HCV negative % tested	p value
Hepatitis A	36% (35/98)	22% (41/185)	0.041
Hepatitis B	55% (54/98)	16% (30/185)	<0.0001
Hepatitis C	56% (55/98)	8% (15/185)	<0.0001

4.5.5 HCV Treatment History

In those who were aware that they had HCV (n=52), only two had been treated with interferon (in 1993 and 1995) and none with interferon/ribivirin combination therapy. Both subjects had received interferon for less than 12 weeks in total, at dose of 1 million units three times per week. Neither had responded to treatment, both were currently HCV RNA positive and were genotype 1a.

Five subjects had tried homeopathic remedies such as milk thistle or silymarum for varying lengths of time. Most felt symptomatic benefit from this and had combined the treatment with abstinence from alcohol.

4.6 Morbidity Outcomes

4.6.1 Serology

4.6.1.1 Unexposed (Anti-HCV Negative) Cohort

Seventeen unexposed subjects whose 1971-75 sera tested anti-HCV negative, were anti-HCV positive at follow-up and excluded from further analysis to avoid misclassification bias. Of these, 60% (n=10) had a history of IDU prior to admission to FIDH. The mean length of time subjects had been injecting was 9 months (SD 8.4). After admission to FIDH, 94% (n=16) admitted to a history of IDU for a mean period of 14 years (SD 25). Twelve subjects shared needles during this time. It is likely that subjects had become infected with HCV during this period of IDU after admission to FIDH.

The remainder of the unexposed group (n=185) remained anti-HCV negative at follow up.

Four unexposed subjects, who had been admitted with a clinical diagnosis of hepatitis B in the early 1970s, were HBsAg positive at follow up. Testing of stored sera indicated that three had been HBcAb positive at the time of discharge from FIDH. At follow up two also had HBeAg and DNA status measured, and were negative for both. Only one had an elevated ALT level, which was just above normal at 57 u/l (NR 25-50 u/l)

Two unexposed subjects were HIV positive at follow up.

4.6.1.2 Exposed (Anti-HCV Positive) Cohort

Seven exposed individuals, who were strongly anti-HCV positive on testing of stored sera from the early 1970s, were anti-HCV negative on follow-up testing, while a further subject had an equivocal result (with a previously positive anti-HCV result in 1996). This group (n=7, 7%), who were also negative for HCV RNA by PCR, appear to have 'lost' anti-HCV during the period 1971/75 to 1997/98, consistent with 'natural' clearance of HCV infection. None of these subjects had been treated with interferon therapy.

None of the anti-HCV positive group had evidence of chronic HBV infection (HBsAg) at follow-up.

One subject, who died in 1994 of cirrhosis, had HIV and HCV co-infection. None of the other exposed subjects were known to have HIV at follow up. HIV status was not routinely measured on study subjects due to ethical constraints. It is unlikely to be significant issue in this cohort as the risk of acquiring HIV through injecting drug use in Australia is very low[27] and no exposed subjects were identified as homosexual.

4.6.2 Virology

PCR testing was completed in 97% (95/98) of anti-HCV positive subjects at follow-up. Fifty one (54%) of the anti-HCV positive group were HCV RNA positive indicating chronic HCV infection. Genotyping was performed on HCV RNA positive samples. The most common genotypes and subtypes were 3a (45%), followed by 1a (24%), 1b (10%) and 2b (9%). Another 6% of samples were genotype 1, but could not be further subtyped by the Innogenetics assay.

Thus at follow up, 7% of the exposed cohort had unequivocal (anti-HCV negative) resolution of HCV infection, 42% had probable resolution (anti-HCV positive, but HCV RNA negative), while 51% were chronically infected with HCV (anti-HCV and HCV RNA positive).

Risk Factors for Chronic HCV Infection

Factors predictive for chronic HCV infection among exposed subjects who remained anti-HCV and HCV RNA positive at follow up, were examined by comparing HCV RNA positive subjects to HCV RNA negative subjects (*Table 4.11*).

On univariate analysis, HCV RNA positive subjects were more likely to have had histories of IDU. In those who had a history of IDU, HCV RNA positive subjects were likely to have injected more frequently - more than 3 times per week - and to have injected for longer periods of time than were those who were HCV RNA negative at follow-up.

Average lifetime consumption of alcohol - in average gms per week - was also a predictor for chronic HCV infection, although total duration of alcohol consumption was not. On multivariate analysis, frequency of injecting (adjusted OR 2.98, 95% CI: 1.2 to 7.3, p=0.017) remained significantly associated with chronicity and total average lifetime consumption of alcohol was of borderline significance (adjusted OR 1, 95% CI: 1 to 1.01, p=0.055). Having a history of IDU, duration of injecting and frequency of injecting were too highly correlated and could not all be included in the model.

Table 4.11 Factors associated with chronic HCV infection in the anti-HCV positive cohort comparing HCV RNA positive and negative subjects

	RNA positive (n =51)		RNA negative (n = 44)		OR	p value	95% CI	Adjusted OR*	p value	95%CI
Age										
Mean age (SD)	43	(3)	45	(4)	0.89	0.083	0.78, 1.01			
Gender										
Male (%)	31	(61%)	33	(75%)	0.79	0.579	0.34, 1.84			
Country of birth										
Australia (%)	41	(82%)	38	(83%)	0.91	0.860	0.32, 2.56			
Co-infection with HBV										
HBcAb positive (%)	36	(71%)	36	(81%)	0.78	0.658	0.27, 2.30			
HBsAg positive (%)	0	(0%)	0	(0%)	n/a					
Injecting drug use history										
History of IDU (%)	50	(98%)	38	(86%)	12.9	0.002	1.6, 106.1			
Mean no of years IDU (SD)	8	(8)	11	(9)	1.06	0.045	1.01, 1.11			
% Shared Needles	79%		63%		2.14	0.165	0.73, 6.25			
% Injected >3 times week	59%		32%		3.70	0.003	1.57, 8.71	2.98	p=0.017	1.22,7.31
Alcohol intake history										
Drunk alcohol in past 12 months (%)	27	(53%)	28	(64%)	0.68	0.366	0.29, 1.58			
Ever drank at least once per week	50	(98%)	44	(100%)	3.66	0.269	0.37, 36.51			
Mean duration of intake in yrs (SD)	17	(9)	19	(10)	0.98	0.441	0.93, 1.03			
Lifetime average gms/wk (SD)	342	(310)	223	(262)	1.01	0.028	1.00, 1.03	1.00	p=0.055	1.00, 1.01
Reported binge episodes ever	32	(63%)	21	(48%)	2.00	0.144	0.79, 5.09			

* Adjusted for age, sex, frequency of injecting, total average lifetime consumption of alcohol

4.6.3 Non Liver Related Morbidity

Fifty five percent (n=54) of the exposed group had at least one other medical condition requiring ongoing clinical care compared to 42% (n=77) of the unexposed group (RR 1.4, 95% CI: 1.1 to 1.9, p=0.045). Exposed subjects, with active medical conditions, were significantly more likely than the unexposed group to have psychological or alcohol-related conditions and significantly less likely to suffer from gastrointestinal or genitourinary conditions (*Table 4.12*).

Table 4.12 Non liver related morbidity in exposed and unexposed cohort subjects who reported ongoing medical conditions

	Anti-HCV positive (n =54)	**Anti-HCV negative (n=77)**	**RR (95% CI)**	**p value**
Cardiac	11%	21%	0.59 (0.26 to 1.3)	0.153
Respiratory	13%	6%	1.61 (0.9 to 2.9)	0.158
Arthritis	17%	12%	1.32 (0.72 to 2.9)	0.427
Gastrointestinal	15%	36%	0.45 (0.22 to 0.92)	0.013
Malignant neoplasms	2%	5%	0.56 (0.96 to 3.30)	0.475
Neurological	6%	7%	0.92 (0.36 to 2.39)	0.866
Genitourinary	27%	46%	0.57 (0.32 to 0.99)	0.036
Psychological	23%	10%	1.79 (1.12 to 2.85)	0.033
Alcohol related	4%	0%	2.92 (2.31 to 3.78)	0.007

Both exposed and unexposed subjects had an average of 2.5 surgical operations requiring a general anaethestic.

4.6.4 Liver Related Morbidity

4.6.4.1 Unexposed (Anti-HCV Negative) Cohort

Six percent (n=11) of the anti-HCV negative cohort had elevated ALT levels at follow-up (*Table 4.13*). Median ALT was 23u/l (NR 25-50 u/l) and median AST was 22u/l (NR 20-40 u/l). All had a serum albumin concentration within the normal range and 97% had a bilirubin level within the normal range (*Table 4.13*)

Three percent (n=2) had evidence of chronic liver disease (spider naevi, firm hepatomegally) on clinical examination - as documented by the examining doctor using the study clinical proforma. One had biopsy proven cirrhosis and evidence of portal hypertension (ascites, splenomegaly and caput medusa); alcohol was the likely aetiology. No cases of HCC were detected.

4.6.4.2 Exposed (Anti-HCV Positive) Cohort

In the anti-HCV positive cohort, LFTs at follow up were suggestive of relatively benign disease (*Table 4.13*). Forty one (42%) had elevated serum ALT levels (*Figure 4.3*). Subjects who were HCV RNA positive were significantly more likely to have elevated ALT levels (69%) than those who were HCV RNA negative (14%). Median ALT in those who were HCV RNA negative was 21 mmol/l (IQ range 17 to 35) compared to 95 mmol/l (IQ range 36 to 137) in HCV RNA positive subjects. Only one subject had a serum albumin level below the normal range, and the same individual had a bilirubin level greater than 40 umol/l. In total 7% of the exposed group had abnormal bilirubin levels (*Table 4.14*).

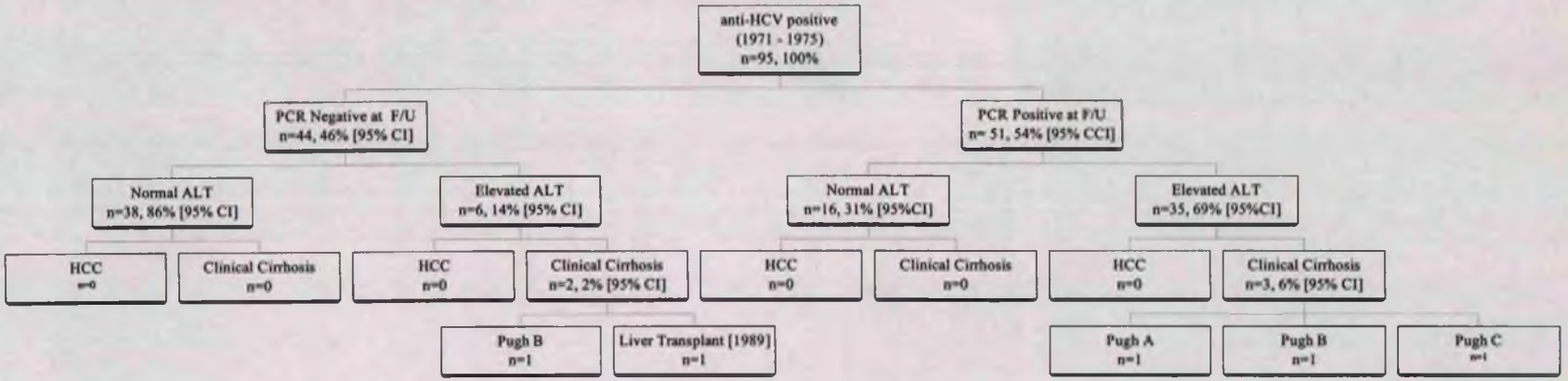
Table 4.13 Median values of liver function tests at clinical follow up in the anti-HCV positive and anti-HCV negative cohorts

	Anti-HCV positive (n=98)		**Anti-HCV negative (n=185)**		**p value**
	Median	(IQ Range)	Median	(IQ Range)	
Albumin (NR 30-50 g/l)	41	(38-44)	40	(37-43)	0.57
Alkaline phosphatase (NR 80-120u/l)	67	(64-69)	77	(72-81)	0.07
ALT (NR 25-50 u/l)	36	(20-78)	23	(17-32)	<0.001
AST (NR 20-40 u/l)	28	(21-58)	22	(18-26)	<0.001
GGT (NR 30-63 u/l)	32	(16-53)	22	(16-37)	0.016
Bilirubin (NR 10-20 umol/l)	10	(7-12)	10	(7-13)	0.17
Total protein (NR 65-80 u/l)	74	(71-77)	72	(70-75)	0.13

Table 4.14 Proportion of the anti-HCV positive and anti-HCV negative cohorts with abnormal albumin and bilirubin liver tests at clinical follow up

	Anti-HCV positive (n=98)	**Anti-HCV negative (n=185)**	**p value**
Albumin (NR 30-50 g/l)			
<30 g/l	1 (1%)	0 (0%)	0.236
31-35 g/l	11 (11%)	20 (11%)	
36-40 g/l	34 (35%)	82 (44%)	
>40 g/l	52 (53%)	83 (45%)	
Bilirubin (NR 10-20 umol/l)			
<20 u/l	90 (92%)	180 (97%)	0.77
21-25 u/l	3 (3%)	4 (2%)	
36-40 u/l	4 (4%)	1 (0.5%)	
>40 u/l	1 (0.4%)	0 (0%)	

Figure 4.4: Outcomes in exposed (anti-HCV positive) cohort who completed study follow up including HCV PCR (n=95)



In those who were HCV RNA positive at follow up, 14% (7/52) had evidence of chronic liver disease (firm hepatomegally) on clinical examination. Of these, 6% (3/52) had clinical manifestations of cirrhosis (i.e. portal hypertension), and 8% (4/52) had biopsy proven cirrhosis.

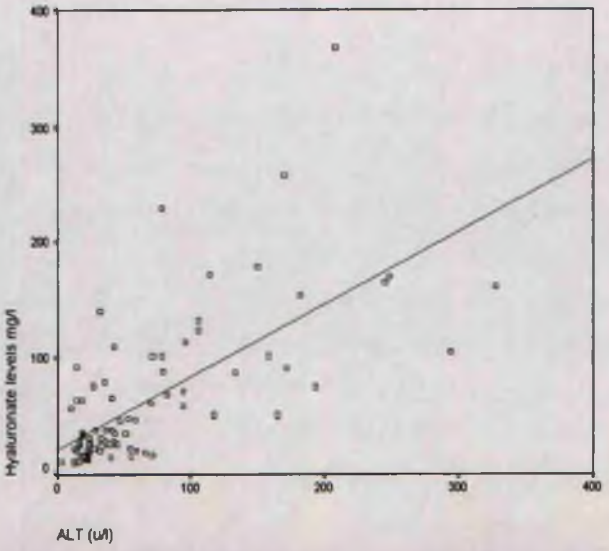
The HCV genotypes in those with evidence of chronic liver disease were – 1a, 1b, 1b, 1b, 3a, 3a, 3a - and in those with cirrhosis; 1a, 1b and 3a.

In subjects who were HCV RNA negative, 5% (2/44) had progressed to cirrhotic liver disease during the period of follow up; one underwent liver biopsy in 1996 with histological findings more suggestive of alcohol as the aetiology rather than HCV. A further subject had liver transplant in 1989 for cirrhosis and is currently asymptomatic. All other RNA negative subjects were clinically well. No cases of HCC were detected in either group.

Serum Hyaluronate (non invasive marker of liver fibrosis)

In total, 14% (14/98) HCV seropositive subjects underwent liver biopsy in the five years preceding study follow up. In view of the lack of histological data on study subjects, serum hyaluronate (HA) was measured in the HCV seropositive group as a surrogate marker for hepatic fibrosis. *Figure 4.5* demonstrates the correlation between serum ALT and serum HA levels.

Figure 4.5 Correlation between serum ALT and serum HA levels in exposed cohort subjects



Although serum HA principally measures hepatic fibrosis and serum ALT hepatic inflammation, in chronic HCV both processes are often evident and hence the noted correlation in our subjects. (Pearson's correlation coefficient was significant at 0.627, p <0.0001).

Forty four percent of the exposed cohort had abnormal serum HA levels (>37μg/l) suggestive of some degree of hepatitis fibrosis. Those who were HCV RNA positive were twice as likely as HCV RNA negative subjects to have elevated HA levels (64% vs 24%, RR 2.2, CI: 1.3 to 3.7; p=0.003).

All with biopsy proven cirrhosis (n=5) had HA levels >170μg/l. Another study, which used the same methodology to measure HA levels, reported mean HA levels in those with biopsy proven cirrhosis of 219μg/l.[237] In this study only 3 subjects (5%) had HA levels >200μg/ml.

4.6.4.3 Rate Ratio Liver Morbidity at Follow Up

The rate of chronic hepatitis in the exposed group at follow up was 3.7 that of the unexposed group (*Table 4.15*). Exposed subjects were 11.9 times more likely to have cirrhosis at follow up.

Table 4.15 Rate ratio of liver morbidity in exposed versus unexposed cohorts

	Rate per 1,000PY in anti-HCV positive cohort	**Rate per 1,000 PY in anti-HCV negative cohort**	**Rate ratio (95% CI)**	**p value**
Chronic hepatitis	17.3	2.3	3.7 (2.7-4.8)	p<0.0001
Cirrhosis	2.5	0.21	11.9 (5-19)	p=0.04
HCC	0	0	-	-

4.7 Mortality Outcomes

4.7.1 All Cause Mortality

Thirteen percent (20/152) of the HCV seropositive group and 7% (21/285) in the HCV seronegative were found to have died since the time of discharge from FIDH to the end of the follow up period (31/12/98). The proportion of liver related mortality was 1.3% (2/150) in the exposed group and 0% in the unexposed.

The two liver related deaths in those anti-HCV positive were hepatic cirrhosis, in 1996 and 1998.

The main cause of death in the anti-HCV positive group was drug overdose (4%, 6/152) and suicide (2%, 3/152). Anti-HCV positive subjects were 4 times more likely to die from suicide or overdose than from HCV related liver disease (*Table 4.16).* None of those who committed suicide had been treated with interferon therapy.

Table 4.16 Underlying cause of death in anti-HCV positive and anti-HCV negative cohorts

Cause of death	Anti-HCV positive (n=20)	Anti-HCV negative (n=21)
Liver cirrhosis	2 (10%)	0 (0%)
Cardiovascular disease	2 (10%)	4 (19%)
Malignant neoplasms	4 (20%)	5 (24%)
Opiate overdose	6 (30%)	1 (5%)
Suicide	3 (15%)	3 (14%)
HIV/AIDS	0 (0%)	1 (5%)
Motor vehicle accident	2 (10%)	1 (5%)
Other	1 (5%)	6 (30%)

4.7.2 Standardised Mortality Ratios

The all cause mortality rate was significantly higher in the exposed cohort than the general Australian population (SMR 333, 95% CI: 121 to 912, p<0.001), but not in the unexposed group (SMR 116, 95% CI: 93 to 143, p=0.479), *Table 4.17*. In the exposed cohort the mortality rate due to liver disease was 2.3 times higher than the general population, although this increased rate was not significant. The suicide rate in the exposed group was significantly greater at six times that of general population.

Deaths from liver disease or suicide in the unexposed group were no higher than that found in the general population.

Table 4.17 Standardised Mortality Ratio by ICD 9 classification categories in exposed (anti-HCV positive) and unexposed (anti-HCV negative) subjects

	Anti-HCV positive cohort					Anti-HCV negative cohort				
Cause of death*	**Obs**	**Exp**	**SMR**	**95% CI**	**p value**	**Obs**	**Exp**	**SMR**	**95% CI**	**p value**
All Cause	20	6	333	121 - 912	< 0.001	21	18	116	93 - 143	0.479
Liver	3	1.3	230	96 - 548	0.1658	0	1.2	-	-	-
Suicide	3	0.5	600	252 - 1428	0.0004	2	0.87	229	95 - 2189	0.227

*ICD-9

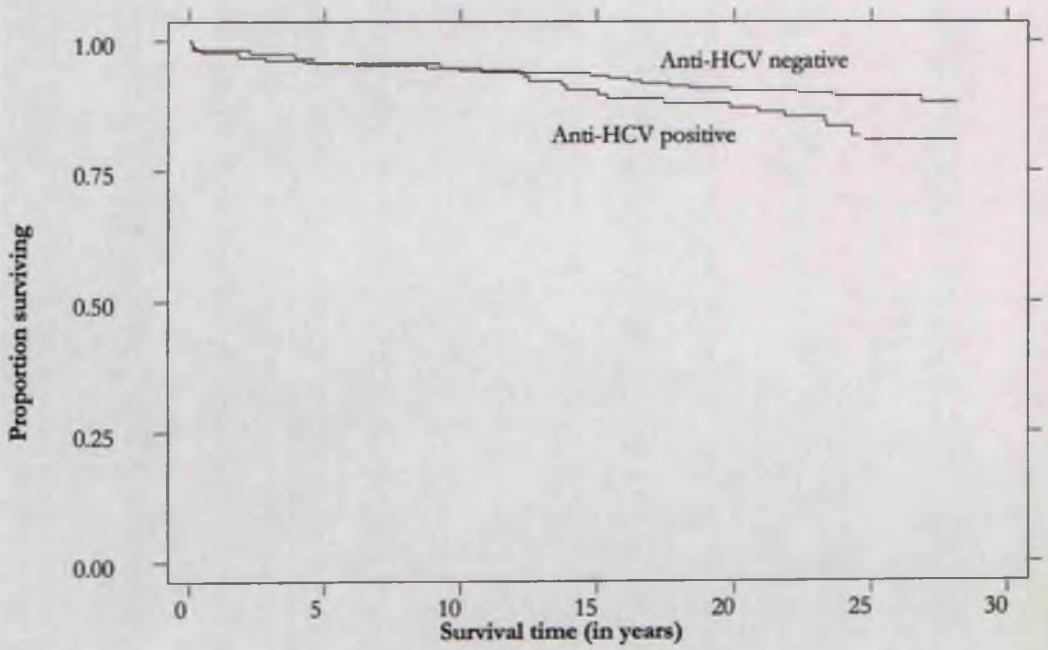
Obs = observed, Exp = expected number of deaths based on Australian age and calendar-year specific rates

4.7.3 Survival

Overall survival for the period of follow up from admission to FIDH to death or the end of follow up period (31.12.98), is demonstrated in *Figure 4.6.*

There was no significant difference in the proportion surviving in each group (Log-rank test for equality of survivor functions: chi2 = 3.64, p= 0.0564)

Figure 4.6 Kaplan-Meier survival estimates, by exposure



4.8 Quality of Life Outcomes

4.8.1 Quality of Life

Subjects who were anti-HCV positive had a significantly poorer quality of life scores in six out of the eight scales (*Table 4.18*), indicating that the exposed cohort subjects had a subjective perception of very poor physical and mental health. The only areas with no significant difference were in physical functioning and bodily pain.

Table 4.18 Mean Short Form 36 scores in anti-HCV positive subjects compared to anti-HCV negative subjects

	Anti-HCV positive mean (SE)	**Anti-HCV negative mean (SE)**	**Mean Difference (95% CI)**	**p value**
Physical functioning	75 (2.9)	80 (1.6)	-5.3 (-11.9 to 1.4)	0.119
Role limitation physical	58 (4.9)	74 (2.9)	-16.3 (-27.5 to –5.1)	0.004
Bodily pain	63 (2.7)	69 (1.6)	-6.1 (12.4 to 0.14)	0.056
General health	50 (3.0)	66 (1.7)	-15.8 (-22.5 to –8.9)	0.000
Vitality	49 (2.6)	60 (1.7)	-10.9 (-16.9 to –4.8)	0.001
Social functioning	66 (3.2)	81 (1.8)	-15.0 (-22.3 to –7.8)	0.000
Role limitation emotional	59 (5.0)	75 (2.7)	-15.3 (26.5 to –4.0)	0.008
Mental health	63 (2.3)	74 (1.4)	-10.6 (-15.9 to –5.8)	0.000

4.8.2 Impact of a Diagnosis of Hepatitis C on Quality of Life

To measure the impact of a diagnosis of hepatitis C on quality of life we compared the SF-36 scores of chronically HCV infected subjects who were aware of their serostatus at the time of completion of the SF-36 questionnaire, to those who were unaware.

Subjects were excluded from this analysis if found to be currently anti-HCV or HCV RNA by PCR negative, have cirrhosis or clinically detectable liver disease, if symptomatic at the time of their recent anti-HCV testing or with medical conditions other than HCV which could affect quality of life.

Of the 34 chronically HCV infected subjects (anti-HCV positive and HCV RNA by PCR positive at follow up) included in the QOL analysis, 15 (44%) already knew of their HCV status prior to follow-up contact. Testing for anti-HCV had been undertaken for a variety of reasons including; patient request due to a previous history of IDU or an ex-injecting partner being diagnosed with HCV (5), GP recommendation due to a history of IDU (4), screening prior to blood donation (2), routine health screen (2) or during participation in other research studies (2). None had been tested because they were symptomatic. The mean length of time since diagnosis was 2 years (SD 1.1 years). The remaining 19 were unaware of their HCV serostatus when they were recruited and completed the SF-36 scale.

Stratified analysis of SF-36 scores found that those aware of their positive HCV serostatus had a significant reduction in QOL scores in seven out of eight scales compared to population norms (*Table 4.19*). These individuals had a subjective perception of extremely poor physical and mental health leading to limitation of daily activities, bodily pain, poor social functioning and emotional problems. In contrast, the group who were unaware of their positive HCV status had significantly lower QOL scores in only three scales (general health, vitality and mental health).

Table 4.19 Mean Short Form 36 scores by awareness of sero-status in anti-HCV positive and PCR positive individuals, compared to population norms

	Population Norms (SE)	anti-HCV &HCV PCR positive study subjects	
		Aware of sero-status n=15 (SD)	Unaware of sero-status n=19 (SD)
Physical functioning	86 (0.5)	82 *(17)*	91 *(14)*
Role limitation physical	83 (0.8)	58 *(45)**	75 *(42)*
Bodily pain	78 (0.6)	67 *(26)***	82 *(23)*
General health	73 (0.5)	43 *(27)****	64 *(25)****
Vitality	66 (0.5)	49 *(20)****	60 *(21)****
Social functioning	86 (0.5)	65 *(18)****	83 *(25)*
Role limitation emotional	85 (0.8)	64 *(43)**	84 *(30)*
Mental health	74 (0.4)	66 *(11)****	71 *(19)***

* $p < 0.05$ compared to population norms

** $p < 0.01$ compared to population norms

*** $p < 0.001$ compared to population norms

Those aware of their positive HCV serostatus did not differ from those who were unaware socio-demographically, on risk history, biochemically or virologically (*Table 4.20*).

The only difference was that those who were aware of their diagnosis were less likely to currently drink, probably reflecting medical advice given at the time of diagnoses. None had been treated or were being treated with standard therapies (interferon +/- ribivirin) or naturopathic remedies. Both groups had comparable histories of previous IDU and no subjects had injected within the previous 24 months. No individuals had a history of depression or any other current illness likely to affect QOL. In addition, there was no link between knowledge of HCV status (and reduced QOL) and objective measures of ill health such as abnormalities on physical examination or elevated ALT. The differences in QOL scores remained after adjustment for age, sex, marital status and serum ALT levels (*Figure 4.7*).

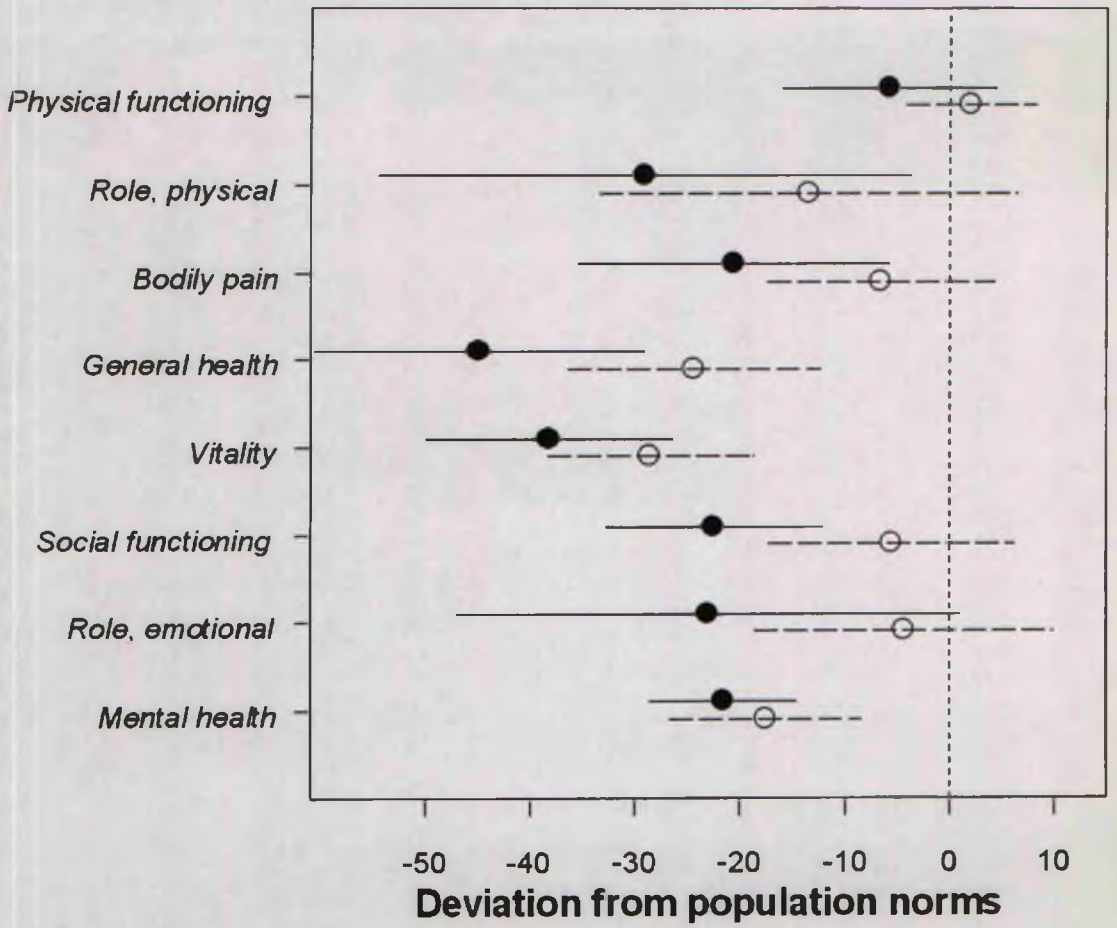
Table 4.20 Sociodemographic, clinical and serological comparison at the time of QOL testing of those aware of positive HCV serostatus and those unaware

	Anti-HCV and PCR positive subjects		p value
	Aware of sero-status (n=15)	Unaware of sero-status (n=19)	
Mean age in years (SD)	44 (2)	42 (2)	0.16
Male (%)	10 (67%)	10 (53%)	0.32
Born in Australia (%)	10 (67%)	16 (84%)	0.37
Never married (%)	2 (13%)	3 (16%)	0.86
Married/defacto (%)	9 (60%)	9 (47%)	
Divorced (%)	3 (20%)	6(32%)	
Other (%)	1 (7%)	1 (5%)	
History of IDU (%)	15 (100%)	19 (100%)	0.07
Mean no of years IDU (SD)	13 (10)	8 (10)	
Injected in past 24 mths (%)	0 (0%)	0 (0%)	
Mean yrs dur'n alcohol use (SD)	18 (8)	19 (9)	0.75
Mean number of gms/day (SD)	31 (32)	32 (33)	0.89
Reported binge episodes (%)	10 (77%)	11 (69%)	0.47
HBcAb positive (%)	10 (67%)	11 (58%)	0.18
HBsAg positive (%)	0 (0%)	0 (0%)	
Mean ALT (SD) [NR 25-50 u/l]	92 (86)	98 (86)	0.83
Mean AST (SD) [NR 20-40 u/l]	59 (37)	57 (47)	0.92
Mean GGT (SD) [NR 30-63 u/l]	74 (144)	62 (75)	0.75
Genotype 1/1a/1b	6 (40%)	9 (47%)	0.67
Genotype 2b	2 (13%)	1 (5%)	
Genotype 3a	7 (47%)	9 (47%)	

Figure 4.7 Mean difference in SF-36 quality of life scores between population norms and anti-HCV and PCR positive individuals aware of serostatus (n=15) and unaware of serostatus (n=19), after adjusting for age, sex, marital status and ALT levels.

● Positive anti-HCV status known

○ Positive anti-HCV status unknown



CHAPTER 5: DISCUSSION

5.1 Implications of the Study

Infection with the Hepatitis C virus (HCV) is widespread and continues to spread at a rapid rate in vulnerable sections of the population, such as IDUs.[48 59 60] An estimated 150,000 people are chronically infected with HCV in Australia as a result of IDU, with a further 11,000 becoming infected each year.[48] Similar scenarios are evident in the UK, the USA and other industrialised nations.[30 57 247 248] As acute HCV infection is generally benign (with estimates that less than 15% are icteric),[249] the major associated medical and public health significance of infection is the development of chronic liver disease.

For policy makers, people with chronic HCV infection, clinicians and others a fundamental question has been how many of those infected will progress to adverse outcomes and over what time period. Answering this question is difficult as knowledge of the natural history of community acquired HCV infection remains limited, primarily due to the methodological difficulties associated with conducting these studies. Such difficulties include the problems of accurately identifying those with incident infection, the long latent period before development of liver-related disease and difficulties in following up the largest affected group, IDUs, over long periods of time.

In the past five years there has been much progress in our understanding of the natural history of HCV. However, the estimated risk of progression to serious sequelae of HCV infection differs depending upon the populations studied, the methods used and the period of follow up. Transfusion related studies report rates of progression to cirrhosis of over 20% at 20 years.[249] Liver clinic series have also estimated rates of progression to cirrhosis similar or greater to those of transfusion related studies,[154] however it is likely such series suffer from considerable referral

bias. There have been very few population-based studies with any significant follow up. Of these, two are based on point source outbreaks from contaminated anti-D immunoglobulin.[74 75]

The picture that is emerging from population based studies such as ours contrasts considerably with that reported in liver clinic patients and transfusion recipients, with few subjects (2-8%) progressing to advanced liver disease at 20 years after infection. It is likely that the Irish and German anti-D studies are at the opposite end of the spectrum from transfusion acquired disease in terms of progression to cirrhosis, with our study identifying rates somewhere in-between. It may be that the findings of studies such as ours may be of greater significance for the future, with clarification of the natural history of relatively rare transfusion or anti-D related HCV infections becoming less clinically important.

In this study we traced and followed up members of a cohort who on retrospective testing, were positive for HCV antibody at the time they were admitted to FIDH with hepatitis during the years 1971 to 1975. This study has overcome many of the methodological problems outlined above, and is unique in its length of follow up and freedom from confounding bias due to treatment, as 98% of subjects studied were interferon-naive and the 2% treated, had interferon therapy for less than 12 weeks in total.

Our results are among the first to challenge the accepted litany of 20 % progression to cirrhosis at 20 years in those infected with HCV. It is also among the first to challenge the accepted chronic HCV carriage rate (quoted at 80%[250]) in those infected with community acquired HCV, as we found that just over half of our cohort had chronic HCV infection (by anti-HCV and PCR results). Our study is also unique in terms of follow up length, having already accrued a follow up period of 25 years, which is greater than any other published study, except for one that had only 17 subjects.[171]

Excess mortality was evident in the anti-HCV positive cohort, but was predominantly due to overdose or suicide rather than liver related disease and there was no significant difference in survival rates between the anti-HCV positive and anti-HCV negative cohorts.

Anti-HCV positive individuals who completed follow up, were found to be at increased risk of liver related pathology, though few (6% overall) had progressed to overt cirrhosis in the 25 years since admission and no cases of HCC were identified.

Our findings suggest that the natural history of community acquired HCV may be more benign than previously thought.

5.2 Power, Bias and Confounding

5.2.1 Power

Recruiting sufficient numbers was difficult as the cohort studied consisted predominantly of people with histories of IDU from at least 25 years ago. Nevertheless, a cohort of this size enabled us to provide useful estimates of disease progression and factors impacting on progression to chronicity, though it may not have been large enough to explore detailed issues such as the influence of specific host (e.g. diet) or viral (e.g. genotype) factors on the development of liver disease. In addition the low numbers of individuals developing liver disease in our cohort made it difficult to determine cofactors influencing disease progression.

5.2.2 Selection Bias

The anti-HCV positive cohort consisted of injecting drug users admitted with acute hepatitis – the majority with acute HAV or HBV - to FIDH in the early 1970s. They are likely to be representative of community acquired HCV infection in Melbourne in the 1970s, as during this time acute hepatitis was common among IDUs and all persons with a clinical diagnosis of hepatitis in the Melbourne Metropolitan area were admitted to FIDH. In line with current concepts of community acquired hepatitis C, more than 80% of the HCV seropositive individuals appear to have sub-clinical HCV infection rather than acute symptomatic HCV on admission, being already anti-HCV positive at the time they were hospitalised for acute HAV or HBV. Indeed only a small proportion of the 17% of the anti-HCV positive cohort with no evidence of other acute viral hepatitis markers at the time of admission were likely to have had acute HCV. This is due to the fact that clinically evident acute HCV is uncommon in IDUs and that the appearance of anti-HCV in serum often occurs after symptom onset.[251]

The possibility of introducing bias by selecting participants on the basis of co-infection with other hepatitis viruses is unknown, but unlikely to be significant as acute HAV is a self-limiting infection, and none of the anti-HCV positive group with acute HBV on admission became chronic carriers. However, the impact of an acute episode of non-C hepatitis or occult HBV infection on long-term outcomes in subjects with chronic HCV infection is unknown and the selective nature of our cohort in this respect should be noted.

IDU was the most probable source of HCV infection in the majority of subjects and as the mean time of IDU prior to admission to FIDH was 1.5 years, it is very likely that acquisition of HCV infection occurred during this time.

The recruitment rate was good and as such we believe that selection bias is unlikely to be significant in this study. Serological markers, basic socio-demographic data, and risk behaviours were all similar between those recruited and those who did not participate. We were less likely to trace those with a history of IDU and this is doubtless due to the intentional invisibility of this section of the community. However as the majority of the traced anti-HCV positive cohort were injecting drug users, it is likely that the cohort is representative.

In this cohort, an internal comparison group was used i.e. those admitted to FIDH with clinical hepatitis who did not test anti-HCV positive on stored sera. They were realistically the only available control group due to the fact that they had sera stored from the same time period as the cases. Both groups had identical follow up and assessment for outcomes. The groups were comparable except that the unexposed control group was slightly older and less likely to have a history of injecting drug use than the exposed group. It is unlikely that a history of IDU increases the risk of severe liver disease except by its associations with HBV and increased alcohol use, both if which were controlled for in the analysis. The increased age of the control group was significant, but after being controlled for in the analysis it was considered unlikely to affect study outcomes, although advanced age is associated with a higher incidence of liver disease, in particular HCC.[252]

5.2.3 Measurement Bias

Significant misclassification of hepatitis status appears unlikely as there was moderate to good agreement on comparison of original results for hepatitis A and B with results of current testing. In addition, a more stringent cut off was used to classify HCV antibody status and this increased specificity and reduced misclassification bias.[234]

The use of clinical examination and LFTs alone, without histological diagnosis, to assess the degree of liver injury is fraught with difficulties. Standardised clinical assessment forms were used to try and limit variation in clinical observations, although the worth of clinical examination *per se* in assessing liver status is debatable. To compensate for this, clinical examination was supplemented by more reliable means of assessing outcomes including laboratory indices, the use of ultrasound and where possible examination of liver histology.

It is accepted that elevated ALT does not directly correlate with the degree of hepatocellular injury, though in one study peak ALT levels and biopsy scores (Knodell and Sheffield) were strongly correlated.[253] Several studies have demonstrated that chronic liver disease is also likely in those who have antibodies to HCV, but normal ALT concentrations and no biochemical or radiological evidence of liver disease.[158 254] Liver biopsy is well established as the gold standard for staging HCV related liver disease in terms of severity of fibrosis and cirrhosis.

Unfortunately, a minority of participants underwent liver biopsy due to the nature of the study, the cohort involved, and the fact that responsibility for the ongoing care of many subjects was with the treating physicians and not the investigators. This may have led to an underestimation of the percentage of subjects with cirrhosis. To reduce this possibility, we measured serum hyaluronate levels, which have been shown to correlate well with fibrosis in numerous studies and which are relatively sensitive and specific for detecting cirrhosis in hyaluronate levels highly predictive of cirrhosis, and in all of these, the diagnosis of cirrhosis was already established.

In other studies, laboratory indices such as serum bilirubin, albumin and platelets have been demonstrated to indicate the severity of underlying liver disease and the likelihood of progression to liver related complications.[132 186] In one study, patients with a serum albumin concentration <30g/l had an 85% chance of progression to severe liver related complications after 5 years.[186] In our study only one individual had a serum albumin less than 30g/l and they

had already been diagnosed with cirrhosis. The same individual was the only study subject to have a bilirubin level >40mol/l, which is associated with a 25% chance of progression to severe liver disease.[186]

Thus, while an under-reporting bias cannot be excluded, the effect is likely to be relatively small.

5.2.4 Confounders

Information was collected on potential cofounders of the effect of HCV infection on outcomes, and study risk estimates were adjusted to take account of known confounders. The most important confounder is lifetime consumption of alcohol. Underestimation of alcohol consumption may have led us to over estimate the impact of HCV on outcomes. It is also possible that unknown confounding factors were not taken into account.

Lifetime alcohol consumption: Alcohol is one of the major causes of severe liver disease including HCC.[257 258] There is strong evidence that HCV and alcohol act synergistically to increase the risk of liver disease.[185 192 193] It is possible in this study that subjects under reported alcohol consumption because of social stigma. However in this cohort the candour with which they discussed an illicit activity such as IDU makes it unlikely that they were less that truthful about their alcohol habits. It is more likely that some subjects simply could not remember details of consumption during certain periods of their lives and may have under or overestimated alcohol habits.

Co-infections: HBV is independently associated with severe liver disease and also acts synergistically with HCV, in particular in the development of HCC.[200 259] We measured HBcAb and HBsAg in both the exposed and unexposed cohorts to assess previous exposure and current

carriage of HBV. None of the exposed cohort were HBsAg positive at follow up but we did not measure HBV DNA in HBcAb positive subjects. We may therefore not have detected subjects with so called 'occult' HBV infection, although the clinical significance of this remains unclear.

HIV is not associated with chronic liver disease, although it does act synergistically with HCV to increase progression to severe liver disease.[68] We did not measure HIV status in study subjects due to ethical constraints, unless it was otherwise indicated. It is unlikely that any would have tested HIV positive as the majority of subjects had no risk factors for acquisition of HIV having ceased IDU over a decade ago, and in any case the risk of acquiring HIV through injecting drug use in Australia is very low.[27]

Smoking: Smoking is implicated as a risk factor for HCC.[260-263] There is evidence that it may also act as a cofactor with HCV to increase the severity of HAI scores and also in the development of HCC.[264] Information on lifetime smoking habits were obtained from study subjects.

Sociodemographics: Age and gender are independently associated with liver disease, in particular HCC.[252 259 265 266] There is a higher incidence of HCC in males than in females with a male: female ratio between 2 and 4. Most studies hypothesise that differences in hormone status between males and females plays a significant role, but the exact mechanism remains poorly understood. The incidence rate of HCC also increases with age.

Other: Information was collected on patterns of oral contraceptive and anabolic steroid use as both are associated with an increased risk of HCC.[252] Information was also collected on other co-morbidities that may impact on the development of liver disease, including iron overload conditions.

5.3 Seroprevalence of HCV in Melbourne in the Early 1970s

Measurement of anti-HCV in the stored sera confirms that anti-HCV was present among adults admitted with acute hepatitis to FIDH in Victoria in the early 1970s and also that prevalence increased markedly during the period between 1971 and 1975. Most cases were in young men who had a history of IDU.

The original admission of the majority of individuals with HCV appears to have been precipitated by subsequent infection with hepatitis A or B. The actual proportion is unclear, but is likely to be significant, as only 16% of HCV seropositive individuals had no evidence of other acute hepatitis markers. This implies that individuals with subclinical acute HCV infection were admitted and therefore included in the study. The significant association between the presence of HCV antibody and documented contact with a hepatitis case probably results from a spurious association with the HAV or HBV infection that precipitated admission. This also reflects that those infected with HCV were likely to have risk behaviours that exposed them to other hepatitis viruses, in particular HBV.

The presence of tattoos and a history of blood transfusion were not identified as significant risk factors for HCV seropositivity probably reflecting the low prevalence of HCV in the general population at this time.

The significant increase in the prevalence of HCV over the five year period studied suggests that infection became firmly established within the Australian community, particularly the IDU population, as early as the mid 1970s. One potential source of infection could have been American servicemen who used Australia regularly as a 'rest and relaxation' base during the Vietnam War. It is known that over 20% of the troops were IDU, [267] and studies have documented the presence of HCV in American Vietnam [268] and Korean [171] war veterans. It is

likely that serviceman gained access to local Australian injecting networks and unsafe injecting practices would have ensured rapid spread of the virus once introduced.[48] Another possible source of infection was the migration of a population infected as a result of unsafe vaccination practices in Europe after the Second World War.

It is evident that the Australian IDU population expanded dramatically in the late 1970s and early 1980s and it is likely that the majority of the 140,000 Australians currently infected with HCV became infected in the last two decades.[48] This is supported by our results, which documents a two-fold increase in the recording of IDU among all admissions with viral hepatitis for the period between 1971 and 1975.

5.4 Tracing and Recruiting Subjects with a History of IDU

This study is unique in examining the feasibility of establishing a cohort of individuals, including a high proportion of IDUs, 25 years after hospitalisation with hepatitis. Completeness of case finding and recruitment was essential to avoid inaccuracy in measurement of outcomes of infection with hepatitis C.

Study funds were limited and two-thirds of the final cohort were located through relatively inexpensive means with important outcomes identified. We found that IDUs were less likely to be traced than non-IDUs possibly due to the often-intentional invisibility of this section of society. Other studies have found IDUs undertake frequent name changes and use aliases, nicknames or street names to avoid detection.[269] This practice severely limits the ability to trace participants especially after almost three decades have elapsed.

Unlimited study funds and time may have allowed us to detect further subjects, principally through systems tracking.[270] It is unlikely however that in Australia we could have easily gained access to social security, vehicle registration or penal records, as our initial inquiries were met with firm refusals by the organisations concerned. In addition, the ethical issues involved in accessing this non-health information are not insignificant.

We found that accurate ascertainment of birth date and full name - including middle name - from original case records was essential for tracing purposes. The practice of destroying inactive patient files, which has become common practice in Public Hospitals, may adversely affect future retrospective study initiatives. In addition, if false details were given at the time of admission or inaccurately transcribed there is little hope of tracing the subject.

Correct identification using the electoral roll and CD-ROM telephone directory was the norm, with less than 5% of attempts proving to be mismatches. This was a relatively inexpensive and productive means of locating study subjects. Telephone directories for many countries are also available free of charge on the World Wide Web,[271] although the ability to search on different fields is less well developed than on CD-ROM. We had extremely few complaints from subjects contacted, principally due to the discreet and sensitive manner by which the initial contact was made with the subject.

Advertisements in major daily newspapers were an expensive and unproductive exercise. Only 1% of study subjects were located through this method, and numerous calls resulted from individuals with wide ranging queries unrelated to the study. Approaches to doctors recorded in the FIDH medical records met with little success, mostly due to the now common practice of destroying inactive patient files. Next of kin tracing became crucial to finding women, who had possibly married and changed their surname.[270] Word of mouth played a very minor role in the search, but made many recruitment phone calls easier.

Health Insurance Commission records were used as the last resort to find those not located by other means. Computerisation of Health Insurance records occurred in 1982 and it was unclear at what point from that date the contact address referred to, as the Commission would release only the subjects address and not any details of date of last contact with services. The speed with which the Commission searched their data-base and returned contact details to the study centre, in addition to the small cost required, makes this a very cost effective method of tracing if the aims of the study are judged justifiable by the Health Insurance Commission.

Participation rate was high in those traced. This did not differ between the exposed and unexposed groups. It perhaps demonstrates the willingness of individuals to participate in a study that may not directly benefit them, although the opportunity to have their current health status assessed was appealing to many. The use of experienced study personal to make the initial contact is essential,[269 272] and extra care and discretion had to be employed due to the sensitive nature of the study.

The study questionnaires determined detailed and personal information including a history of IDU. For many of these subjects such histories were unknown to their families and the willingness of subjects to trust the study team to maintain the confidentiality of such information would appear to be due to the positive relationship developed with the staff member responsible for the initial contact and recruitment. Once more this factor has been identified as crucial for study success with 'hard to reach' populations.[269 272 273]

We demonstrated that it is possible to trace a population - including a large proportion of subjects with histories of IDU - 25 years after last contact. Methods of tracing used do not have to be overly expensive or sophisticated although the use of a skilled and experienced tracer is essential.

5.5 Outcomes of Infection with HCV 25 Years after Initial Infection

5.5.1 Quality of Life

Our study results indicate that QOL is markedly reduced in subjects with hepatitis C compared to population controls. This finding has been documented in other studies that also report disabling fatigue and a reduced sense of well-being in many patients with chronic HCV infection.[220 222 274 275] These studies could not however determine the impact of HCV diagnosis *per se* on QOL as all patients were aware of their diagnosis at the time of completion of the QOL scales.[276]

We wished to explore the hypothesis that subjective health perceptions are influenced by diagnosis with HCV. To assess the impact of a diagnosis of HCV on QOL in our anti-HCV positive cohort we compared QOL scores between those aware of their serostatus at the time QOL scores were measured, and those that were not aware.

Individuals with chronic HCV who were aware of their serostatus prior to interview had a significantly worse subjective QOL compared to population controls than those who were not aware of their serostatus. Both groups had been infected with HCV for an average of 25 years.

The reduction in QOL scales in those aware of serostatus was almost universal and affected variables measuring both emotional and physical health, and also those measuring the impact of health on activities of daily living. Those unaware of their diagnosis scored poorly in only three modalities; in addition they did not perceive that their emotional or physical health impacted in any way on daily activities.

The fact that the unaware group did differ from population norms in the areas of vitality and general health suggests that the commonly reported symptoms of fatigue and tiredness in those with HCV may be secondary to a physiological, but as yet unclear, mechanism rather than any psychological process. A recent study identified elevations in basal ganglia and white matter choline/creatine ratios in patients with histologically mild HCV, compared with healthy volunteers and patients with HBV.[225] The clinical significance of this is far from clear however.

In this study it is not clear how much of the reduction in QOL in either group is attributable to chronic HCV and how much it may be associated with psychological disturbance, previously documented in IDUs,[227 277] that can adversely affect QOL. It was not possible in this study to independently assess the impact of chronic HCV (without a history of IDU) on QOL, as all subjects were ex IDUs. Any impact however would have affected both groups equally and cannot be held responsible for the noted differences in QOL between the groups.

What is clear from this study is that subjects who have been diagnosed with HCV have a global and significant reduction in QOL compared to individuals with chronic HCV who have not yet been diagnosed. There is a growing body of literature indicating the impact of HCV infection on QOL. These results provide evidence of a possible major aetiology reduced QOL, namely the potentially adverse consequences of the knowledge of a diagnosis of HCV, unrelated to objective measures of pathogenicity.

Though unexamined confounding factors may remain as explanations for this observation it is unlikely that any factors other than the diagnosis of HCV could have led to this observed difference. Those aware of their positive serostatus did not differ sociodemographically, clinically, virologically or serologically from those who were unaware. All had asymptomatic liver disease and none had chosen to be treated with interferon. There was no link between QOL scores and objective measures of ill health and in all cases HCV diagnosis had been made

incidentally and not because of the presence of symptoms. All were ex IDUs though none had injected in the previous 24 months.

A diagnosis of HCV raises significant concerns about current and future health status, compounded by the current inability to confidently assess prognosis, as the natural history of community-acquired HCV is poorly defined. The ominous prediction that 60% of those infected will develop chronic liver disease with 20% progressing over 15 to 30 years to cirrhosis and a subsequent 2-7% developing HCC is derived mainly from studies relating to transfusion-acquired disease, and may not be true in those with community acquired disease. It may therefore not be surprising that a diagnosis of a chronic illness with an uncertain prognosis will affect individuals QOL.

In addition many of our subjects were infected with HCV as young adults through injecting drug use. The majority ceased injecting drugs some time previously, and in many cases this part of their lives remained unknown to family and others till HCV was diagnosed. Discrimination or recrimination from family, friends, employers and others would also affect psychological and emotional well-being which is recognised to be a major determinant of overall QOL.

Therefore, although infection with HCV and/or a history of IDU may be associated with impaired QOL, the globally reduced QOL in the diagnosed group in our study may also be an effect of 'labelling,' by which diagnosis of chronic disease *per se* affects subjective psychological well being.[278]Appropriate and sensitive management of the process of imparting a diagnosis of HCV may help to reduce the negative effects of diagnosis.

5.5.2 Morbidity and Mortality

Only about half the cohort (54%) had evidence of chronic HCV infection based on positive anti-HCV and HCV RNA results. Other studies have reported rates of chronic infection with HCV (defined as persistence of HCV RNA for greater than six months) of over 80%.[249 250] Low chronic carrier rates of HCV have been reported in children,[183] and subjects infected as young women[75] raising the possibility that the patients age at the time of infection is a factor in determining whether chronic infection occurs. Our study subjects had a mean age of less than 19 years at infection and may be a contributing factor to the low chronic HCV carriage rates observed. It may also indicate that chronic HCV carriage in community acquired HCV may be less frequent than previously thought.

Factors associated with progression to chronic HCV infection in our subjects were being an IDU, increased frequency of IDU, increased total duration of IDU and higher average weekly alcohol intake than those who did not progress. In those with increased frequency of injecting and a longer overall duration of injecting, repeated exposures to small doses of the virus or infection with multiple HCV genotypes may have promoted progression to chronicity. Our results, in line with numerous other studies,[153 164 185 192 193 279] also suggest that excess alcohol intake promotes chronicity of infection, possibly through alcohol's effect on viral replication, or by its effect on the immune system.

We found that 31% of those with chronic HCV infection had normal serum ALT levels and no biochemical or clinical evidence of chronic liver disease. Although individuals with chronic HCV infection and initially normal ALT levels can develop abnormal LFTs, the overall progression rate among this group appears to be low. Our results support the suggestions that the vast majority of HCV infected people with normal LFTs appear at low risk of long-term complications of chronic HCV infection.[121-123 280 281]

Only 8% of the chronically HCV infected group (and 6% of the overall HCV seropositive group) had progressed to clinically apparent cirrhosis after a mean follow up time of 26 years and no cases of HCC were detected.

There was no difference in survival between the exposed and unexposed cohorts. However, excess mortality was evident in the HCV seropositive group who were four times more likely to die from drug overdose or suicide than from HCV-related disease. Increased mortality in IDUs from suicide and overdose have been previously documented,[282 283] as has the presence of psychiatric morbidity which in many cases is unidentified and untreated.[227 283] The risk of overdose is ever present for IDUs, who by the illegal nature of their activity are forced to operate without the support of medical or counselling facilities. Our results highlight the fact that efforts to reduce mortality in those at risk of HCV infection should not neglect factors that appear to have overall a greater impact on survival than HCV.

The estimated risk of progression to serious sequelae of HCV infection differs depending on the populations studied and the methods used. Transfusion related studies report rates of progression to cirrhosis of over 20% at 20 years. The apparently more rapid progression among people with HCV infection acquired through transfusion of contaminated blood products compared to our subjects, may be due to the higher initial HCV inoculum relative to other modes of transmission such as IDU as well as the generally advanced age of transfusion recipients. Liver clinic series have also estimated rates of progression to cirrhosis similar or greater to those of transfusion related studies, however it is likely such series suffer from considerable referral bias. There have been relatively few population-based studies such as ours on the natural history of chronic HCV infection.[74 75 284] Nonetheless the picture which is emerging from such studies contrasts considerably from that of liver clinic series, with few subjects progressing to advanced liver disease.

Although the milder clinical picture in our study group may be due in part to the lack of histological diagnosis of liver disease or differential follow up, it appears to indicate that those with community acquired HCV follow a more benign clinical path than previously thought and that the majority of HCV infected people may not progress to advanced liver disease.

5.6 Summary of Study Findings

Our study makes a significant contribution to knowledge in this area.

1. This manner in which this study was designed has overcome many of the methodological problems encountered in studying the natural history of HCV, and is unique in its length of follow up and freedom from confounding bias due to treatment.

2. There was no difference in survival between the anti-HCV positive and anti-HCV negative cohorts although excess mortality was evident in the HCV seropositive group who were four times more likely to die from drug overdose or suicide than from HCV-related disease in the 25 years from infection.

3. Only about half the cohort (54%) had evidence of chronic HCV infection based on positive anti-HCV and HCV RNA results. Other studies have reported rates of chronic infection with HCV (defined as persistence of HCV RNA for greater than six months) of over 80%.

4. Factors associated with progression to chronic HCV infection in our subjects were being an IDU, higher frequency of IDU, longer total duration of IDU and higher average weekly alcohol intake than those who did not develop chronic infection.

5. We found that one third of those with chronic HCV infection had no biochemical or clinical evidence of chronic liver disease. Our results support the suggestions that the vast majority of HCV infected people with normal LFTs appear at low risk of long-term complications of chronic HCV infection.

6. Only 8% of the chronically HCV infected group (and 6% of the overall HCV seropositive group) had progressed to clinically apparent cirrhosis after a mean follow up time of 25 years and no cases of HCC were detected. Our study provides the most significant evidence to date that community acquired HCV does follow a more benign path than previously predicted.

7. Quality of life measures were significantly worse for HCV seropositive individuals aware of their serostatus, compared to those unaware. We were able to investigate the impact of diagnosis of HCV *per se* on quality of life, and found that reduced quality of life in those diagnosed with HCV may be unrelated to any pathogenic effect of the virus and may partially be an effect of 'labelling'. The impact of the diagnostic process alone on quality of life in individuals with HCV requires further evaluation.

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