Hepatitis C virus infection
Ann-Sofi Duberg

Hepatitis C virus infection
A nationwide study of associated morbidity and mortality
ABSTRACT


The hepatitis C virus (HCV) was characterised in 1989. HCV was transmitted through transfusion of blood/blood products, but injection drug use is now the most common route of transmission. The infection is usually asymptomatic but becomes chronic in about 75%, and in 20 years 15-25% develops liver cirrhosis, with a risk for liver failure and liver cancer. HCV has also been associated with lymphoproliferative disorders.

The aim of this thesis was to study morbidity and mortality in a national, population-based cohort of HCV-infected individuals. The study population consisted of all persons with a diagnosed HCV-infection recorded in the national surveillance database. This file was linked to other national registers to obtain information of emigration, deaths, cancers, and inpatient care. All personal identifiers were removed before analysis.

In Paper I the standardized incidence ratios (SIR) for Hodgkin’s and non-Hodgkin’s lymphoma (NHL), multiple myeloma, acute and chronic lymphatic leukaemia, and thyroid cancer were studied. In the HCV-cohort (n: 27,150) there was a doubled risk for NHL and multiple myeloma in patients infected for more than 15 years, compared with the general population (age-, sex- and calendar-year specific incidence rates). The results strengthened these earlier controversial associations.

The SIR and also the absolute risk for primary liver cancer were estimated in Paper II. In the HCV-cohort (n: 36,126) the individuals infected for more than 25 years had a more than 40 times increased risk for liver cancer compared with the general population. The absolute risk of primary liver cancer was 7% within 40 years of HCV-infection.

Mortality and cause of death were studied in Paper III. The standardized mortality ratio (SMR) demonstrated a 5.8 times excess mortality in the HCV-cohort (n: 34,235) compared with the general population, and a 35.5 times excess mortality from liver disease. Deaths from illicit drugs and external reasons were common in young adults.

Paper IV presents a study of inpatient care. The HCV-cohort (n: 43,000) was compared with a matched reference population (n: 215,000). Cox regression was used to estimate the likelihood, a hazard ratio, for admission to hospital, and frequencies and rates to estimate the total burden. In the HCV-cohort inpatient care was high and about 50% was psychiatric, often drug-related care. The likelihood for liver-related admissions was very high, and serious liver complications increased in the 2000s, indicating that HCV-associated liver disease will increase the next decade. In the 2000s, about 1000 individuals per year were treated with HCV-combination therapy.

To conclude, the risk for NHL and multiple myeloma was doubled, and liver- and drug-related morbidity and mortality was very high in the HCV-cohort. Serious liver complications increased in the 2000s and will probably increase the coming decade.

Keywords: HCV; hepatitis C; epidemiology; non-Hodgkin’s lymphoma; NHL; multiple myeloma; primary liver cancer; HCC; mortality; inpatient care; hospitalization

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Populärvetenskaplig sammanfattning


Syftet med denna avhandling var att studera sjuklighet och dödlighet hos alla personer med diagnostiserad HCV-infektion i Sverige. Studiepopulationen hämtades från det nationella smittskyddsregistret och samkördes med andra nationella register för information om emigration, död, cancer och sjukhusvård. Registerfilerna aidentifierades innan de analyserades.

I det första arbetet studerades risken för lymfkörtelcancer, s.k. non-Hodgkin’s lymfom (NHL) och Hodgkin’s lymfom, akut och kronisk lymfatisk leukemi, multipelt myelom samt sköldkörtelcancer. Hos de 27150 HCV-infekterade fann man en dubblerad risk för NHL och multipelt myelom hos dem som varit HCV-infekterade mer än 15 år, jämfört med den allmänna befolkningen (korrigerat för ålder, kön och år). Detta styrkte det tidigare omdebatterade sambandet.

I det andra arbetet studerades hur stor risken för levercancer var hos 36126 HCV-infekterade. Efter mer än 25 år med HCV-infektion var risken för levercancer 40 gånger högre än i normalbefolkningen och den absoluta risken för att utveckla levercancer var 7% under 40 års tid med HCV-infektion.

Död och dödsorsaker studerades i det tredje arbetet. Den totala dödligheten var närmare sex gånger högre, och dödligheten i leverinfektion 36 ggr högre, bland de 34235 HCV-infekterade än i normalbefolkningen. Drogmissbruk och yttre orsaker (olyckor, självmord, m.m.) var vanliga dödsorsaker i yngre åldrar.

ABBREVIATIONS

ALT alanine amino transferase
ALL acute lymphatic leukaemia
anti-HBs antibodies to hepatitis B surface antigen
anti-HCV antibodies to hepatitis C virus
ATC Anatomical Therapeutic Chemical Classification System
CI confidence interval
CLL chronic lymphatic leukaemia
DDD defined daily doses (dose per day of a drug)
DNA deoxyribonucleic acid
EIA enzyme immunoassay
HAV hepatitis A virus
HBsAg hepatitis B surface antigen
HBV hepatitis B virus
HCC hepatocellular carcinoma
HCV hepatitis C virus
HIV human immunodeficiency virus
HL Hodgkin’s lymphoma
HR hazard ratio
ICD International Statistical Classification of Diseases
IDU injection drug use
IU international units
MM multiple myeloma
NANBH non-A non-B hepatitis
NHL non-Hodgkin’s lymphoma
ORF open reading frame
peg-IFN pegylated interferon alfa
PCR polymerase chain reaction
PLC primary liver cancer
PLD liver-related principal discharge diagnoses
RIBA recombinant immunoblot assay
RNA ribonucleic acid
RVR rapid viral response
SIR standardized incidence ratio
SLC serious liver complications
SMI Swedish Institute for Infectious Disease Control
SMR standardized mortality ratio
<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>STAT-C</td>
<td>specifically targeted antiviral therapy for HCV</td>
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<tr>
<td>SVR</td>
<td>sustained viral response</td>
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<tr>
<td>TC</td>
<td>thyroid cancer</td>
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<tr>
<td>UTR</td>
<td>untranslated region</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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LIST OF PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


(*contributed equally to the article)


INTRODUCTION

Historical aspects on hepatitis C virus (HCV) infection

When specific diagnostic tests for hepatitis A virus (HAV) and hepatitis B virus (HBV) became available in the 1970s a post-transfusion hepatitis without serological markers of these viruses, non-A non-B hepatitis (NANBH), was recognized. The NANBH also occurred sporadically within the community and early studies of the natural history suggested a prolonged, asymptomatic course with risk for chronic hepatitis and liver cirrhosis.

Searching for the NANBH agent for more than a decade finally resulted in the characterization of the hepatitis C virus (HCV) in 1989. The subsequent development of sensitive and specific diagnostic assays revealed that more than 90% of NANBH was caused by HCV. Furthermore, it was disclosed that chronic HCV-infection was a global problem, estimated to affect about 170 million individuals, corresponding to a global prevalence of 3%.
HCV virology

The hepatitis C virus belongs to the Flaviviridae family, genus Hepacivirus. HCV is a spherical, enveloped RNA virus, approximately 50 nm in diameter. The single positive-strand RNA genome of approximately 9,500 nucleotides contains a large translational open reading frame (ORF) flanked by highly conserved untranslated regions (UTR) at both the 5' and the 3' termini (Figure 1). The major open reading frame encodes a large polyprotein of about 3,000 amino acids. The polyprotein processing yields at least 10 different structural and non-structural proteins: the core protein (C) which forms the viral nucleocapsid, two envelope glycoproteins (E1 and E2), the short membrane peptide p7 probably promoting assembly and release of infectious virions, and six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) involved in polyprotein processing and viral replication.

Figure 1. Schematic diagram of the HCV-genome.

The HCV strains are classified into six major genotypes and an increasingly number (>80) of subtypes or variants, designated by Arabic numerals for the genotype and small letters for the subtype. The genotypes differ from each other by more than 30% at the nucleotide level, compared with 20% to 25% between subtypes.

The understanding of the viral life cycle and the structural details of HCV have been hampered by the lack of a satisfactory cell culture system. Only recently were infectious HCV virions produced in cell cultures and studied by electron microscopy. The virions isolated from cultured cells had a rather uniform diameter of around 50 nm and a smooth or spike-less outer surface, thus very similar to other Flaviviridae such as dengue and West Nile virus.

Mathematical modelling of viral dynamics reveals high turnover rates of viral production and clearance, approximately $10^{11}$ to $10^{13}$ virions per day, and an estimated half-life of a few hours for free virions.
Diagnosis of HCV infection

Antibodies to hepatitis C virus

Diagnostic methods for HCV-infection became available in 1990. The detection of specific antibodies in body fluids is based on the use of enzyme immunoassays (EIAs) and recombinant immunoblot assays (RIBAs). In 1991 the first assays were replaced by the more sensitive and specific second (and subsequently the third) generation tests 1,19. These tests are based on four recombinant HCV antigens, c22-3, c100-3, 5-1-1, and c33c (representing the core, NS3, NS4, and NS5 sequences), to capture circulating antibodies.

In EIA the antigens are coated onto the wells of micro titre plates or micro beads, adapted to automated devices. The presence of antibodies in the sample is revealed by anti-antibodies with an enzyme that transform a substrate to a coloured compound. In RIBA the four antigens are attached as four separate bands on a nitrocellulose strip and reactivity with two or more of the antigens are considered a positive test.

The currently used EIA and RIBA detect HCV-antibodies with a high sensitivity and specificity. However, patients with an acute HCV-infection can be negative as the serological window (HCV-RNA in plasma in the absence of antibodies) can be 60 days on average 23. HCV antibodies usually appear 2–8 weeks after the acute phase of infection (Figure 2). Exceptionally, patients with profound immune-suppression such as HIV, agammaglobulinaemia, or in haemodialysis could be negative for HCV-antibodies in spite of an HCV-RNA positive infection. The antibody-tests do not allow distinguishing an acute or chronic infection from a resolved infection. The specific antibodies persist for life in patients with a chronic infection, but also persons with a resolved infection (spontaneously or by treatment) usually have antibodies for many years or life-long. Thus, in these individuals HCV-RNA detection is required to discriminate a resolved infection from a chronic.

The EIA is the mainstay in HCV-diagnostics, being cheap, easy to use, fully automated, and well adapted for large volume testing. The RIBA, separating the four antigens, has been used as a confirmation test, but is nowadays often replaced by RNA-tests.
**Hepatitis C virus RNA detection and quantification**

Assays for the detection of HCV-RNA are used to disclose viraemia. HCV-RNA detection can be achieved using target amplification such as polymerase chain reaction (PCR) or signal amplification such as the branched DNA assay. The classical techniques for viral genome detection and quantification are now being replaced by real-time PCR assays. These assays have a broad dynamic range of quantification and are more sensitive than classical PCR, with lower limits of detection of 10–15 IU/ml, and an upper range of quantification of 7–8 log_{10} IU/ml. Real-time PCR can be fully automated and has become the technique of choice to detect and quantify HCV-RNA in clinical practice.

For quantification the preferred unit is international units per millilitre (IU/ml), conversion factors can be used for the relationship between the IUs and the earlier used non-standardized copies/ml.

HCV-RNA becomes detectable within 1–2 weeks after initiation of infection (Figure 2). Detection and quantification of HCV-RNA is useful in clinical practice to detect and confirm HCV-infection and to monitor the virological response to antiviral therapy. However, in untreated patients the HCV-RNA level has no prognostic value and monitoring with repeated analyses is not recommended.

*Figure 2. The virological markers during chronic hepatitis C virus infection.*
Hepatitis C virus genotype determination

The HCV genotype is determined through viral genome sequence analysis, generally based on direct sequencing or reverse hybridization. The reference method is phylogenetic analysis of sequences generated after PCR amplification. Direct sequence analysis is the gold standard for genomic sequencing; however, it only identifies viral variants representing at least 20–25% of the circulating viral populations, with a risk for overlooked dual infections. Reverse hybridization is more sensitive to detect minor variants representing as few as 5% of the viral population, and the new versions have improved the accuracy for the subtype determination 23. There is also a serological test for HCV-genotypes, detecting antibodies against genotype-specific antigens. This test allows detection of the genotypes but not the subtypes 110.

The geographic distribution of genotypes and subtypes varies. In the United States, Japan, and the main part of Europe genotype 1b and 1a are predominant followed by 2b and 3a. In Sweden genotype 3 is the predominant, followed by 1a and 2 151. Genotype 4 is common in Africa and the Middle East 131.

Genotype identification is clinically important because of the varying resistance to the currently recommended therapy for hepatitis C. The HCV genotype should be determined before treatment, as it has an influence on the indication, dosing and duration of treatment, and the virological monitoring procedure 82.
Epidemiology and routes of transmission

The global burden of hepatitis C virus infection

Hepatitis C virus infection is a global health problem, in the 1990s WHO estimated the global prevalence to 3% \(^{155,156}\), more recent data suggested 2.2%, corresponding to 150 million individuals worldwide \(^{59}\). An update is needed and the work on estimating the global burden of disease is currently in progress \(^{59,84}\).

There is a large degree of geographic variability in the distribution; the highest prevalence rates of HCV-antibodies are reported in Asia and Africa \(^{127}\), in Egypt the overall seroprevalence is around 15–20% as a result of parenteral antischistosomal therapy \(^{51}\). Areas with lower prevalence include the United States, Japan, Australia and Central Europe with reported rates of 1–2%, Southern Europe with overall prevalence rates 2.5–3.5%, and Northern Europe <1%, with the lowest rates in the United Kingdom and Scandinavia \(^{6,8,10}\).

It has been suggested that the spread of HCV in Southern and Central Europe started during the last century as an epidemic of iatrogenic nature through the use of unsafe injections, medical and surgical procedures, and transfusion of blood products. This led to high prevalence in older individuals, followed, about 30 years later, of a still on-going IDU-related epidemic in younger people \(^{38}\). Similar patterns with high prevalence in older age groups were reported from other countries where iatrogenic spread has been important \(^{6}\).

In Northern Europe HCV was mainly transmitted by IDU, resulting in an overall prevalence between 0.1 and 1%, with the infected predominantly 30–49 years old. This indicated that the transmission occurred in the last 20–40 years and primarily among young adults. A similar pattern was observed in the United States, Australia, and other countries with similar HCV epidemiology \(^{6,10,33,34}\).

Blood transfusions used to be a leading cause of HCV-infection but the availability of diagnostic assays, the subsequent introduction of blood donor screening and the rapid improvement of healthcare conditions have almost eliminated transfusion-associated transmission in industrialized countries. However, nosocomial outbreaks still occur \(^{3,152}\), and the iatrogenic spread continues in developing countries \(^{115}\). In Western countries IDU has become the main transmission mechanism of HCV, often acquired already the first year with IDU. The risk is associated with the sharing of injection equipment as needles and syringes, but also spoons, cottons, and other paraphernalia \(^{38,55,91}\).

Transmission from an infected mother to the newborn has been estimated to about 5%, with no protective effect of elective caesarean section delivery, however, there is no evidence of mother-to-infant transmission from breast-feeding \(^{39}\).
Hepatitis C virus infection in Sweden

In Sweden (population 9 million) the seroprevalence was estimated to ≤0.5% in the beginning of the 1990s when blood donor screening revealed that 0.2–0.5% of blood donors, and 0.4% of a middle-aged urban population in Southern Sweden were anti-HCV positive. These studies probably included a healthier part of the population and few injecting drug users, resulting in an underestimation, but there are no recent population-based studies from Sweden.

Hepatitis C virus infection is a notifiable disease since 1990 when diagnostic methods became available. All clinicians as well as laboratories are obliged to report diagnosed HCV-infections, both positive HCV-antibody and/or HCV-RNA analyses, to the Swedish Institute for Infectious Disease Control (SMI). The notifications include information of epidemiological relevance. Individuals with a resolved infection, spontaneously or by treatment, will still be in the register.

Since 1990 about 45,000 HCV-infections have been notified. In a study of the HCV-infected population notified 1990–2006 (43,000 individuals) the mortality rates were high and 16% had died by the end of 2006, leaving about 36,000 living, diagnosed HCV-infected individuals (paper IV). This could agree with an overall prevalence rate of 0.5% (i.e. 45,000) with about 20% of the infections undiagnosed.

Approximately 90% of the individuals notified with HCV-infection originate from the Nordic countries, and 69% are men. More than 80% were born 1950 or later, 60% in the 1950s and 1960s. This resulted in an age-distribution where 30–39 years were the predominant ages in the 1990s and 40–49 years in the 2000s, with a rapid increase of age group 50–59 years. Consequently, the HCV seroprevalence differs by age group, in year 2006 the living HCV-notified population constituted about 0.4% of the Swedish population, however, about 0.3% of age group 20–29 years, 0.5% of 30–39 years, 1.0% of 40–49 years, 0.7% of 50–59 years, 0.2% of 60–69 years, and <0.1% of 70+ years (results from the work with Paper IV).

According to the notifications to SMI the probable routes of transmission were IDU in 65%, transfusion of blood/blood products 6%, sexual transmission 2%, a few reports of mother-to-child, occupational, or nosocomial transmission, and unknown or not stated in 26%.
In Sweden, NANBH existed but was rare in the 1950s; the large spread probably started in the mid 1960s and increased in the 1970s as a result of the increase of IDU. In a Swedish study, analyses of stored frozen serum samples from patients with acute hepatitis in 1969–1972 revealed that 52% of the patients with IDU were anti-HCV positive at that time. In more recent studies it was found that about 90% of injecting drug users were anti-HCV positive by the age of 30, and also short-term use was associated with a high risk of HCV-infection.

Since the introduction of blood donor screening in 1991 the risk for HCV-transmission by blood transfusion is minimal. However, those who had blood transfusions from the mid 1960s to 1992 were at risk for HCV-transmission. In 2007 the National Board of Health and Welfare recommended that everyone who during childhood in the years 1965–1991 had received blood transfusions because of heart surgery, neonatal exchange transfusion, prematurity, or cancer should be identified and HCV-tested. This national campaign has resulted in an increase of diagnosed HCV-infections, the previously declining number of notifications increased with about 400 in 2008.

Transmission through medical procedures have been reported also in Sweden, in some of these the most likely route of transmission was contamination of saline multidose vials, but also contaminated batches of immunoglobulin has been reported. Among those with unknown route of transmission there is probably an overrepresentation of persons with a former IDU, supported by the finding of a high proportion of inpatient care with psychiatric/drug-related diagnoses in this group (Paper IV).

The HCV-epidemiology in Sweden, with the increase of individuals that have been infected for 25-35 years, indicates that there would be an increase of HCV-associated liver complications in the 2000s. This was verified in the study presented in Paper IV, but this increase has still not had any major impact on the national rates of serious liver complications such as liver cancer. However, in Sweden the inpatient care for ascites and liver failure has slowly increased. This could be an effect of HCV, since in 2006 oesophageal varices, liver failure/encephalopathy, ascites, and primary liver cancer in HCV-infected individuals constituted 15% of all admissions with these diagnoses in Sweden, in 2001 this was 8% (Paper IV). Also the number of HCV-associated HCC has increased, in the 2000s constituting 10% of the about 500 HCCs annually reported in Sweden (Paper II and IV), but the national HCC incidence rates are still low, with a decreasing trend from the 1980s. However, 20-30% of all liver transplantations in Sweden were performed in patients with HCV-infection.
Figure 3. The annual number of HCV notifications, deaths/emigrations, and the resulting annual observation time in person years as an approximation for individuals alive.

Figure 4. The age structure in the HCV-cohort over time, presented as person years (approximately equal to the number of individuals alive) by age group and calendar year.
The natural course of HCV infection

Already when NANBH was first described it was apparent that this hepatitis often persisted for a long time and that symptoms were mild, the diagnosis more often based on elevated alanine aminotransferase (ALT) levels. After the initial exposure, the HCV-infection can be identified already within 7–10 days by detection of serum HCV-RNA. Some weeks later the liver-associated serum enzymes such as ALT become elevated, the peak is often noted about two months from infection date. Only about 20% of persons with acute hepatitis C develop symptoms with jaundice, and the incubation period is usually about 7 weeks. Fulminant hepatitis is rare in hepatitis C. The HCV-specific antibodies appear on average 2-8 weeks after the acute phase of infection, therefore HCV-RNA analysis is preferred for diagnosis of acute HCV-infection.

Chronic HCV-infection is defined as persistence of HCV-RNA for 6 months or more, but is often possible to predict earlier with stable HCV-RNA and fluctuating ALT levels. Progression to chronic HCV-infection occurs in about 75%. However, in studies this differs between 50–85%, probably dependent on host factors as age, race, immune status, and to some extent viral factors. Patients who develop acute infection with jaundice and also children and young women tend to have a higher rate of spontaneous clearance than others, and old and immunosuppressed persons more often develop chronic infection.

Chronic infection usually remains asymptomatic for decades. However, some studies have indicated that these patients have a reduced quality-of-life also in the absence of liver cirrhosis. This could be related to the HCV-infection, but also to the psychological impact of the knowledge of the infection, or a psychiatric condition related to drug use.

Chronic hepatitis C progress to liver cirrhosis in 15–25% of the infected in 20 years. In early studies even higher rates were found, possibly an overestimation due to referral bias in tertiary care centres. In general, lower rates have been reported from community-based studies and in patients who were young at the date of infection. There is little information regarding progression over periods longer than 30 years, but there is increasing evidence that disease progression does not follow a linear path and that it is likely to increase as the infected person ages. Co-factors as alcohol, obesity, and HBV or HIV co-infection may worsen the prognosis. In those who develop cirrhosis the progression of liver fibrosis begins and can be identified many years in advance, and when cirrhosis is present the progression continues to end-stage liver disease in 2–4% annually. Also patients with cirrhosis have few signs and symptoms be-
fore culmination in end-stage liver disease with thrombocytopenia, prolonged protrombinkomplex values, hypoalbuminemia, followed by ascites, oesophageal varices, encephalopathy and/or HCC.

Liver biopsy is used for liver histology, to evaluate the grade of inflammation and the stage of fibrosis. To minimize the risk for sampling error a biopsy longer than 25 mm is considered optimal. The biopsy is invasive with potential adverse effects, therefore other non-invasive fibrosis markers have been studied, however, liver biopsy is still considered the golden standard.

Hepatocellular carcinoma is a late complication, typically appearing after more than 25 years with HCV infection, in patients with advanced cirrhosis. Clinical findings could be worsening of symptoms and signs of cirrhosis and right-upper-quadrant pain. Serum alfa-fetoprotein is sometimes elevated but ultrasound or computed tomography could better reveal the tumour. It has been estimated that 1–4% of cirrhotic patients develop HCC annually, and among patients with HCV-infection the total risk for developing HCC was estimated to 7% in 40 years or lifetime.

The incidence and mortality rates of HCC have been reported to increase in several developed countries. In contrast, the HCC incidence rates in Sweden have slowly decreased since the 1980s, and the last years there have been an annual incidence of about 500 HCC. However, also in Sweden an increasing proportion of all liver cancers occur in patients infected with HCV, and HCV-associated liver disease is a major cause of liver transplantation.

Extra-hepatic manifestations

HCV-infection has been associated with several extra-hepatic manifestations, preferably immunological disorders. An association with essential mixed cryoglobulinaemia has been established. Also, an association with malignant non-Hodgkin’s lymphoma (NHL) has been suggested, though debated. A geographic variation was proposed as high HCV prevalence rates were found in NHL patients in Italy, Japan and the United States, but not in Northern Europe. Later on several studies have found evidence for an association, with an almost doubled risk for NHL in HCV-infected individuals, also in HCV low-prevalence countries. Moreover, HCV has been associated with other non-hepatic neoplasm as multiple myeloma (MM) and thyroid cancer (TC).

Hepatitis C virus infection has also been linked to glomerulonephritis and end-stage renal disease, and an association with several other disorders have been proposed.
Mortality

Early studies of mortality in HCV-infected individuals focused on mortality due to liver disease, and were often performed in selected populations [126, 144]. A few community-based studies on mortality and causes of death were performed in HCV low-endemic countries. These studies demonstrated a 3–6 times increased all-cause mortality in HCV-infected individuals, and very high mortality rates from liver disease and liver cancer compared with the general population [9, 36, 104]. However, the high proportion of IDU also influenced the mortality rates, with a high excess mortality from drug-related and external reasons in younger age groups, contributing to the high all-cause mortality rates (Paper III).

Health care resource use

Hepatitis C virus infection has been expected to cause an increasing demand on the health care systems when the infected population ages. Several simulation models of the future burden and temporal trends have found evidence for a rapid increase in health care resource use [17, 61, 71, 138]. In the United States, from 1994 to 2001, HCV liver-related hospitalizations were estimated to increase at average annual rates exceeding 20% [61], and in England and Scotland models estimated a rapid increase the next decade [71, 138].

In Sweden, 80% of the HCV-infected individuals are born in 1950 or later, 60% in the 1950s and 1960s, probably HCV-infected since the 1970s and early 1980s. They have now been infected for 25–35 years, some of them very likely at risk for HCV-associated morbidity. This was the reason for the study of health care resource use defined as inpatient care (Paper IV). This study verified an increase of inpatient care from serious liver complications in the 2000s, with evidence for a further increase the next decade.

Therapy for HCV infection

The main treatment goal in chronic hepatitis C is usually the prevention of liver cirrhosis and HCC. Eradication of HCV improves liver histology and patient outcome [105, 116, 148] and sustained viral response (SVR), defined as undetectable serum HCV-RNA by a sensitive assay 24 weeks after the end of treatment, is the short-term goal of antiviral therapy [82, 88].

The first report of antiviral treatment with interferon-alpha for NANBH was published in 1986 [70]. It was followed by several studies of alpha-interferon treatment for chronic hepatitis C; however, the outcome was poor as only about 20% of the patients treated achieved a SVR [32, 112]. Prolonged therapy resulted in higher
SVR \(^{117}\), but in the late 1990s when interferon was combined with ribavirin the SVR rate was approximately 40\% \(^{97, 114, 118}\). Since then, the standard interferon-alpha has been modified to a pegylated interferon (peg-IFN) by adding a poly-ethyleneglykol molecule to increase the half-life and obtain a more efficient viral suppression.

The current standard therapy is a combination of peg-IFN and ribavirin for 24 or 48 weeks \(^{53, 62, 90}\). The dosing and duration of treatments are guided by the HCV genotype, the HCV-RNA levels before treatment, and the viral kinetics during treatment \(^{82}\). Approximately 80\% of patients infected with HCV genotype 2 or 3 achieve a SVR after 24 weeks of combination therapy. However, of those infected with genotype 1 only 40–50\% achieve a SVR after 48 weeks of therapy. Treatment for 48 weeks is recommended also in patients infected with HCV genotype 4.

A low baseline HCV-RNA is more often associated with a rapid viral response (RVR) defined as undetectable HCV-RNA after 4 weeks treatment. In some patients with a RVR the duration of treatment can be shortened to 12 or 16 weeks for genotype 2 and 3 \(^{27, 81, 82}\), and to 24 weeks for genotype 1 and 4. In a recent trial, patients with HCV genotype 1 and 4 and RVR had a SVR of 80\% \(^{46}\). On the contrary, patients with genotype 1 and a slow viral response (detectable HCV-RNA at week 12 but undetectable HCV-RNA at week 24) benefit from lengthening the treatment to 72 weeks. However, the SVR rates are still low, in a recent study of slow responders treated for 72 weeks the SVR was 29\% \(^{14}\). Shortening the duration of therapy is associated with a lower SVR in patients older than 50 years, or with high pre-treatment viral load, and/or cirrhosis. Other important factors for optimal treatment result are adherence to treatment and dose maintenance \(^{42, 140}\).

The unsatisfactory treatment results in HCV genotype 1 infections, and also the substantial side effects of the currently recommended treatment, point to the need for new treatment options. The development of new compounds under the name of specifically targeted antiviral therapy for HCV (STAT-C), protease and polymerase inhibitors, but also nucleoside and non-nucleoside analogues, is ongoing. Promising results have been reported with two protease inhibitors that are currently in phase III studies \(^{67, 96}\). However, to reduce the risk for resistance to the STAT-C drugs, the peg-IFN and ribavirin combination will probably be the recommended treatment also in the near future, and when several STAT-C drugs become available a combination of anti-virals will probably be used \(^{11}\).
AIMS

The overall aim was to study hepatitis C virus (HCV) infection and associated morbidity and mortality. The specific aims were to:

- Study the association between HCV infection and non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, acute and chronic lymphatic leukaemia, multiple myeloma, and thyroid cancer in a large, population-based HCV-infected cohort in Sweden (Paper I).

- Study the association between HCV infection and primary liver cancer (PLC), and to estimate the absolute risk for PLC, in all diagnosed HCV-infected individuals in Sweden (Paper II).

- Study mortality and cause of death in all individuals with diagnosed HCV, chronic HBV, or combined HCV-HBV infections in Sweden (Paper III).

- Examine the use of health care resources defined as inpatient care in all diagnosed HCV-infected individuals in Sweden, to demonstrate the total burden, the temporal trends, and to assess the risk for inpatient care compared with a non-infected population (Paper IV).
CONFIDENTIALITY AND ETHICS

The linkage between registers was possible using the personal identification numbers, all personal identifiers were then removed and the analyses were performed on anonymous data.

The studies were approved by the Regional Ethical Boards in Örebro (Paper I) and Stockholm (Paper I-IV).
MATERIALS AND METHODS

Study population

All four studies were register-based cohort studies and the study populations were identified from the national surveillance database at the Swedish Institute for Infectious Disease Control (SMI) \(^{132}\). Hepatitis C virus infection was classified as a notifiable disease in 1990, when diagnostic methods became available, and since then all clinicians as well as laboratories are obliged to report all diagnosed HCV-infections to the SMI. Also hepatitis B virus (HBV) infection is, since 1969, a notifiable disease. The notifications include information of epidemiological relevance and are merged using the unique personal identification number issued to all Swedish residents and used in all health care contacts \(^{86}\).

The study populations in the four studies were identified from all HCV-notifications, and in paper III also from the HBV-notifications, with the start in 1990. Statistics Sweden (Paper I and IV) or the National Tax Board (Paper II and III) made a check up and excluded duplicates and notifications with incorrect personal identification number \(^{135}\). The recruitment periods and the study populations were:

- **Paper I:** All HCV-notifications 1990–2000 were identified, and the *study population* consisted of 27,150 individuals notified for HCV-infection.

- **Paper II:** The HCV-notifications 1990–2004 were identified. The 3,238 individuals notified for both HCV and acute or chronic HBV-infection were excluded. Thus, the study population, *the HCV-cohort*, consisted of 36,126 individuals notified for HCV-infection but no HBV-infection.

- **Paper III:** All HBV- and HCV-notifications 1990–2003 were identified. The notifications for acute hepatitis B were excluded as the aim was to study chronic hepatitis. The study population constituted three cohorts; the *HCV-cohort* consisted of 34,235 individuals notified for HCV-infection but no HBV-infection, the *HBV-cohort* of 9,517 individuals notified for chronic HBV but no HCV-infection, and the *HBV-HCV cohort* of 1,601 individuals with both HCV and chronic HBV-infection. HBV-notifications before 1990 were not included, therefore some co-infected with HBV-infection diagnosed before 1990 could be in the HCV-cohort.
• **Paper IV**: All HCV-notifications 1990–2006 were identified. The *HCV-cohort* consisted of 43,000 individuals notified for an HCV-infection, including the 3,556 also notified for an acute or chronic HBV-infection. A *matched comparison-cohort* consisting of 215,000 never HCV-notified persons from the general population was identified by Statistics Sweden. For each member of the HCV-cohort, five individuals were selected at random from among those with the matching characteristics: birth-year, sex, and county of residence on December 31st the year of HCV-notification.

In the four studies the characteristics of the HCV-infected individuals were almost similar. About 80% were born after 1949, 69–70% were men, and approximately 90% originated from the Nordic countries (mostly Sweden). The likely routes of HCV-transmission reported on the notifications were injection drug use (IDU) in about 60%, transfusion of blood/blood products in 6%, sexual 2%, and unknown or not stated in approximately 30%.

In the HBV-cohort (Paper III) 85% were born in 1950 or later, 53% were men, and >90% were immigrants with a probable HBV-transmission at birth (mother-to-child) or early in life. In the HBV-HCV co-infected cohort 87% were born after 1949 and 78% were men, and the IDU transmission route constituted 67%. Chronic HBV-infection is uncommon when HBV-infected as an adult, though it seems as the HBV-HCV co-infected cohort consisted predominantly of injection drug users probably infected as adults.

The HCV-cohort in Paper IV; the annual number of notifications, deaths and emigrations, the annual observation time, and the age structure by calendar year, was presented in *Figure 3* and *Figure 4* (see Epidemiology and routes of transmission). The age distribution over time demonstrated the fast growth of age groups 40–49 and 50–59 years during the last decade, a result of the HCV-epidemic in the 1970s and early 1980s.
Linkage to other registers

The personal identification numbers 86 were used to link the notification datasets to other national registers. Statistics Sweden (Paper I and IV) and the National Tax Board (Paper II and III) added information on dates for emigration, immigration and deaths, country of birth and educational level (Paper IV). Statistics Sweden also identified the non-infected, matched comparison group for Paper IV 135.

The National Board of Health and Welfare added information from the Swedish Hospital Discharge Register (Inpatient Register), Cancer Registry, Cause of Death Register, and in Paper IV also the Prescription Register 134.

Since 1987 the Inpatient Register covers all inpatient care in Sweden, dropouts are estimated to between one and two percent. Each in-hospital episode is recorded with (among other things) dates of admission and discharge, surgical procedures, and the discharge diagnoses with up to 8 medical conditions coded according to the International Statistical Classification of Diseases (ICD). The ninth revision (ICD-9) was used in 1987–1996 and the tenth revision (ICD-10) was introduced in 1997. Validations revealed a correct ICD-code at the four-digit level in 86% of all principle diagnoses 107.

All incident cancers in Sweden should be reported to the Cancer Registry both by the clinician and pathologist/cytologist, and it has been estimated that more than 95% of all tumours are reported and approximately 99% histologically or cytologically verified 92. The cancers are coded according to the seventh revision of ICD (ICD-7).

Swedish statistics on causes of deaths are among the oldest in the world, a nationwide report system was first introduced in 1749. Since the 1940s the World Health Organisation (WHO) is responsible for the international coordination. In the Swedish Cause of Death Register the cause of death was coded according to ICD-9 in 1987–1996 and ICD-10 was implemented in 1997. The underlying cause of death is taken from the death certificate and is defined as the disease or injury that initiated the chain of diseases that finally resulted in death.

The Prescription Register includes information regarding prescribed pharmaceuticals, the code according to the Anatomical Therapeutic Chemical Classification System (ATC), the name, dose, size of package, cost, date of prescription and dispatch, and since July 2005 the personal identification number of the customer. Therefore the data on prescribed pharmaceuticals was of limited use and only in Paper IV.
Modelling date of infection

Because of a mild or asymptomatic initial course the actual date for the primary HCV-infection is seldom known, and the infection could remain undetected for many years. The risk for HCV-related complications probably increases with time and a model for date of infection was needed to make it possible to stratify according to duration of infection (Paper I and II).

A model to approximate the date of infection was constructed based on birth year, route of transmission, and available data on the HCV-epidemic in Sweden indicating that HCV existed but was rare in the 1950s and that the spread of HCV in Sweden started in the mid 1960s 16, 55, 103, 146, 153.

The same model was used for individuals infected through IDU, sexual, or unknown routes of transmission. Persons born before 1930 were considered infected in 1965; persons born in 1930 infected at the age of 35 years, linearly falling to the age of 20 years when born in 1955; and when born in 1955 or later considered infected at the age of 20 years. If notified before the age of 20 years the age at notification was used.

For those with transfusion-associated HCV infection (before blood donor screening in 1991) the date of infection was approximated to 1980. When reported route of transmission was mother-to-child or adopted child, the date of birth was considered the date of infection, and when nosocomial or occupational route of transmission the date of infection and notification was equalled.
Analyses

Paper I

The study period was 1990–2000. For each subject the observation time (expressed as person years) started at date of HCV-notification and ended at the first date of any malignancy reported to the Cancer Registry, emigration, death, or end of study, whichever occurred first.

To reduce the possibility that the HCV-infection was diagnosed as a result of the cancer diagnosis only patients with the HCV-notification more than 3 months before cancer diagnosis were included in the analyses.

All non-Hodgkin’s lymphoma (NHL), multiple myeloma (MM), chronic lymphatic leukaemia (CLL), acute lymphatic leukaemia (ALL), Hodgkin’s lymphoma (HL), and thyroid cancer (TC) in the HCV-cohort were identified. Data were stratified according to estimated duration of HCV-infection, less than 15 years and 15 years or more, calculated according to the infection date model.

The risk for malignancy in the HCV-cohort compared with the general population was expressed as a standardized incidence ratio (SIR), the ratio of the observed number of malignancies compared with the expected number. The expected number was calculated using the age-, sex- and calendar year specific incidence rates from the Cancer Registry.

Paper II

The study period was 1990–2004. For each subject the observation time started at date of HCV-notification and ended at date of primary liver cancer (PLC) reported to the Cancer Registry, emigration, death, or end of study, whichever came first.

All PLC were identified, patients with PLC diagnosis the first 3 months after HCV-notification were excluded, to reduce bias evolving from the possibility that the HCV-infection was diagnosed as a result of liver cancer.

Time with HCV-infection was estimated according to the date of infection model, and the cohort was divided in six strata: <10 years, 10–20, 20–25, 25–30, 30–35, and ≥35 years duration of HCV-infection.

The risk for PLC in the HCV-cohort compared with the general population was expressed as SIR, the ratio between the observed and the expected numbers calculated from the age-, sex-, and calendar year specific incidence rates from the Cancer Registry and the observation time (person years) in the HCV-cohort.

The absolute risk of PLC within 40 years of HCV-infection was estimated with the Kaplan-Meier method.
Paper III
The study period was 1990–2003. For each subject the observation time started at date of HBV or HCV notification (first notification if co-infected) and ended at death, emigration, or end of study, whichever occurred first.

All individuals who died less than 6 months after the hepatitis notification were excluded from the analyses to reduce possible bias from HBV and/or HCV infections diagnosed as a result of the disease that led to death.

To categorize the causes of death, the ICD-codes were used to identify deaths from the diagnoses forming the ICD-chapters, but also other diagnoses of interest, i.e. liver-related, drug-related, external reasons, HIV, NHL, MM, and other malignancies.

The mortality in the study population was compared with the mortality in the general population by calculating standardized mortality ratios (SMR), the observed number of deaths divided by the expected number of deaths. The mortality rates for the general population were obtained from the Cause of Death Register. For the calculation of the expected number of deaths the sex- and age-specific mortality rates in the calendar year 1999 were used.

Paper IV
The study period was 1990–2006. For each subject the observation time (person years) started at date of HCV-notification (same date for the five matched comparison individuals) and ended at date of death, emigration, or end of study, whichever occurred first.

The risk for admission to hospital, a hazard ratio (HR), was estimated using Cox regression to compare the HCV-cohort with the non-infected comparison-cohort. The time from HCV-notification to first admission was used and the studied episodes were “all episodes”, “psychiatric care” (principal diagnoses, mostly drug-related), “principal liver-diagnoses” and “all liver cancer” (principal and non-principal diagnoses). To evaluate the effect of selection and surveillance bias these analyses were also performed excluding all subjects who had been inpatients the year before and/or the year after HCV-notification. The analyses were also stratified by sex, period of notification, and age at notification, and adjusted by age at notification, sex, HIV, and performed without the HBV-infected, and by route of transmission.

The total burden was estimated by the frequency of admissions and days in hospital, totally and by groups of diagnoses, and by sex, age groups, calendar years, and annual rates per 1000 person years. The person years were an approximation for the number of individuals alive in the cohorts. The age- and sex-
adjusted rate ratios for admissions and hospital days were calculated. The changes over time were also demonstrated by the differences in inpatient-care in the years 1996, 2001 and 2006.

In all studies the ninety-five percent confidence intervals (CI) were calculated assuming a Poisson distribution.
RESULTS AND DISCUSSION

HCV, non-Hodgkin’s lymphoma and multiple myeloma – Paper I

After exclusion of the 464 individuals who had a cancer diagnosis before or within 3 months after HCV-notification the observation time for the remaining 26,686 was 122,272 person years, 35,982 in the stratum with HCV-duration <15 years and 86,290 in the stratum ≥15 years with HCV-infection.

During follow-up, with the start 3 months after HCV-notification, there were 20 NHL, 7 MM, 4 CLL, 5 TC, 1 ALL, and 1 HL diagnosed in the HCV-cohort. The risk of NHL was significantly increased in the stratum ≥15 years of HCV-infection (SIR= 1.89; 95% CI: 1.10–3.03) and in the overall NHL group (SIR= 1.99; 95% CI: 1.21–3.07). However, when excluding 4 patients with HIV-infection the SIR was 1.6 (95% CI: 0.91–2.59). The risk of MM was significantly increased in the ≥15 year stratum (SIR= 2.54; 95% CI: 1.11–5.69).

Table 1. Standardized incidence ratios (SIR) for B-cell non-Hodgkin lymphoma, multiple myeloma, chronic lymphatic leukaemia, and thyroid cancer in 26,686 HCV-infected

<table>
<thead>
<tr>
<th>Diagnosis and Stratum (duration of HCV-infection)</th>
<th>Observed</th>
<th>Expected*</th>
<th>SIR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-cell non-Hodgkin’s lymphoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14 years</td>
<td>3</td>
<td>1.08</td>
<td>2.78</td>
<td>0.57–8.11</td>
</tr>
<tr>
<td>≥15 years</td>
<td>17</td>
<td>8.98</td>
<td>1.89</td>
<td>1.10–3.03</td>
</tr>
<tr>
<td>All</td>
<td>20</td>
<td>10.06</td>
<td>1.99</td>
<td>1.21–3.07</td>
</tr>
<tr>
<td><strong>Chronic lymphatic leukaemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14 years</td>
<td>0</td>
<td>0.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>≥15 years</td>
<td>4</td>
<td>1.98</td>
<td>2.03</td>
<td>0.54–5.04</td>
</tr>
<tr>
<td>All</td>
<td>4</td>
<td>2.07</td>
<td>1.93</td>
<td>0.53–4.95</td>
</tr>
<tr>
<td><strong>Multiple myeloma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14 years</td>
<td>0</td>
<td>0.13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>≥15 years</td>
<td>7</td>
<td>2.76</td>
<td>2.54</td>
<td>1.11–5.69</td>
</tr>
<tr>
<td>All</td>
<td>7</td>
<td>2.89</td>
<td>2.42</td>
<td>0.97–4.99</td>
</tr>
<tr>
<td><strong>Thyroid cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14 years</td>
<td>0</td>
<td>0.75</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>≥15 years</td>
<td>5</td>
<td>2.47</td>
<td>2.03</td>
<td>0.80–5.75</td>
</tr>
<tr>
<td>All</td>
<td>5</td>
<td>3.22</td>
<td>1.55</td>
<td>0.50–3.62</td>
</tr>
<tr>
<td><strong>Analysis excluding patients with HIV-infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B-cell non-Hodgkin’s lymphoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14 years</td>
<td>2</td>
<td>1.07</td>
<td>1.86</td>
<td>0.12–6.75</td>
</tr>
<tr>
<td>≥15 years</td>
<td>14</td>
<td>8.95</td>
<td>1.56</td>
<td>0.86–2.62</td>
</tr>
<tr>
<td>All</td>
<td>16</td>
<td>10.02</td>
<td>1.60</td>
<td>0.91–2.59</td>
</tr>
</tbody>
</table>

*The expected number of cases was calculated using age, sex and calendar year specific incidence rates from the Swedish Cancer Registry.
The role of HCV as an etiological factor or cofactor in the pathogenesis of NHL has been controversial. Earlier studies were often case-control studies with diverging results and geographical differences had been suggested, an increased risk for NHL was reported from Italy, Japan, and the United States, but not in HCV low-prevalence countries in Northern Europe. In our study we used a new approach and studied the incidence in the entire cohort of notified HCV-infected individuals in Sweden, a country with low HCV prevalence. Furthermore, other non-hepatic malignancies in which an association had earlier been described (MM, TC) or with a theoretic background consistent with an association were studied.

The study was complicated by the very mild initial course of HCV-infection, the onset rarely identified. The chronic phase may last for decades without symptoms (and diagnosis) and also the virus was not discovered until 1989. The risk for HCV-related complications might increase with time of infection, therefore the model for date of infection and the stratification was performed. The SIR estimates were as high in the strata with shorter duration of HCV-infection, but the numbers were small and no firm conclusions may be drawn.

Sometimes HCV-infection is diagnosed late and in the relation to other disease, in this study demonstrated by the cluster of 14 NHL and 6 MM in close relation to the HCV-notification (±3 months), therefore not included in the analysis. These patients had probably been HCV-infected for many years and pointed to the undiagnosed HCV-infections in society.

The results indicated an increased risk for B-cell NHL and MM in HCV-infected in Sweden compared with the general population; the association with NHL was still increased but not statistically significant when HIV-infected were excluded. HIV-infection is a well-recognized risk factor for malignant lymphoma, however, the NHL incidence has decreased since the introduction of highly active antiretroviral therapy in 1996. Also organ transplantation with immune suppressive therapy increases the risk for NHL, however, in this study there were no transplanted patients in the SIR analyses.

This study has been succeeded by studies supporting the findings of a low-grade association between HCV and NHL but there are only few studies on the association HCV and MM. The almost doubled risk for NHL was verified in a recent study on methodology using the HCV-cohort in Sweden 1990–2006 (Törner A, et al. Submitted). This study supported the use of a 3 month time window after HCV-notification but recommended the observation-time for all subjects to start at that date, reducing the observation time with 6,500 person years, resulting in lower number of expected, and a somewhat higher SIR.
HCV and hepatocellular carcinoma – Paper II

The study population contributed an observation time of 246,105 person years and there were 234 patients (180 men, 54 women) with incident primary liver cancer (PLC). Out of these cancers 219 (94%) were histologically verified; 211 as HCC, eight as intrahepatic cholangiocellular cancers, and 15 were not histologically examined (most of them probably HCC). The mean time from HCV-notification to PLC diagnosis was 5 years (range <1–13 years), and the mean estimated time from onset of HCV-infection to PLC diagnosis was 29 years (range 14–39 years). In the studied HCV-cohort another 120 PLC were diagnosed before or less than three months after HCV-notification, and therefore not included in the analyses in spite of an estimated onset of HCV-infection long before PLC diagnosis.

The risk for PLC in the HCV-cohort compared with the general population was significantly increased in all strata with more than 10 years with HCV-infection, the highest SIR in the stratum 25–30 years (SIR= 46; 95% CI: 36–56) and 30–35 years with infection (SIR= 40; 95% CI: 31–51). The results were similar for men and women. The absolute risk of PLC within 40 years of infection was 7% in the HCV-cohort.

Table 2. Standardized incidence ratios (SIR) for primary liver cancer in 36,126 individuals notified with HCV- but no HBV-infection.

<table>
<thead>
<tr>
<th>Duration of HCV-infection</th>
<th>Observation time (person years)</th>
<th>Observed</th>
<th>Expected*</th>
<th>SIR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>27,466</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0–57</td>
</tr>
<tr>
<td>10-20</td>
<td>80,997</td>
<td>16</td>
<td>0.9</td>
<td>18</td>
<td>11–30</td>
</tr>
<tr>
<td>20-25</td>
<td>64,600</td>
<td>37</td>
<td>1.4</td>
<td>27</td>
<td>19–37</td>
</tr>
<tr>
<td>25-30</td>
<td>50,782</td>
<td>85</td>
<td>1.9</td>
<td>46</td>
<td>36–56</td>
</tr>
<tr>
<td>30-35</td>
<td>18,545</td>
<td>69</td>
<td>1.7</td>
<td>40</td>
<td>31–51</td>
</tr>
<tr>
<td>≥35</td>
<td>3,715</td>
<td>27</td>
<td>0.8</td>
<td>34</td>
<td>22–49</td>
</tr>
<tr>
<td>Total</td>
<td>246,105</td>
<td>234</td>
<td>6.7</td>
<td>35</td>
<td>31–40</td>
</tr>
</tbody>
</table>

*The expected number of cases was calculated using age, sex and calendar year specific incidence rates from the Swedish Cancer Registry.

This study confirmed a very high risk for liver cancer in the HCV-cohort (HBV-infected excluded) compared with the general population. Earlier studies has estimated a progression to liver cirrhosis in about 20% of HCV-infected in 20-30 years, an annual incidence of HCC about 1–4% in cirrhotic patients, and an estimated overall risk for HCC of 7% in all patients with chronic hepatitis C. However, most previous studies were performed in selected populations,
in tertiary care units, or in short follow-up periods and the high chronicity rate and cirrhosis progression rate have been debated.

The here presented study used all HCV-notifications representing a population based cohort as close as possible to all HCV-infected (HBV co-infected excluded) in Sweden, however, the size of the undiagnosed cohort is not known. The proportion of undiagnosed HCV-infections was high in the beginning of the 1990s but has decreased with time, and at the end of study probably more than 75% were diagnosed. The 120 PLC reported before or in close relation to the HCV-notification (and not included in the analysis) have not been under observation and represent HCV-infected who had been undiagnosed for a long time.

The SIR analysis was stratified according to estimated date of infection (see modelling date of infection) to relate the cancer incidence to duration of infection. An alternative could have been stratifying by age, but the model is probably more relevant, being influenced both by age and HCV-epidemiology.

The stratification revealed that the highest relative risk for HCC was in patients who had been HCV-infected for more than 25 years, and the mean age at PLC diagnosis was 60 years. Duration of HCV-infection in Sweden is highly related to age as the majority were infected already the first year with IDU, as very young adults. In the HCV-cohort the majority was born in the 1950s and 60s and infected in the 1970s and early 80s. In the general population PLC is a disease of the elderly, the incidence rates increasing with age. In spite of that the risk for PLC in the HCV-cohort compared with the general population increased with duration of infection (and with higher age). This indicates that the incidence of HCV associated PLC will increase when the HCV-cohort ages (the majority still younger than 60 years) – if not prevented by more efficient treatments for HCV. In the 2000s about 10% of PLC in Sweden was in patients with a diagnosed HCV-infection, however, there is still no increase in the Swedish PLC incidence rates that actually have decreased since the 1980s.

The SIR results were confirmed in a recent study of the methodology using the HCV-cohort 1990–2006 (Törner A, et al. Submitted). This study also concluded that to avoid bias when studying HCV and PLC the time window after HCV-notification should be one year, however the predominant effect was the first months, indicating that in the here presented study the SIR could be somewhat too high due to selection bias, but also that the SIR could be a little too low as a result of not excluding the first three months of observation time (resulting in a lower number of expected PLC) for all individuals. However, none of these adjustments would have any major impact on the results.
HCV and mortality – Paper III

The mean observation times per subject in the HBV, HCV, and HBV-HCV cohorts were 6.4, 6.3, and 7.9 years, constituting totally 60,697, 214,602, and 12,667 person years respectively. There were 425 (4.5%) deaths in the HBV-cohort, 4651 (13.6%) in the HCV-cohort, and 209 (13.1%) in the co-infected cohort. The most frequently reported underlying cause of death in the HBV-cohort was neoplasm (38%), but external causes (e.g. injuries, intoxication, suicide) were the predominant in the HCV and the co-infected cohort, 29% and 34% respectively.

To reduce bias all individuals who died the first six months after notification were excluded, this was 116 (27%), 744 (16%) and 21 (10%) in the HBV, HCV and co-infected cohort, leaving 311, 3907, and 188 deaths respectively, for the standardized mortality ratio (SMR) analyses.

The “all cause” mortality was significantly increased with SMR 2.3 in the HBV, 5.8 in the HCV, and 8.5 in the HBV-HCV cohort. In the HCV and the co-infected cohort there was a great excess risk for death from causes related to IDU, e.g. HIV, psychiatric diagnoses (98% drug-related) and external reasons, compared with the general population. However, liver-related mortality was highly increased in all three cohorts. Some of the results are presented in Table 3.

Table 3. Cause of death in the HBV, HCV and HBV-HCV cohorts. The risk is expressed as Standardized Mortality Ratio (SMR), the observed deaths / expected deaths.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>HBV (311 deaths)</th>
<th>HCV (3,907 deaths)</th>
<th>HBV-HCV (188 deaths)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SMR*</td>
<td>95% CI</td>
<td>SMR*</td>
</tr>
<tr>
<td>All cause</td>
<td>2.3</td>
<td>2.0-2.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Infection</td>
<td>13.7</td>
<td>8.7-20.6</td>
<td>28.7</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>2.8</td>
<td>2.3-3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>3.1</td>
<td>1.8-5.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Circulatory</td>
<td>1.4</td>
<td>1.1-1.8</td>
<td>2.6</td>
</tr>
<tr>
<td>External reasons</td>
<td>1.7</td>
<td>1.2-2.4</td>
<td>12.4</td>
</tr>
</tbody>
</table>

Subgroups:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>SMR*</th>
<th>95% CI</th>
<th>SMR*</th>
<th>95% CI</th>
<th>SMR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral hepatitis</td>
<td>78.9</td>
<td>46.8-124.7</td>
<td>133.0</td>
<td>114.3-153.9</td>
<td>168.6</td>
<td>84.2-301.7</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>31.2</td>
<td>21.9-43.2</td>
<td>34.9</td>
<td>30.1-40.2</td>
<td>65.2</td>
<td>33.7-113.9</td>
</tr>
<tr>
<td>Liver disease</td>
<td>10.7</td>
<td>6.8-15.9</td>
<td>25.1</td>
<td>22.3-28.0</td>
<td>24.4</td>
<td>13.0-41.8</td>
</tr>
<tr>
<td>“Total, all liver”</td>
<td>21.7</td>
<td>17.1-27.0</td>
<td>35.5</td>
<td>32.9-38.3</td>
<td>46.2</td>
<td>31.5-62.3</td>
</tr>
<tr>
<td>HIV</td>
<td>11.4</td>
<td>2.4-33.2</td>
<td>41.2</td>
<td>31.4-53.2</td>
<td>23.7</td>
<td>2.9-85.5</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>1.4</td>
<td>0.6-2.7</td>
<td>1.8</td>
<td>1.3-2.3</td>
<td>4.4</td>
<td>1.2-11.2</td>
</tr>
</tbody>
</table>

*SMR = observed/expected, the expected number of deaths were calculated using age and sex specific mortality rates in the general population.
The SMR in relation to age disclosed excess “all cause” mortality at all ages. In the HCV and HBV-HCV cohorts the greatest excess mortality was at age 15–35 years, then slowly declining, reflecting the high risk of death from psychiatric (drug-related) and external reasons at younger ages in HCV-infected individuals. The excess liver-related mortality increased with age, the maximum SMR was 26 at age 60-69 years in the HBV, and 42 at age ≥70 years in the HCV cohort.

Liver-related death was highly increased in all cohorts, though a little higher in the HCV-infected than in the HBV-cohort, this could possibly be related to a higher risk for alcohol induced liver damage in HCV-infected 20, 150, also there could be a synergistic effect in HBV-HCV co-infection. However, also in the 2,154 individuals infected through transfusion of blood/blood products the SMR analyses revealed an excess all cause mortality of 2.9 (95% CI: 2.6–3.2) and a great excess “all liver-related” mortality of 29.2 (95% CI: 22.7–37.0) including liver cancer 19.2 (95% CI: 11.7–29.6), but no increased mortality from psychiatric disease indicating a low prevalence of drug abuse in this subgroup.

The HCV and the HBV-HCV co-infected cohort consisted of a large proportion of active or former injection drug users, reflected in the high risk from lifestyle related mortality in younger ages, consistent with earlier findings in drug addicts 54, 99. This suggests that IDU is a larger threat than hepatitis C in young drug addicts, with a greater need of drug preventive interventions than HCV treatment. However, there is a high risk of HCV-related complications later in life and treatment of the HCV-infection should be considered in patients without active IDU.

In this study, HCV-infected (in contrast to the HBV-infected) had an excess mortality from almost all diagnoses. This should be interpreted with the mind that the SMRs represent mortality, not incidence of a disease, and it is possible that some excess mortality represent treatment failure due to lack of co-operation or bad adherence to therapy of an underlying disease. The increased risk to die from congenital disease (mostly organic heart disease), genitourinary disease and renal failure rather reflects the former risk of HCV-transmission through blood transfusion and haemodialysis. Previous studies demonstrated an excess mortality also from diagnoses not related to HCV in blood recipients, i.e. probably from the underlying disease that required transfusion 108, 126.

More than 90% of the HBV-cohort was immigrants, predominantly from HBV high-endemic countries, probably infected at birth or early childhood 83 and the “all cause“ and the liver-related mortality in HBV-infected in Sweden were high, very similar to the HBV associated mortality in a high-endemic country as Taiwan 72.
When studying the total burden and the temporal trends of HCV-associated inpatient care the size and the age structure of the HCV-cohort have major impact (Figure 3–4). The majority of the HCV-infected individuals in Sweden were born in the 1950s and 60s, with an increase of individuals aged 40-59 years in the 2000s. During observation time 16% in the HCV-cohort and 3% in the matched comparison-cohort died, resulting in a shorter mean observation time/subject in the HCV-cohort than in the comparison group, 7.75 and 8.54 years, respectively.

In the HCV-cohort 72% and in the comparison group 34% had been patients. The estimated likelihood, a hazard ratio (HR), for admission to hospital was 4.03 (95% CI: 3.98–4.08) in the HCV- compared with the comparison-cohort. The HR for liver-related care was 77.52 (95% CI: 71.03–84.60) and liver cancer care 40.74 (95% CI: 30.58–54.27). Age- and sex-adjustment did not notably change the results.

The burden of inpatient care in the HCV-cohort was high, the age- and sex-adjusted rate ratio for admissions was 5.91 (95% CI: 5.87–5.94) and for hospital days 8.78 (95% CI: 8.76–8.80), when compared with the comparison-group. Psychiatric (mostly drug-related) care constituted 45% of admissions and 62% of hospital days in the HCV-cohort. As expected, inpatient care increased with the growth of the cohorts, however, inpatient care/person decreased over time in the HCV-cohort. This was partly a result of a decrease in psychiatric care (more common in younger age groups) but also due to selection bias, the first years patients with pre-existing morbidity were more likely to be tested for HCV. Also, hospital beds in Sweden decreased with 50% during the study period, this affected both the HCV and the comparison-cohort, preferably the length of the hospital stays, with a 60% decrease in hospital days but no change in admission rates in the comparison group.

Inpatient care with liver-related (including HCV-infection) principal discharge diagnoses (hepatitis A and B not included) were very common in the HCV-cohort (6.4% of episodes) compared with the comparison cohort (0.6% of episodes), the age-adjusted rate ratios were: admissions 70.05 (95% CI: 66.06–74.28) and hospital days 47.73 (95% CI: 41.3–57.15). In the HCV-cohort diagnostic liver biopsies constituted 20% of the principal liver diagnosis (PLD) admissions but 1% of the hospital days as the majority did not stay over night. Serious liver complications (SLC) defined as oesophageal varices, liver failure/encephalopathy, ascites, cirrhosis, liver cancer, and liver transplantation constituted 42% of the PLD episodes and 63% of the hospital days. In the 2000s inpatient care from SLC increa-
Figure 5. The HCV and the comparison cohort, episodes with liver-related diagnoses at discharge, the admission rates (/1000) by calendar year (with 95% confidence intervals).

Figure 6. The HCV and the comparison cohort, episodes with liver-related diagnoses at discharge, the admission rates (/1000) by age group (with 95% confidence intervals).
sed, also per person, with a 74% increase in admission rates from 2001 to 2006 (Figure 5). Both PLD and SLC rates increased with age, and SLC were uncommon before the age of 40 years with the highest rates in the 60–69 years age group (Figure 6).

In the HCV-cohort the prescription register identified 1,611 individuals who were prescribed combination therapy with pegylated alfa-interferon and ribavirin during the last 18 months of the study. Based on these results and national data on annual prescribed DDDs of ribavirin, an estimated 6,000 patients (about 1,000 annually) had been prescribed HCV-treatment in 6 years (2001–2006).

The first years of the study there was probably a selection of more sick individuals into the cohort with a high probability that asymptomatic individuals remained undiagnosed. Initially the undiagnosed population was considerable, but the last years of the study probably >75% of all HCV-infected individuals in Sweden were diagnosed, and the total burden defined as inpatient-care likely rather correct (undiagnosed presumed to use less inpatient-care). The rates (inpatient-care per person) decreased when a larger part of the HCV-cohort was diagnosed.

To evaluate the bias the likelihood for admission (expressed as HR) was estimated excluding inpatients the year before and/or the first year after HCV-notification. This reduced associations somewhat, suggesting some bias, however, the magnitude of the associations remained substantial.

In the subgroup infected through transfusion of blood/blood products the HR estimates for liver care and liver cancer care was equally high, but the HR for psychiatric care was much lower, compared with those infected through other routes of transmission. This indicated that there was a high risk for liver disease also in the absence of drug abuse. The HR for psychiatric (mostly drug-related) care was almost as high when route of infection was IDU, sexual, unknown, or tattoos, indicating that IDU probably was common in all these groups.

Liver-related care, especially from SLC, increased in the 2000s. The SLC admission rates by age group were stable over time, demonstrating that the increase in SLC was an effect of the growth of the older age groups, resulting from the spread of HCV in the 1970s and early 80s, and the large group of individuals infected for 25–35 years. In the coming decade age group 60–69 years will about four-double and inpatient care from SLC will likely more than double if not prevented by treatments. The last six years of the study approximately 1000 patients annually were treated with combination therapy curing about 50% of the patients treated. If treated before serious liver damage this could prevent complications 10–20 years later, however, as the patients with cirrhosis have a lower treatment response rate, and also when successfully treated will be at higher
The fast increase in HCV liver-related hospitalizations estimated in the United States 1994-2001 \(^6\) was not confirmed in the Swedish HCV-cohort. However, in the next 10 years there could be a faster increase, also consistent with the predictions from England and Scotland \(^7\),\(^{138}\).

In spite of very high rates of SLC in the HCV-cohort these episodes were too few to significantly influence the national rates \(^{134}\). However, the Swedish admission and hospital day rates for ascites and liver failure have slowly increased, possibly related to HCV. In 2006 oesophageal varices, liver failure/encephalopathy, ascites and primary liver cancer care in the HCV-cohort constituted 15% of all admissions with these diagnoses in Sweden, in 2001 this was 8%. The HCV-cohort liver cancers in 2006 constituted 10% of all liver cancers in Sweden, but the HCC incidence rates in Sweden have slowly decreased since the 1980s.

This study of the HCV-cohort in Sweden confirmed a high demand for inpatient care, with a considerable proportion psychiatric care for drug-related conditions in younger age groups, and a very high demand for liver-related care in all ages. Inpatient care from serious liver complications increased in the 2000s, and will probably increase the next decade as a result of the HCV-epidemic in the 1970s.
CONCLUSIONS

This thesis includes register-based cohort studies of morbidity and mortality in all diagnosed and notified HCV-infected individuals in Sweden and can be summarized with the following conclusions:

- A low-grade association between B-cell non-Hodgkin lymphoma and HCV was supported.

- An association between Multiple Myeloma and HCV was found.

- The risk for primary liver cancer was 40–45 times increased after more than 25 years with HCV-infection, compared with the general population.

- The absolute risk for primary liver cancer was 7% within 40 years of HCV-infection.

- All-cause mortality was significantly increased in the three cohorts of HBV, HCV, and HBV-HCV co-infected.

- Liver-related mortality was highly increased in the HBV, HCV, and HBV-HCV cohorts, especially after the age of 50 years.

- The HCV and HBV-HCV cohorts were similar with a high risk for lifestyle-related mortality in young adults.

- The total burden of inpatient care was high in the HCV-cohort and about half of the episodes were from psychiatric, drug-related conditions, especially in younger ages.

- Inpatient care from serious liver complications in HCV-infected individuals started to increase in the 2000s, and will probably increase the next decade, as a result of the HCV-epidemic in the 1970s.
ACKNOWLEDGEMENTS

The work with this thesis has occupied my thoughts for a long time by now, and I wish to express my sincere gratitude and appreciation to all who have supported me and made this work possible. In particular I wish to thank:

Erik Bäck, my supervisor, for always being enthusiastic and supportive, and for your pioneer ideas when planning the first paper – the spark that set it all off, and also for, already in 1988, encouraging my interest in viral hepatitis.

Karl Ekdahl, assistant supervisor, for the collaboration with SMI, your interest, and your excellent support and instructions in manuscript writing.

Rolf Hultcrantz, assistant supervisor, for sharing your knowledge in hepatology, and for always stressing the simple but important questions.

Anders Blaxhult, assistant supervisor, for the collaboration with SMI, and your valuable comments.

Anna Törner for your enthusiasm, ideas, and statistical knowledge, and for your special engagement in the mortality study, enabling this thesis.

Marie Nordström for excellent collaboration regarding the first study, and for encouraging me to continue this work.

Scott M Montgomery for sharing your knowledge in epidemiology and answering my numerous questions.

Helena Pettersson for your hard work with the register files for paper IV.

Ragnhild Janzon for supplying data from the National Surveillance database.

All other co-authors for support and advice and for the collaboration in our study group, hopefully to be continued.

Margareta Landin for important End-Note assistance.

Jan Källman, head of the department, and all my colleagues at the Department of Infectious Diseases for giving me time to carry out this project, for support and patience, and for your nice company during working days.

The Research Committee of Örebro County Council for financial support, and Roche Sweden for the Roche scholarship in 2009.

Family and friends for brighten up my spare time, and for playing golf with my husband while I was working with this thesis. My parents Ulla and Bert who always have supported me. My children (and IT-support) Karl, Jens, and Erik with Guro and little Jacob, you are always in my mind…

Last but not least, Hans – the man in my life, thank you for encouraging and supporting me.

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