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**HIV/HCV CO-INFECTION IN SWEDEN –
EPIDEMIOLOGY, HCV TREATMENT AND THE
IMPORTANCE OF IL28B GENE POLYMORPHISM**

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Epidemiology, HCV Treatment and the Importance of IL28B Gene Polymorphism

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ABSTRACT

Liver disease, mainly due to hepatitis C virus (HCV) infection, is a leading cause of death in HIV positive patients with access to antiretroviral therapy (ART). HCV treatment, which can prevent long-term complications of HCV infection, is available. Despite this, only a minority of HIV/HCV co-infected patients initiate HCV treatment. We aimed to determine key epidemiological features, including HCV treatment uptake, of HIV/HCV co-infection in Sweden.

In 2009, the IL28B genotype was shown to be strongly associated with spontaneous and treatment induced clearance of HCV genotype 1. Patients with HCV genotype 2/3 and HIV co-infection were less studied. We therefore aimed to study the role of IL28B genotype in these sub-groups of HCV infected patients.

In paper I and II, 13 HIV/HCV co-infected and 100 HCV mono-infected patients with HCV genotype 2/3 were treated with a lower-than-standard dose of pegylated interferon and weight-based ribavirin. Early viral kinetics and treatment outcome were studied according to IL28B genotype. In HCV mono-infected patients, IL28B genotype CC was associated with a steeper first phase decline during HCV treatment. This did not translate into higher sustained viral response (SVR) rates, hence the value of pre-treatment IL28B genotyping can be questioned in patients with HCV genotype 2/3. The small number of HIV/HCV co-infected patients included prevents firm conclusions regarding this group.

In paper III and IV, data from InfCare HIV, where all known HIV infected persons in Sweden (n=5315) are included, were extracted. Factors associated with spontaneous clearance of HCV and HCV treatment were studied in uni- and multi-variate analyses. Furthermore, using a questionnaire given to HIV/HCV co-infected patients and their attending physicians in Stockholm, we investigated reasons for not having initiated HCV treatment yet.

The prevalence of anti-HCV in the total HIV infected Swedish cohort was 14% in 2010. This corresponds to a 9-11% prevalence of chronic HCV. 21% had spontaneously cleared the HCV infection. Spontaneous clearance of HCV was associated with IL28B genotype CC and a chronic hepatitis B virus infection. Interestingly, three cases of spontaneous clearance of a *chronic* HCV infection after immune reconstitution induced by ART were seen, all with IL28B genotype CC. The HCV treatment uptake was 25%, which is in line with European data and possibly also with the treatment uptake in HCV mono-infected patients in Sweden. HCV genotype 2/3, HIV transmission route other than intravenous drug use (IDU) and on-going ART were associated with a higher HCV treatment uptake. No significant differences in treatment uptake according to gender, ethnicity or university clinic or not were found. A major reason for not having initiated HCV treatment was on-going or recent IDU. The higher HIV treatment uptake, and rate of undetectable HIV viral load, indicate that many patients who had not initiated HCV treatment were adherent to their HIV treatment.

When interferon-free direct acting antivirals (DAA) combinations soon will become available, this changes everything. Again. HCV treatment should then expand to include more HIV/HCV co-infected patients in order to prevent morbidity and mortality from HCV in this population.

LIST OF PUBLICATIONS

- I. Nilsson J, Weiland O. **Effect of control selection on sustained viral response rates in genotype 2/3 HCV mono-infected versus HIV/HCV co-infected patients.** Scandinavian Journal of Infectious Diseases, 2010; 42:533-539
- II. Stenkvist J, Sönnernborg A, Weiland O. **HCV RNA decline in chronic HCV genotype 2 and 3 during standard of care treatment according to IL28B gene polymorphism.** Journal of Viral Hepatitis, 2013; 20:193-199
- III. Stenkvist J, Nyström J/ Falconer K, Sönnernborg A, Weiland O. **Spontaneous clearance of chronic HCV in HIV/HCV co-infected patients with ART induced immune reconstitution.** Submitted.
- IV. Stenkvist J, Weiland O, Sönnernborg A, Blaxhult A, Falconer K. **Lower uptake of HCV treatment than HIV treatment in HIV/HCV co-infected patients.** Submitted.

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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
aOR	Adjusted odds ratio
ART	Antiretroviral therapy
CD	Cluster of differentiation molecule
cEVR	Complete early virological response
DAA	Direct acting antivirals
DDI	Drug-drug interaction
EACS	European AIDS clinical society
ELISA	Enzyme-linked immunosorbent assay
ESLD	End stage liver disease
EVR	Early virological response
ETR	End of treatment response
GWAS	Genome wide association search
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
IDU	Intravenous drug use
IFN	Interferon
IL	Interleukin
MSM	Men who have sex with men
OI	Opportunistic infection
OR	Odds ratio
PCR	Polymerase chain reaction
Peg-IFN	Pegylated interferon
PWID	People who inject drugs
RBV	Ribavirin
RVR	Rapid virological response
SMI	Swedish Institute for Communicable Disease Control
SVR	Sustained virological response
WHO	World Health Organisation

1 INTRODUCTION

In my clinical practice at the HIV outpatient department, I met HIV/hepatitis C (HCV) co-infected patients with cirrhosis and end stage liver disease (ESLD). They had been diagnosed with both HIV and HCV a long time ago and were doing well regarding HIV with on-going antiretroviral therapy (ART). Even though there is HCV treatment available, which can prevent cirrhosis and ESLD, these patients had never tried it. There were often reasonable explanations for this, but in some cases, they had just never got around to it. I also learned that my colleagues had promoted HCV treatment and even treated some that other would consider untreatable with the presently available HCV treatment.

International literature stated that about one out of three HIV patients in the West are co-infected with HCV. Among these, liver disease is a major cause of death. Despite this, only few patients initiate HCV treatment.

Considering these factors, I then asked myself: how many HIV/HCV co-infected patients are there, not just at our department but in Sweden totally? How many of them have initiated HCV treatment? Are all HIV/HCV co-infected patients in Sweden given an equal access to HCV treatment? What are the reasons for not yet having initiated HCV treatment? I approached the InfCare HIV database, in which data on all known HIV positive patients in Sweden are included, with these questions.

When treating an infection, not only the treatment and the infective agent are of importance, but also the person who is infected, the host. Several host factors, including genes, can influence treatment response. The study on how genes influence the effect or toxicity of drugs is called pharmacogenomics. A major breakthrough for pharmacogenomics was seen in 2009, when a genome wide association search (GWAS) resulted in the detection of gene variants, single nucleotide polymorphisms (SNPs), strongly associated with the response to interferon (IFN)-based HCV treatment. This interleukin-28B (IL28B) gene polymorphism actually explained some of the difference in response to HCV treatment between ethnic groups that had long been observed. The IL28B genotype was also shown to be associated with spontaneous clearance of HCV infection.

At the time of commencement of our studies, the association of IL28B genotype with sustained viral response (SVR) in patients infected with HCV genotype 1 was established. However, patients with HCV genotype 2 and 3 were less well studied, as were HIV/HCV co-infected patients. We therefore decided to study the role of IL28B gene polymorphism in these subgroups of HCV infected patients.

In this thesis I will introduce you to HCV and HIV infection separately, before attempting to point out the peculiarities of HIV/HCV co-infection. Thereafter, I will discuss the four papers included in this thesis. Paper I and II will be handled together as will paper III and IV, since patients included in these studies are the same. I then hope to conclude something, before you can finally read the acknowledgements.

2 HEPATITIS C

2.1 BACKGROUND

Hepatitis C virus (HCV) was discovered in 1989[1]. The clinical picture of post-transfusion non-A, non-B hepatitis (NANBH), most of which was later shown to be HCV-related, had been recognized since the 1970s[2].

HCV is a blood-borne virus. In the West it is mainly spread by intravenous drug use (IDU), by shared contaminated drug use equipment: needles, syringes and other paraphernalia [3, 4]. Before the first anti-HCV test was introduced in the early 1990s (1992 in Sweden) transmission via blood products was a major route[5]. Iatrogenic HCV transmission continues to occur in low-income countries and sporadic nosocomial outbreaks are still reported from high-income countries [5]. HCV can also be transmitted vertically, from mother to child, but to a low extent (<5%)[6]. There is a very low risk of sexual transmission of HCV in heterosexual couples [7, 8]. Despite an on-going outbreak of HCV in HIV positive men who have sex with men (MSM), there has been no firm evidence of this epidemic in HIV negative MSM[9].

The hepatitis C virus belongs to the Flaviviridae family and the hepacivirus genus. Its genome is about 9600 nucleotides, which encodes a single polyprotein cleaved to three structural proteins, and seven non-structural proteins. The single stranded RNA is surrounded by a nucleocapsid, which is further surrounded by a cell-derived membrane envelope[10].

The HCV RNA polymerase is error prone and the mutation rate very high. HCV has a high rate of replication, 10^{12} particles are produced every day in an infected person[11]. The resulting genetic variability contributes to the high ability of HCV to evade the immune system. There is no vaccine available.

There are 7 genotypes of HCV, with a distinct global distribution. Within each genotype, there are also several subtypes (represented by letters, i.e. 1a, 1b)[12]. Furthermore, quasi-species can be found within infected individuals.

Diagnosis of HCV infection is based on the detection of antibodies against HCV (anti-HCV) or HCV-antigen by an enzyme immunoassay (EIA) test and a polymerase chain reaction (PCR) test for detection of HCV RNA. Until recently, serological methods alone were not able to differentiate a past from present HCV infection, but the detection of HCV core antigen now allows for this distinction. In acute and chronic HCV, the HCV antigen and HCV-RNA tests in blood are positive.

2.2 EPIDEMIOLOGY

HCV causes 499 000 deaths globally every year, and is ranked number 25 in global causes of deaths [13]. End stage liver disease (ESLD) caused by HCV infection is a leading cause of liver transplantation. The mortality due to HCV has increased by 50% between 1999-2007 and exceeds that of HIV in the USA at present[14].

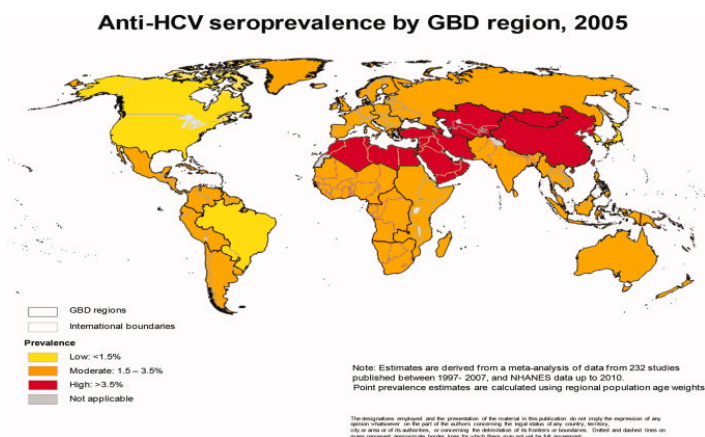
In Sweden, inpatient care due to serious complications of HCV infection increased in the 2000s[15].

HCV infection affects 180 million persons globally [16]. The earlier estimate from the World Health Organisation (WHO) was 130-170 millions, equivalent to a prevalence of 2.8%. Furthermore, 2.3-4.7 million persons are newly infected every year[17]. The HCV prevalence is highest in Central and East Asia, North Africa, and in the Middle East [16]. Egypt has an exceptionally high HCV prevalence, 15%, linked to iatrogenic spread during schistosomiasis treatment campaigns[18]. In Europe, the prevalence varies between 0.13-3.26%, with the highest prevalence found in Italy and Romania (The burden of liver disease in Europe, European Association for the Study of the Liver, EASL 2013, www.easl.eu). The Nordic countries are low endemic with an estimated prevalence of 0.5% [19-21].

It is estimated that 90% of persons with viral hepatitis (including hepatitis B) in Europe are unaware of their disease (The burden of liver disease in Europe, EASL 2013). Similarly, in the USA 45-85% are thought to be unaware of their HCV infection [22].

Figure 1. Estimated global anti-HCV prevalence in 2005.
From Mohd Hanafiah K, Hepatology 2013, with permission.

Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence



In Sweden, HCV is a notifiable disease by the Communicable Diseases Act and is reported by both the diagnosing clinician and the virology laboratory. Every year 2000 new cases of HCV are notified in Sweden. Some 55 000 persons have so far been notified with HCV since 1990 and it is estimated that about 40 000 are currently living with HCV infection (Swedish Institute for Communicable Disease Control, SMI, www.smittskyddsinstitutet.se). The notifications, however, are mainly based on anti-HCV testing alone which means that some of these persons will have cleared their HCV infection spontaneously or as a result of HCV treatment, leading to a overestimation of the prevalence of chronic HCV.

The majority (65%) of persons notified with HCV have been infected via IDU, and the majority of people who inject drugs (PWID) acquire HCV in the first few years after starting injecting[23, 24]. In Stockholm, an anti-HCV prevalence of 86.5% was found after a median injecting duration of 12 years in PWID 2005. Almost 50% was anti-HCV positive within 2 years after first injecting drugs[24]. In the recently started needle exchange program in Stockholm, the anti-HCV prevalence in 2013 is 74% (Martin Kåberg, personal communication).

The peak prevalence in Sweden occurs in persons born 1950-1960. Many of these persons acquired the infection in the late 60ies of 70ies when intravenous drug use became more common in Sweden. There is still on-going transmission in PWID, as indicated by a stable HCV incidence in persons in their 20ies.

Blood transfusion was the probable route of transmission in 6% of persons notified with HCV in Sweden. In 26% the transmission route was unknown, however many of these cases may be IDU-related[15, 23].

2.3 NATURAL HISTORY

An acute HCV infection is spontaneously cleared in 15-50%[25, 26]. Hence, the majority of infected patients develop chronic HCV, defined by a disease duration of more than 6 months. Once the infection has become chronic, spontaneous clearance of HCV is extremely rare[27].

Host factors associated with a higher spontaneous clearance rate include female sex, age less than 40 years, chronic hepatitis B, a favourable IL28B genotype, which affects clearance of HCV genotype 1 and 4, certain human leucocyte antigen (HLA) genotypes, and a strong persistent CD4+ T-cell response[26, 28-33]. Symptomatic patients generally have a higher clearance rate[26].

Host factors associated with a lower clearance rate include HIV infection[34].

Viral factors, such as HCV genotype and HCV RNA viral load may also be of importance[26, 31].

HCV infects hepatocytes, where it replicates, and is also found in peripheral blood. The possibility to infect lymphocytes, monocytes, dendritic cells and also intestinal epithelial cells has been suggested[35]. HCV has also been detected in brain tissue[36]. The entry of HCV into hepatocytes requires an interaction with multiple host proteins, including glycosaminoglycans (GAGs) and low density lipoprotein-receptor (LDL-R)[35]. The process is incompletely understood.

Patients with chronic HCV experience chronic inflammation in the liver and a progression of fibrosis, resulting in cirrhosis in 10-30% after 20-30 years[25]. The fibrogenesis is not linear and seems to be accelerated when more advanced stages of fibrosis are reached[37].

The risk of fibrosis progression is influenced by many factors, including gender, age, alcohol consumption and co-infection with HIV, HBV or schistosomiasis[25]. There is no firm evidence that the HCV genotype or viral load affects fibrosis progression, however, genotype 3 is associated with more pronounced steatosis[25, 38]. The role of the IL28B genotype in fibrosis progression is unclear[39].

Once cirrhosis is present, patients run a 1-4% per year risk of developing hepatocellular cancer (HCC). He/she is also at a 3-8 % yearly risk of developing decompensated cirrhosis: ascites, variceal bleeding, hepatic encephalopathy or severe bacterial infections including spontaneous bacterial peritonitis (SBP)[40-42]. These conditions are referred to as end stage liver disease, ESLD.

Liver transplantation is an established treatment for decompensated liver disease due to HCV, and also for small HCCs. However, the HCV invariably relapses in the transplanted liver and causes accelerated fibrosis progression in the graft[43].

Only 10-20% of patients with acute HCV experience symptoms (i.e. jaundice, fatigue, general flu-like symptoms) and therefore the diagnosis is seldom made in the acute stage[26]. The majority of patients with chronic HCV are also asymptomatic. Hence, the HCV infection can be undiagnosed for decades. A few patients experience extra-hepatic manifestations, such as glomerulonephritis, porphyria cutanea tarda or cryoglobulinemia-associated vasculitis, which may lead to diagnosis of HCV[42]. There is also an association between HCV infection and non-Hodgkin lymphoma and myeloma [44].

2.4 HCV TREATMENT

With antiviral treatment, HCV infection can be cured in a majority of cases. Achieving sustained viral response (SVR), which is regarded as the equivalent of a cure, is associated with halted progression and even regression of fibrosis, and a lower risk of liver related morbidity and mortality[45, 46].

The first available HCV treatment was interferon (IFN)-alfa (a naturally occurring protein, administered by subcutaneous injection), which became available in 1986, resulting in SVR rates of 10-30%[47]. The oral nucleoside analogue ribavirin (RBV) was added in 1998, improving rates of SVR to 40%[48, 49]. Interferon was modified with pegylation (peg-IFN) in 2001; again increasing cure rates and allowing subcutaneous injection once weekly instead of three times per week. The SVR rates with peg-IFN + RBV is 50% in HCV genotype 1, 80% in genotype 2/3 and 60% in genotype 4 [50, 51]. The influence of the IL28B genotype is strong particularly in patients with HCV genotype 1. Patients with the favourable genotype CC achieve SVR in 80% whereas only 38% of those with CT/TT genotype do so [52].

The mechanism of action of peg-IFN is thought to be both direct antiviral and immune modulating[53]. The mode of action of RBV is not clear, it may be immune modulating but its capability of inducing mutagenesis might also be important[54, 55]. RBV seems to be of extra importance in the prevention of relapse.

The recommended doses of peg-IFN + RBV varies according to HCV genotype and underlying host factors. The treatment duration, 16-24-48 weeks, is response-guided. For definitions of virological response, see Table 1.

In general, the highest peg-IFN dose as well as weight based RBV dosing during 48 weeks (shortened to 24 weeks if RVR is achieved) is recommended for patients with HCV genotype 1 or 4. In patients with genotype 2 or 3, a lower flat dose of RBV can be used, and peg-IFN can be given in lower doses with a duration of 24 weeks. Even shorter treatment, 12-16 weeks, can be given in genotype 2 and 3 if RVR is achieved according to current HCV treatment guidelines [56, 57].

Factors affecting treatment response include:

Host factors: gender, age, cirrhosis, insulin resistance, steathosis, BMI, ethnicity, IL28B genotype, IP 10

Viral factors: HCV genotype, baseline HCV-RNA viral load

On treatment response: RVR, EVR

Out of these, IL28B, RVR and HCV genotype are the strongest predictive factors for SVR[32, 52].

Viral kinetics during treatment is a strong predictor of SVR. After the initiation of peg-IFN+RBV treatment, the HCV RNA viral decline usually follows two phases. The first phase, with a rapid decline in the first two days, is thought to reflect the direct antiviral effect of the treatment resulting in a block in production or release of new virions. The second phase, in which the HCV RNA continues to decline but at a lower pace during the following weeks, is thought to reflect the clearing of infected hepatocytes [58].

Table 1. Definitions of virological response of Peg-IFN+RBV

	Time	HCV RNA
Rapid virological response (RVR)	Week 4 on treatment	Undetectable
Early viral response (EVR)	Week 12 on treatment	>2 log ₁₀ drop from baseline
Complete EVR (cEVR)	Week 12 on treatment	Undetectable
Partial EVR (pEVR)	Week 12 on treatment	>2 log ₁₀ drop from baseline, but not undetectable
End of treatment response (ETR)	End of treatment	Undetectable
Sustained viral response (SVR) 12/24	12 or 24 weeks post treatment	Undetectable
Relapse (RR)	End of treatment and 24 weeks post treatment	Undetectable at end of treatment, detectable 24 weeks post treatment
Non response (NR)	24 weeks post treatment	Detectable

While SVR rates of 50-80% of patients are a remarkable achievement, several factors limit the applicability of the peg-IFN+RBV treatment. It has numerous side effects, making patients and providers hesitant to initiate treatment and sometimes causing premature discontinuation of treatment. Also, there are difficult-to-treat patients, including those with advanced cirrhosis, who may decompensate and develop ESLD during treatment. Renal insufficiency, liver transplant and HIV/HCV co-infected patients are also more difficult-to-treat and have lower SVR rates.

In 2011, the first direct acting antivirals (DAAs) became available. In triple therapy, a protease inhibitor: telaprevir or boceprevir, active against HCV genotype 1 only, is given in combination with peg-IFN +RBV for 12-24 weeks, followed by peg-IFN+RBV for another 12-24 weeks[59, 60]. This increased the SVR rate by some 20% and enabled shortened duration in a large segment of patients, however, also markedly increased side effects and cost. This triple therapy is now the current standard of care (SOC) treatment in patients with HCV genotype 1 [56].

In 2014, new DAAs are expected to reach the market, including the nucleoside analogue sofosbuvir and the protease inhibitor simeprevir[61]. Sofosbuvir has achieved exceptionally high SVR rates with shorter treatment durations and much less side effects than the current SOC. Sofosbuvir has a pangenotypic activity and a high barrier to resistance[62, 63]. The development of DAAs is a rapidly evolving field, and all-oral interferon free treatments are expected to cure most HCV infections within the near future [64].

When HCV treatment is given in the stage of acute infection, the SVR rates are higher than when treatment is given in the chronic stage, even with peg-IFN monotherapy[26]. However, since the majority of HCV infections are asymptomatic and hence undiagnosed, treatment in the acute stage is seldom possible. HCV treatment in this section refers to the treatment of chronic HCV.

HCV treatment uptake

When evaluating the HCV treatment uptake, the purpose of HCV treatment must be considered. The main treatment goal today is to prevent HCV related ESLD and death. Given the slowly progressive nature of chronic HCV in many patients, not all will have an indication for HCV treatment. On the other hand, HCV treatment may be seen as prevention and a step towards reducing the HCV incidence and prevalence. Indeed, mathematic modelling has suggested the cost-effectiveness of a more widely used treatment among PWIDs with a potential to influence the HCV epidemic [65, 66]. The “ideal” treatment uptake is thus not defined.

In addition, reliable HCV treatment uptakes estimates are scarce, since many studies may be subject to selection bias[67].

A HCV treatment uptake of 37.5% was reported in 2006 among 10 400 HCV mono-infected patients based on claims data in the USA [68]. This large study only included patients with health insurance, which might lead to an overestimation of the treatment uptake. In a huge (n=99 000) veterans administration cohort in 2005, 12% started HCV treatment [69]. However, the results of veterans are not automatically generalizable to other populations. A 24% HCV treatment rate was reported in a community-based study in Australia [70]. This study was based on self-reported HCV. In a recent meta-analysis of HCV treatment experience outside of clinical trials, a 19% HCV treatment uptake was reported [67].

In an estimation of ever treated prevalent HCV mono-infected patients in 21 European countries from 2005, the numbers ranged from 16% in France to less than 1% in Russia, Greece, Poland and Romania. The average cumulative treatment rate was 3.5%. In Sweden a 12% treatment uptake was reported. This estimate was based on sales of peg-IFN and an estimated national HCV prevalence of 0.5%[71].

In line with this a 14% HCV treatment uptake was reported in Sweden in 2006, based on 1000 peg-IFN prescriptions per year between 2001 and 2006 and an estimated 43 000 HCV infected individuals based on notification data[15].

More recently, Razavi et al estimated HCV treatment rates 2010 in Europe, again based on peg-IFN units sold and estimated national prevalence figures. France had the highest HCV treatment rate, 7% followed by Sweden, 4.3%. Italy and Russia had the lowest rates: 0.6% and 0.5% respectively (Razavi, abstr 50, International Liver Congress 2013).

The cost of the current HCV treatment is high, and the price of the coming DAAs will probably be even higher. This limits the global access to HCV treatment. Mediciens Sans Frontiers (MSF) has launched a call for a scale up of HCV treatment, possibly by the use of biosimilars.

2.5 THE ROLE OF IL28B GENE POLYMORPHISM

In 2009, a genome wide association search (GWAS) revealed a strong correlation between a single nucleotide polymorphism (SNP) at chromosome 19 near the IL28B gene and SVR after peg-IFN+RBV treatment in patients with HCV genotype 1 [32, 52, 72]. The same favourable IL28B genotype was also associated with spontaneous clearance of HCV, at least in HCV genotype 1 infections [73]. These associations were also found in HIV/HCV co-infected patients with HCV genotype 1 [32, 74, 75]. IL28B gene polymorphism influences the very early viral kinetics of HCV during peg-IFN+RBV treatment. This was shown in HCV genotype 1 but not in genotype 2/3 at the time of commencement of our studies (paper II). The importance of the IL28B genotype in predicting SVR was less clear in HCV genotype 2/3 and studies have reported conflicting results[76-79]. Hence, the value of pre-treatment testing of IL28B genotype has been debated in patients with HCV genotype 2/3.

The distribution of SNPs varies geographically, and explains some of the difference in SVR rates noted earlier between ethnic groups[52]. In 2012, 21 different SNPs near or in the IL28B gene had been analysed. Their impact may be different in different populations. So far, the rs 12979860 SNP is the one most commonly studied and most extensive evaluated in Caucasians [80].

The mechanism, through which the IL28B genotype and SVR and spontaneous clearance respectively are associated, is not well understood. The IL28B gene encodes for interferon lambda 3, which induces antiviral activity by itself. It also uses the Janus kinas-signal transducer and activator of transcription (JAK-STAT) pathway and induces interferon-stimulating genes (ISGs), which also have antiviral activity. Favourable IL28B genotypes are associated with lower levels of pre-activated ISGs, but are induced more strongly after IFN-alfa treatment [80].

When more effective DAAs are used, host factors are likely to become less relevant. The IL28B genotype, however, has a predictive influence on the SVR rates of triple therapy including telaprevir or boceprevir[81]. Whether this applies to both IFN-naïve and experienced patients is unclear [82, 83]. The role of IL28B genotype in future interferon-free HCV treatments remains to be seen, but it is likely to be of much less importance.

3 HIV

3.1 BACKGROUND

In 1981, a cluster of unusual pneumonias caused by pneumocystis jiroveci (previously carinii) was noted in MSM in Los Angeles, San Francisco and New York[84, 85]. It soon became clear that a virus caused the immunodeficiency allowing this opportunistic infection (OI) in young MSM as well as in haemophiliacs, PWID and Haitian immigrants in the USA. The virus was isolated in 1983 by the Montagnier/Barré-Sinoussi group and confirmed by Robert Gallo [86, 87]. It was initially called lymphadenopathy associated virus (LAV)/Human T lymphocyte virus III (HTLV3) and later renamed human immunodeficiency virus (HIV). HIV is the causative agent of acquired immune deficiency syndrome (AIDS). HIV is transmitted parenterally, sexually and from mother to child.

HIV is a retrovirus, belonging to the lentiviridae genus. It encodes 3 retrovirus typical proteins; gag (group-specific antigen), pol (polymerase) and env (envelope) as well as 16 accessory proteins with regulatory functions. HIV is an enveloped, single-stranded RNA virus with a DNA intermediate: a provirus that integrates and persists within the host cell DNA.

The reverse transcriptase, an enzyme central to the HIV replication process, is mutagen prone. The virus has a high replication rate of approximately 10^{10} virus particles per day in an infected person. Also, HIV has a high rate of recombination events. Hence, the viral evolution is very rapid. The resulting genetic variation contributes to immunological escape and confers the risk of resistance to antiretroviral drugs[88].

There are two species of HIV, HIV-1 and HIV-2, where HIV-1 is the virus most spread and what is usually referred to when discussing HIV[89].

HIV-1 is classified into four groups: major (M), novel (N), outlier (O) and P, where M is the group dominating the epidemic. There are 9 subtypes, or clades (A, B, C, D, F, G, H, J, K) and numerous circulating recombinant forms (CRFs) of the M group[90]. HIV-1 is believed to have crossed the species barrier from chimpanzee to humans around the year 1900 in Africa[91].

Diagnosis of HIV infection is based on serological methods, detecting HIV antigen and or anti-HIV antibodies in either blood or plasma by enzyme-linked immunosorbent assay (ELISA). Serological methods can also be applied on saliva.

HIV-RNA is detected with a PCR method in blood or peripheral blood mononuclear cells (PBMCs) from HIV-infected persons. The limit of detection varies between assays, but is usually 50 or 20 copies/mL. By ultrasensitive RT-PCR assays, a persistent residual low-level viraemia (1-5 copies/mL) can be detected also in patients on antiretroviral therapy (ART) with undetectable HIV-RNA by conventional methods. This residual viraemia may either arise from latently infected cellular reservoirs or represent on-going viral replication.

3.2 EPIDEMIOLOGY

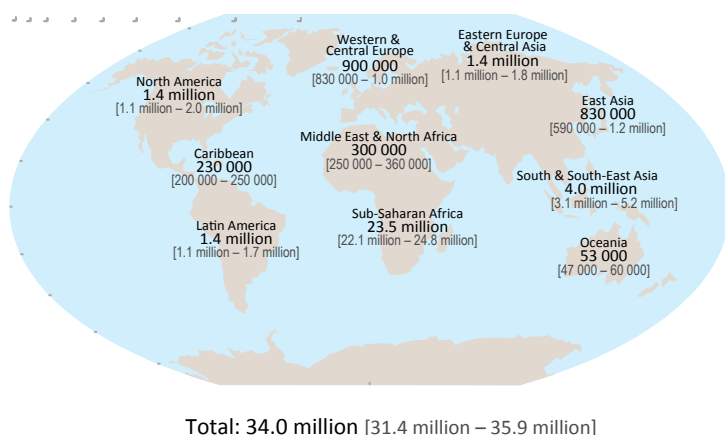
AIDS is a leading global cause of death, ranking number five as a cause of disability adjusted life years (DALY) loss in 2010 Global Burden of Disease [92]. 1.6 (1.4-1.9) million people died because of AIDS in 2012 (UNAIDS Global Report 2013, www.unaids.org). Since the beginning of the HIV epidemic, more than 25 million lives have been claimed by the disease (HIV/AIDS Fact sheet no 360, WHO, www.who.int). In countries with high access to antiretroviral therapy (ART), AIDS is no longer a major cause of death. However, late HIV diagnosis, when there is already advanced disease with immunosuppression, may still result in death from AIDS in the West. About 50% of HIV patients in Europe are “late presenters”, diagnosed when their CD4+ T-cell count is below 350 or presenting with an AIDS defining event[93]. It is believed that 18% of HIV positive patients in the USA are unaware of their infection (Centers for Disease Control and Prevention, CDC, www.cdc.gov).

In 2012, an estimated 35 (32-39) million people were HIV infected worldwide. Furthermore, 2.3 (1.9-2.7) million new infections occur every year, which represents a 33% decline compared to the number of new infections in 2001. (UNAIDS Global Report 2013, www.unaids.org)

The HIV prevalence is highest (up to 25% in persons aged 15-49 years in some countries in Southern Africa) in Sub Saharan Africa and in Asia, where the heterosexual transmission route dominates. SSA alone is home to 69% of all HIV infected persons. The incidence is rising in Eastern Europe and Central Asia, especially in PWID. In Europe, 2.3 (2.0-2.7) million people are estimated to be HIV infected, and the prevalence in persons aged 15-49 years is 0.4% (WHO, www.who.int).

Figure 2. Adults and children estimated to be living with HIV in 2011. WHO (www.who.int)

Adults and children estimated to be living with HIV | 2011



HIV is a notifiable disease in Sweden. Between 1983 and 2012, 10 332 persons have been notified with HIV. Of these, about 6200 are currently alive. The prevalence in Sweden (9600 000 inhabitants) is thus estimated to be 0.06%. The incidence is regarded as stable with about 400-500 new cases reported every year. About half of these were infected before the arrival in Sweden. The MSM group dominates among persons infected in Sweden. In 2007, there was an HIV outbreak in PWID in Stockholm. Since then, however, the reported number of cases in PWID has been low (SMI, www.smittskyddsinstitutet.se).

3.3 NATURAL HISTORY

A few weeks (7-28 days) after encountering the HIV virus, some patients develop symptoms of a primary HIV-infection. These symptoms may include fever, sore throat, and exanthema and are commonly mistaken for other viral illnesses. During this initial phase of the infection, HIV-RNA viral loads are very high which makes this a very infectious stage. In fact, recently infected persons may be driving the HIV epidemic[94].

HIV enters the CD4+ T-cells by attaching to the CD4 receptor and co-receptors, CCR5 or CXCR4. It also infects resting central and translational memory T-cells, thus creating a reservoir of integrated viral DNA. Other reservoirs, including monocytes/macrophages and naïve T-cells have also been suggested [95]. Within days and weeks, there is a pronounced depletion of CD4+ T-cells in the body, mainly in the gut associated lymphoid tissue (GALT) but also in peripheral blood. The mechanism for HIV killing of CD4+ T- cell is not entirely clear. HIV is also capable of infecting other cells such as monocytes/macrophages, dendritic cells, astrocytes in the CNS, and stellate cells in the liver[96, 97].

The pathogenesis of HIV is characterized by a progressive loss of CD4+ T-cells while HIV-RNA increases from a set point. Initially the patient is asymptomatic but may later develop constitutional symptoms, such as weight loss, diarrhoea, fever, and lymphadenopathy. When the CD4+ T-cells reach a certain level, the risk of opportunistic infections (OIs) gradually increases. The risk of malignancies, especially those driven by viruses, is also increased.

AIDS refers to advanced stages of HIV infection and is defined by any of more than 20 OIs or related malignancies, for example pneumocystis pneumonia, cerebral toxoplasmosis and Kaposi sarcoma. The median time from HIV infection to AIDS is 8 years (6-10), however there are large inter-individual variations in the progression rate (1-20 years) [98]. A few so-called elite controllers are able to maintain HIV-RNA below detection limit and have normal CD4+ T-cell counts in the absence of ART[99]. Also, long-term nonprogressors maintain stable CD4+ T-cell counts and are asymptomatic but are viraemic[100]. Certain HLA alleles have been found to be protective against progression[101].

The immunosuppression caused by HIV can be partially reversed by ART. With ART, most HIV infected persons can lead a healthy life with an average life expectancy just below that of a general population [102]. However, there is a residual excess morbidity

and mortality even in ART treated individuals [103]. This increased mortality, in cardiovascular disease, comorbidities and cancer, seems to be associated with inflammation. The mechanism is not clear, but on-going immune activation, possibly driven by microbial translocation has been proposed[104].

3.4 HIV TREATMENT

ART is a life-saving treatment, which dramatically reduces morbidity and mortality. ART can achieve undetectable levels of HIV RNA in blood. When HIV replication is suppressed the CD4+ T-cell count gradually increases and is sometimes restored to normal levels.

ART is also effective as prevention. HIV infected patients with on-going ART have a dramatically reduced risk of transmitting HIV to their sero-discordant sexual partners [105].

However, a cure is not achieved with ART, since the interruption of ART almost invariably leads to the re-emergence of viral replication and progression towards AIDS. Rare cases of persons maintaining an undetectable HIV-RNA viral load after coming off ART have recently been reported [106](Persaud D, abstr 48LB 20th Conference on Retroviruses and Opportunistic Infections, CROI 2013). These persons all initiated ART soon after HIV infection.

Also, ART is not capable of eliminate HIV proviruses integrated in resting memory T-cells. Presently, reservoir-cell activating drugs, including histone deacetylase (HDAC) inhibitors, are being investigated. The hope is that these drugs may be part of a strategy to achieve at least a functional cure of HIV[107].

The first ART was zidovudin in 1987, followed by other nucleoside reverse transcriptase inhibitors (NRTI) and non-NRTIs (NNRTI). With the advent of protease-inhibitors (PI) in 1996, Highly Active Antiretroviral Therapy (HAART) was possible. With this treatment, mortality dramatically decreased. Today, more than 20 different antiretroviral drugs from 4 classes with different modes of action are available. The established treatment principle is combination ART, consisting of 3 active drugs to decrease the risk of resistance. The ART drugs of use today in the West are generally potent, have a low pill burden and relatively few side effects.

In Sweden, 87% of all known HIV infected patients were on ART in 2012, according to the InfCare HIV register.

Globally, there has been a scale-up in HIV treatment access with a goal to reach 15 million people with ART in 2015 set up by the UN. In 2012, 9.7 million people in low- and middle-income countries had access to ART, representing 61% of those eligible according to the WHO 2010 guidelines. However, under the WHO guidelines from 2013, in which ART is recommended at CD4+ T-cell count <500, this represents only 34% (UNAIDS Global Report 2013, www.unaids.org).

Despite continuing efforts, there is still no vaccine available against HIV.

4 HIV/HCV CO-INFECTION

4.1 EPIDEMIOLOGY

Liver diseases, mainly attributable to HCV, have become a leading cause of death in HIV infected persons with access to ART. In several cohort studies liver diseases rank among the top three causes of death [108-115].

ESLD in the HIV/HCV co-infected population has increased over the last 15 years in the USA, and is presently an important clinical problem [14].

However, Weber et al reported a decreasing proportion of deaths attributed to liver diseases in the D:A:D cohort 1999-2011 (Weber, abstr THAB0304 AIDS 2012). The relative importance of liver diseases as a cause of death must be analysed in connection with the prevalence of HCV and hepatitis B in the cohort under study.

Due to shared routes of transmission, especially the blood-borne route, HIV/HCV co-infection is common with an estimated total anti-HCV prevalence in HIV infected persons of 15-30 % in the West [116, 117]. Compared with HIV, HCV is more rarely sexually or vertically transmitted.

The prevalence of HIV/HCV co-infection is depending upon distribution of HIV transmission routes and varies in different countries and epidemiological settings. PWID generally have the highest HCV prevalence, 72-95%, haemophiliacs intermediate, heterosexuals (9-27%) and MSM (1-12%) the lowest [116]. The highest co-infection rates are thus reported from countries and settings with a high prevalence of blood-borne HIV infection. Up to 70% of HIV infected patients from Eastern Europe (Ukraine, Belarus) and 85% among Chinese plasma donors are anti-HCV positive [118, 119]. In Sub Saharan Africa and Thailand, where the heterosexual HIV transmission route dominates, the HIV/HCV co-infection rate is much lower, below 5-10% [120-122]. Reliable data on HIV/HCV co-infection prevalence are missing from many parts of the world. Also, the prevalence may vary within countries, i.e. from 3.7% in rural areas to 18% in urban Tanzania [123, 124].

An anti-HCV prevalence of 33% (1960/5957 patients) was reported from the large EuroSIDA cohort, which includes patients from about 100 centres in Europe, Israel and Argentina in 2005. There were marked regional differences within Europe, with the highest prevalence in the East (47%) and South (41%) and a lower prevalence in the West (23%) and North (20%) of Europe [119]. In 2008, an anti-HCV prevalence of 24% (3375/14310 patients), whereof 77% had a chronic HCV infection was reported from the same cohort [125]. In a recent publication, the anti HCV prevalence in EuroSIDA was reported to be 31% (4044/13025) with 74% being HCV RNA positive [126].

The prevalence of HIV/HCV co-infection in Sweden has not been studied in detail. In the EuroSIDA study from 2005, the reported prevalence in Sweden was 29% based on a sample of 205 Stockholm patients of known HCV serostatus [119]. When sampling only from Stockholm, the IDU transmission route might be overrepresented and hence, the co-infection prevalence overestimated.

Since 2004, there is an on-going HCV epidemic in HIV-positive MSM in Europe, the USA and Australia. In this group the prevalence and incidence is increasing but from a low level. Acute HCV infection in this setting has been associated with mucosal damage, non-injected drugs and other sexually transmitted infections (STI). The HCV genotypes seen are commonly genotype 1 and 4, and large transmission networks related to perimucosal injury have been identified using phylogenetic analysis[127, 128].

4.2 NATURAL HISTORY

4.2.1 The effect of HIV on HCV infection

HIV alters the natural history of HCV, accelerates the rate of fibrosis progression and aggravates the complications of HCV.

HIV infected persons have an increased rate of sexually transmitted HCV infection. This is the case for HIV-infected MSM (see above), but also the heterosexual transmission rate might be increased even though evidence is weak [117, 129, 130]. In addition, an increased rate of mother to child transmission of HCV has been reported in HIV infected persons [6, 131].

A lower rate of spontaneous clearance of an acute HCV infection is generally seen in patients with HIV infection [34]. The association of IL28B genotype with spontaneous clearance of HCV has been reported also in HIV/HCV co-infected patients [32]. However, at the time of initiation of our studies, this was less well established than in mono-infected patients.

Clearance of *chronic* HCV infection without specific HCV treatment is rarely seen, but several case-reports in HIV/HCV co-infected patients have previously been published[132-138]. Most of these cases, if not all, have been associated with immune reconstitution after initiation of ART.

Interestingly, there are also a few case reports of spontaneous clearance of chronic HCV in the post-transplant setting in HCV mono-infected persons[139, 140].

HIV/HCV co-infected patients tend to have a higher HCV-RNA set point, on average one log higher than mono-infected patients [141, 142]. The effect of ART on the viral load is somewhat unclear. Several studies report a lower HCV RNA viral load after successful ART, sometimes following a short increase in HCV RNA after initiation of ART [141, 143]. In a recent study, ART was associated with stable HCV RNA levels whereas HCV RNA increased in the absence of ART[144].

Fibrosis progresses more rapidly in HIV/HCV co-infected than HCV mono-infected patients [145-150]. This is evident even with moderately lowered CD4+ T-cell counts [151]. The mechanism remains unclear. HIV related immunosuppression, ART related liver toxicities, a direct effect of HIV on hepatic stellate cells or amplified microbial

translocation has been proposed.[96, 117, 141]. The faster rate of fibrosis progression is partially, but probably not fully, reversed by ART although the data are conflicting [37, 152-154]. ART should therefore be started early in co-infected patients [155](European AIDS Clinical Society, EACS, guidelines 2013 v7.0, www.eacsociety.org). Also in patients with decompensated liver cirrhosis, there is a clear survival benefit of ART [156].

Liver cancer has been increasingly reported in HIV/HCV co-infected patients [14, 110, 157-161]. A shorter time from HCV infection to HCC and a higher prevalence of metastatic disease at the time of diagnosis has been suggested in HIV/HCV co- versus HCV mono-infected patients [162-164]. However, other studies did not find an increased risk of HCC in co-infected patients, and the effect of HIV on development of HCC is not clear [165]. International guidelines recommend that HIV/HCV co-infected patients with established liver cirrhosis are included in HCC-surveillance programs (EACS, guidelines 2013 v7.0, www.eacsociety.org).

If liver decompensation occurs, the prognosis of HIV/HCV co-infected patients is very poor, and hence, these patients need to be evaluated at a liver transplant unit [166]. The reported post transplantation 5 years survival is 55%, which is lower than in HCV mono-infected patients, 71-75% [167, 168]. There is no accelerated progression of HIV in the post-transplant setting. Drug interactions can be problematic but are usually manageable. However, there is invariably a HCV recurrence, which is associated with accelerated liver fibrosis and a risk of fibrosing cholestatic hepatitis[169].

4.2.2 The effect of HCV on HIV infection

This is an area of uncertainty. The current understanding is that HCV infection does not have any major effect on HIV disease progression or the effect of ART.

An increased all cause mortality in HIV/HCV co-infected patients versus HIV mono-infected has been reported [170, 171]. This increased mortality, however, seems to be driven by excess liver related and IDU related deaths and not by HIV disease progression[126, 172-174]. A nationwide cohort study from Denmark reported a higher mortality in HIV/HCV co- versus HCV mono-infected patients, but this was exclusively driven by HIV-related immunosuppression[175].

Some studies report a lower rate of CD4+ T-cell increase after the initiation of ART in co-infected patients [170, 176]. One possible explanation would be that cirrhosis per se is associated with lower CD4+ T-cell counts [177]. Adherence to ART may also be lower in co-infected patients due to IDU[178].

The risk of chronic kidney disease was also increased in HIV/HCV co-infected versus HIV mono-infected patients and associated with HCV RNA viraemia[179, 180].

HIV/HCV co-infected patients have an increased risk of hepatotoxicity following ART initiation. For this reason certain antiretrovirals, such as nevirapine, tipranavir, didanosine and stavudine, should be avoided in co-infected patients. Hepatotoxicity, however, is reported with other ART drugs as well. The advantages of ART outweighs the risk of hepatotoxicity, which is usually manageable [155].

4.3 HCV TREATMENT IN HIV/HCV CO-INFECTED PATIENTS

4.3.1 General principles

The main principles of, indications and contra-indications for HCV treatment are the same in HIV/HCV co-infected as in HCV mono-infected patients. Given the faster progression of fibrosis in HIV/HCV co-infected patients the treatment indication is sometimes considered stronger than in HCV mono-infected. Accord to this view, HCV treatment should be considered regardless of fibrosis stage[181]. Current guidelines though recommend HCV treatment in co-infected patients with fibrosis stage 2 or higher [56] (EACS guidelines 2013 v7.0, www.eacsociety.org). At present, the development of new DAAs must be taken in account, and many patients might benefit best from awaiting these.

On-going opportunistic infection/AIDS is an added contra-indication.

The predictors of SVR to peg-IFN and RBV are the same as in HCV mono-infected patients, including IL28B gene polymorphism in patients with HCV genotype 1 [80, 182]. Recently, an association of human leucocyte antigen E (HLA-E) polymorphism with SVR was reported in HIV/HCV co-infected patients [183].

Patients with lower baseline CD4+ T-cell count or percentages tend to have a lower SVR rate. If HIV-RNA is suppressed and CD4+ T-cells still remain low, HCV treatment can still be initiated. The CD4+ T-cell absolute count decreases substantially during IFN treatment due to IFN induced neutropenia, but the percentage of CD4+ T-cells increases slightly. There are limited data on the risk of opportunistic infections in this setting, but it is probably low [184, 185].

HIV/HCV co-infected patients have lower rates of SVR after peg-IFN+RBV treatment compared with HCV mono-infected. Approximately 30 (17-36%) in HCV genotype 1 and 70 (44-82)% in HCV genotype 2/3 co-infected patients achieve SVR[186-193]. The reason for this is not fully understood. Inadequate RBV doses, frequent dose-reductions, treatment discontinuations, drug-drug interactions (DDIs) with ART, higher baseline HCV RNA viral load, higher proportion of cirrhosis and other co-morbidities have been suggested. As peg-IFN is dependent of the patient's immune response, the impairment of cellular responses associated with HIV may also be a factor.

It is not clear if DAA treatment also will result in lower SVR rates in co-infected patients. In the phase 2 studies of telaprevir and boceprevir higher SVR rates, which were comparable to historical controls of HCV mono-infected patients, were achieved[194, 195]. However, the number of patients included has so far been very small.

Sustained viral response (SVR) after HCV treatment is associated with a lower incidence of ESLD and death also in co-infected patients [196, 197].

In principle, the same peg-IFN, RBV doses and treatment durations as in HCV mono-infected patients are recommended[56] (EACS guidelines 2013 v7.0, www.eacsociety.org) However, weight-based RBV is recommended in all HCV genotypes, even though a large study failed to confirm the importance of this [188]. The higher peg-IFN dose is recommended. The use of response-guided therapy of less than 24 weeks has not been evaluated in co-infected patients and hence should not be used. Patients with genotype 2/3 can be treated for 24 weeks if they achieve RVR. For treatment of acute HCV infection, RBV is recommended in combination with peg-IFN in HIV-infected patients. The use of protease-inhibitors for treatment of an acute HCV genotype 1 infection is not established. The same dose of telaprevir or boceprevir is recommended, but the treatment duration cannot be shortened.

Modifications of ART are sometimes necessary to avoid added toxicities and drug-drug interactions (DDI). For example, zidovudine or didanosine should not be given in combination with ribavirin due to aggravated anaemia and risk of lacticidosis, respectively. These nucleoside-analogues are not recommended parts of ART anymore, due to their mitochondrial toxicities.

There has been controversy as to whether abacavir interacts with ribavirin, possibly due to competition of intracellular phosphorylation, resulting in lower SVR rates [198, 199]. However, the use of abacavir seems not to be a problem provided that weight-based ribavirin is used[200, 201] (EACS, guidelines 2013 v7.0, www.eacsociety.org). The arrival of the first DAAs highlighted the importance of DDI with ART. Telaprevir and to a certain extent boceprevir are inhibitors and substrates of CYP3A4, and hence interactions with both PIs and NNRTIs, which use the same pathway, is common. An important lesson from the first DAAs is that DDIs are difficult to predict, therefore DDI studies need to be performed.

For most patients, unless they have accumulated HIV resistance mutations, ART can be modified to avoid clinically relevant interactions with the first generation DAAs.

4.3.2 Timing of HCV treatment versus HIV treatment

Previously, it was recommended that HCV treatment should be given before ART, in order to avoid interactions and added toxicities. This was provided that the CD4+ T-cell count was high enough not to warrant ART initiation.

However, in more recent guidelines, the importance of early ART initiation (<500) in co-infected patients, in order to prevent progression of fibrosis and to optimize the chance of SVR has been highlighted (EACS guidelines 2013 v7.0, www.eacsociety.org). The current trend is to initiate ART earlier in all HIV patients, regardless of co-infections (WHO guidelines 2013, www.who.int and Department of Health and Human Services, HHS, guidelines 2012, www.aidsinfo.nih.gov/guidelines). In clinical practice, as only few patients present with very high CD4+ T-cell counts when diagnosed with HIV, the option to treat HCV first is seldom available.

4.3.3 HCV treatment uptake

In general, the HCV treatment uptake has been reported to be low in HIV/HCV co-infected patients, and regarded lower than in mono-infected patients [67, 202-205]. Varying HCV treatment uptake, from 2% to 60%, has been reported in HIV/HCV co-infected patients, partly depending on the denominator used.

Only 2% of co-infected patients started HCV treatment in a prospective study in Boston [206]. Similarly, only 3% of co-infected patients at Johns Hopkins HIV clinic had initiated HCV treatment [207]. A HCV treatment uptake of 7.6% in the EuroSIDA cohort was reported in 2004 [208]. However, this was based on anti-HCV positive patients and therefore most likely underestimated the treatment uptake. From the Swiss cohort a HCV treatment uptake of 12.5% among HCV RNA positive patients was reported [209]. In an overview based on surveys to WHO representatives, patient advocates and biomedical industries, HCV treatment uptake in 2004 in 23 European countries was estimated to be 10% on average, ranging from 0-23%. The reported uptake in Sweden was 10% [210]. These reports all concern the period up until 2004, a time when evidence of HCV treatment safety and efficacy was available only in HCV mono-infected patients.

More lately, a multicentre cohort from USA reported a 20% HCV treatment uptake until 2007, with an increasing proportion initiating treatment within the first year [211]. Also, a single centre study from Ireland reported a 28% HCV treatment uptake until 2008 [212]. The higher uptake rates, 30-60%, may have been based on already selected patients [213-216]. For example, a 41% treatment uptake was reported from a reference HIV/AIDS clinic in Madrid 2008, including only co-infected patients on regular follow-up [217].

A cumulative HCV treatment uptake of 25% in 2010 was recently reported in co-infected patients in the Euro-SIDA cohort [218]. The Euro-SIDA cohort is huge, however, it may still be subject to selection bias since usually only a few centres in a country report to Euro-SIDA. The HCV treatment rates at these centres may not be representative of the whole country.

In contrast with previous studies, a similar HCV treatment uptake of 21% (n=246) in co-infected versus 22% (n=15 163) in mono-infected patients with incident HCV in 2006-2009 was reported by Kirbach (abstr 838, International Liver Congress 2013). Although large, this study was based on insurance claims data and hence possibly not representative of all co- and mono-infected patients in the USA.

In a recent meta-analysis, the treatment uptake in HIV/HCV co-infected patients (n=1522) was reported to be 16% versus 19% in HCV mono-infected patients (n=13 583) [67]. Thus, even though the treatment uptake in co-infected patients was lower, the difference might not be that great.

5 AIMS

The overall aims were to

1. Determine epidemiological key features of HIV/HCV co-infection in Sweden
2. Correlate the IL28B genotype with spontaneous clearance of HCV and early viral kinetics during HCV treatment

More specifically, I aimed to

1. Study the early HCV kinetics and HCV treatment outcome according to IL28B genotype in HCV genotype 2/3 mono and co-infected patients (Paper I and II)
2. Study spontaneous HCV clearance in HIV infected patients according to IL28B gene polymorphism and baseline demographic factors (Paper III)
3. Describe the epidemiology of HIV/HCV co-infection in Sweden (Paper IV)
4. Determine the HCV and HIV treatment uptake in co-infected patients in Sweden according to HIV transmission route (Paper IV)
5. Define factors associated with initiation of HCV treatment (Paper IV)
6. Define barriers to HCV treatment in co-infected patients in Stockholm (Paper IV)

6 MATERIALS AND METHODS

6.1 PAPER I AND II

6.1.1 Patients

In an investigator initiated HCV treatment study 100 HCV mono-infected and 13 HIV/HCV co-infected naïve patients with HCV genotype 2 and 3 were recruited at the hepatitis and HIV outpatient departments at Karolinska University Hospital in Huddinge in 2003-2006. Patients with chronic hepatitis B, other concomitant liver diseases, on-going substance abuse or severe psychiatric disease were excluded. Ten of 13 co-infected patients had had undetectable HIV RNA levels on ART for a minimum of 6 months prior to inclusion. Three co-infected patients had not yet initiated ART, all with CD4+ T-cell counts above 350.

Patients were treated with a lower than standard dose of peg-IFN-alfa (135 micrograms/week) in combination with weight-based RBV (11 mg/kg/day) for 24 weeks. Experienced nurses monitored patients during treatment.

6.1.2 Estimation of fibrosis

According to current Swedish consensus guidelines, HCV treatment was allowed in all HCV genotype 2/3 patients regardless of fibrosis stage and hence a liver biopsy was not regularly performed. At the time of the study, Fibroscan had not yet been introduced at the Karolinska University Hospital. However, in order to estimate the fibrosis stage we calculated the AST to platelet ratio index (APRI) and Gothenburg University Cirrhosis index (GUCI).

6.1.3 Viral outcome definitions

The standard definitions of viral responses: RVR, cEVR, pEVR, ETR, SVR and NR (see Table 1 in the introduction) were used. Patients who failed to achieve pEVR stopped treatment.

In paper II, the decline in HCV RNA levels was analysed during the 1st phase, from baseline to treatment day 2, and 2nd phase, from day 2 to treatment week 2 or 4.

6.1.4 Virological methods

HCV RNA levels were analysed using the Roche Taqman Real-Time PCR (detection limit of 15 IU/ml). HCV genotyping was performed with a line probe assay (Inno-LiPA HCV II, Innogenetics NV, Gent, Belgium) or an in-house method

6.1.5 IL28B genotyping (Paper II)

All patients were tested for IL28B rs 12979860 SNP with a Taqman-based allele-specific PCR method (Applied Biosystems Inc, Foster City, CA,USA), using the ABI 7500 Fast equipment. For details, see paper II.

The SNP was defined as rs 12979860 genotype CC, CT or TT genotype. We did not assess deviations from the Hardy-Weinberg equilibrium.

6.1.6 Statistics

In paper I, the 13 HIV/HCV co-infected patients were matched to 2 HCV mono-infected control patients. Matching for genotype, baseline viral load, and age (+/-5 years), was done in that order by an investigator blinded to all other baseline demographic factors and treatment outcomes.

In the analysis, the HIV/HCV co-infected patients, their 26 HCV mono-infected controls, and the total 100 HCV mono-infected patients were compared.

Differences between groups were compared with chi-square test or Fisher's exact two-tailed for categorical variables. The Wilcoxon Rank Sum test was used for continuous variables.

In the univariate analysis of baseline factors associated with RVR or SVR, we included disease duration, APRI score, gender, weight, baseline HCV RNA viral load, HCV genotype 2 versus 3 and IL28B SNP CC versus non-CC. As no baseline factor was significantly associated with SVR (at the <0.10 level), no multivariate analysis was performed (paper II).

A p-value of < 0.05 was considered statistically significant.

All data were analysed using JMP software version 9.0.0.

6.1.7 Ethics

Study I and II were performed in accordance with the Helsinki declaration and were approved by the Regional Ethics committee (Dnr 250/03, including an amendment, and 2010/1782-31).

6.2 PAPER III+IV

6.2.1 Patients

6.2.1.1 *InfCare HIV database*

More than 99% of all known HIV positive patients in Sweden have been prospectively included in the InfCare HIV research database since 2009. Retrospective data from the beginning of the 1990-s have also been included from patients in Stockholm and Gothenburg.

On the 28th of September 2010, 5315 adult living persons were known to be HIV infected in Sweden.

Demographical data of all living anti-HCV and anti-HIV positive adults (n=652) (>= 18 years) were extracted including age, sex, ethnicity, HIV transmission routes, clinical data (prior AIDS diagnosis, CD4+ T-cell count), virological data (anti-HCV serology, HCV-RNA levels, HCV genotype, HIV-RNA levels, HBV serology), and HCV and/or HIV treatment data (HCV treatment and ART).

6.2.1.2 *The Stockholm cohort (Paper IV)*

In Stockholm, two outpatient departments provide specialised health care for HIV infected adult patients: the Karolinska and Venhälsan at Södersjukhuset. Together they provided care for 2732 (51%) of the 5315 HIV infected patients in Sweden in 2010.

In total, 322 patients in Stockholm were HIV/HCV co-infected. The Stockholm cohort thus constituted 69% of the total HIV/HCV co-infected cohort in Sweden.

82 (25%) had received HCV treatment whereas 240 had not. These 240 persons were considered eligible for inclusion in the questionnaire-based study of barriers to HCV-treatment.

As a comparison, 77 HCV mono-infected patients at the Karolinska hepatitis outpatient department were included in the same study.

6.2.2 Viral outcome definitions

Chronic hepatitis C was defined as a positive HCV-RNA test in an anti-HCV positive individual. Spontaneous clearance of HCV was defined as a treatment naïve patient with a positive anti-HCV test but a negative HCV-RNA test.

A patient was defined as having a chronic hepatitis B virus (HBV) infection if HBsAg was positive, and to have had a previous HBV infection if anti-HBc (+/- anti-HBs) were positive with a negative HBsAg test, and to be immune to HBV from vaccination when anti-HBs was positive as an isolated test.

Hepatitis C treatment had been given with IFN or peg-IFN alone or in combination with RBV. The standard definitions of SVR and NR were used (see Table 1, page 6). Persons who had received HCV treatment for an acute hepatitis C (n=5) were excluded from the analyses of factors associated with spontaneous clearance and treatment of chronic HCV.

An undetectable HIV RNA viral load was defined as <20 HIV RNA copies/ml.

6.2.3 Virological methods

Analyses of HIV-RNA, anti-HCV antibodies, and HBV serology were performed at the local virological laboratories by routine techniques. HCV RNA was analysed in anti-HCV positive individuals with the Roche TaqMan Test (detection limit of 15 IU/ml). HCV genotyping was performed with a line probe assay or an in-house method.

6.2.4 IL28B genotyping (Paper III)

Anti-HCV positive patients from the Stockholm area (n=263) were tested for IL28B rs 12979860 SNP. For details, see paper III.

6.2.5 Questionnaires (Paper IV)

A questionnaire was given to HIV/HCV co-infected patients in the Stockholm area who had not yet initiated HCV treatment investigating the underlying reasons. The attending physician was given a separate questionnaire for every patient he/she cared for whom had not yet initiated HCV treatment.

Reasons for not having initiated treatment were categorized as IDU/alcohol (on-going or recent abuse), patient desire not to start (including not motivated to initiate HCV treatment, fear of side effects, loss of income in case of sick leave and unstable social situation), doctors recommendation (including no indication due to mild fibrosis or recommendation to postpone treatment until better HCV treatments become available), psychiatric disease, comorbidities (somatic contraindication to HCV treatment, including ESLD, excluding HIV-related contra-indications), HIV/AIDS (uncontrolled HIV infection, on-going opportunistic infection), don't know or other.

The questionnaires from every patient-doctor pair were assessed for agreement or discrepancies in reasons stated for not having initiated HCV treatment.

For comparison, HCV mono-infected patients and their attending physician at the Karolinska University Hospital hepatitis outpatient department were given the same questionnaires.

6.2.6 Statistics

Differences between groups were compared with chi-square test for categorical variables and the Wilcoxon Rank Sum test for continuous variables.

In the univariate analysis of factors associated with spontaneous HCV clearance, we included age, sex, ethnicity, HIV transmission route, prior AIDS diagnosis, CD4+ T-cell count, present ART, plasma HIV-RNA level, HBsAg status, and IL28B genotype. (Paper III)

In the univariate analysis of factors associated with initiation of HCV treatment we included age, sex, ethnicity, born in Sweden or not, HIV transmission route, prior AIDS diagnosis, CD4+ T-cell count, present ART, plasma HIV-RNA level, HBsAg status, HCV genotype and department (university clinic or not). (Paper IV)

Factors with a p-value < 0.10 were included in a multivariate model. Logistic regression was used to identify variables associated with spontaneous HCV clearance or initiation of HCV treatment, respectively.

The results are presented as odds ratio (OR) and adjusted OR (aOR) with 95% confidence intervals (CI).

A p-value of < 0.05 was considered statistically significant.

All data were analysed using JMP software version 9.0.0.

6.2.7 Ethics

The studies were performed in accordance with the Helsinki declaration and were approved by the Regional Ethics committee (Dnr 2010/1782-31 and 2011/514-31/1).

7 RESULTS AND DISCUSSION

7.1 PAPER I+II

7.1.1 HCV treatment outcome (Paper I and II)

The baseline demographics in the 13 HIV/HCV co- and 100 HCV mono-infected patients are shown in table 1, paper I.

The tolerability of HCV treatment was high: only one of the HCV mono-infected patients withdrew prematurely from treatment due to an adverse effect. All 13 HIV/HCV co-infected patients completed treatment. This was probably due to the low peg-IFN dose used and support from the treatment staff.

The HCV treatment outcome, according to RVR or not, is illustrated in Figure 1 paper I. In total 77% of co-infected patients, 77% of their matched mono-infected controls and 86% of all mono-infected patients achieved SVR. The ability of RVR to predict SVR was high.

This small study suggests that patients with well controlled HIV and HCV genotype 2/3 can be treated with lower-than-standard doses of peg-IFN with satisfactory results. However, this would need to be evaluated in a larger randomized trial comparing the higher with the lower dose of peg-IFN.

In a single-arm trial, 58 HIV/HCV co-infected patients with HCV genotype 3 were treated with a lower-than-standard peg-IFN and RBV (800 mg). A 58% SVR rate was reached [219]. In a non-randomized trial in 106 HIV/HCV co-infected patients with HCV genotype 3, the lower-than-standard dose was compared with the higher peg-IFN dose in combination with RBV (800 mg). The HCV viral decline during the first 4 weeks was less in the lower dose group, even though this did not result in significantly lower RVR rates (73 vs. 60%, $p=0.17$). SVR rates were not reported [220]. Of note is that both these trials used a flat dose of RBV (800 mg), thereby changing two parameters at the same time. They also included only HCV genotype 3 patients, which generally have lower SVR rates than genotype 2 patients.

The strikingly complicated title of paper I reflects our efforts to interpret the previously published viral kinetic part of the study. There was no difference in early viral kinetics between HIV/HCV co-infected and their matched HCV mono-infected patients [221]. Did the matching process, including the matching on baseline viral load, introduce some bias? We added estimates of fibrosis and disease duration and then compared the co-infected patients with the total HCV mono-infected group, without finding any major differences. The more general question behind the study was whether HIV/HCV co-infected patients with well controlled HIV are really that different from HCV mono-infected patients regarding response to HCV treatment? To answer this question, however, a large study comparing HCV treatment outcomes in co- and mono-infected patients would need to be designed. Due to the currently changing treatment landscape of HCV, this is no longer relevant to do with an interferon-based treatment, but would be interesting if a combination of the coming DAAs were used.

7.1.2 Prevalence of IL28B genotypes in the study population (Paper II)

The result of IL28B genotyping was available in all but one HCV mono-infected patient. In the HCV mono-infected patients, the overall prevalence of IL28B genotype CC, CT and TT was 44%, 52% and 4%, respectively. In the HIV/HCV co-infected patients, the corresponding figures were 46%, 46% and 8%. This is similar to a North European study of mainly Caucasian patients with HCV genotype 2/3, where the corresponding allele frequencies of 44%, 45% and 11% were found [222].

7.1.3 Baseline factors according to IL28B genotype (Paper II)

Baseline factors according to IL28B genotype are outlined in Table 1, paper II. Baseline HCV-RNA viral load was significantly higher in HCV mono-infected patients with IL28B genotype CC than CT/TT (non-CC), 9 750 000 versus 2 000 000 IU/mL ($p=0.02$). This is in line with other reports showing higher HCV-RNA levels in HCV genotype 1, even though the association is less firmly established in HCV genotype 2/3 infections [52, 76, 222].

7.1.4 Early viral kinetics according to IL28B genotype (Paper II)

The early viral kinetics according to IL28B genotype in HCV mono-infected patients is shown in Figure 3. Of note is that Figure 3b and 3c actually shows the difference in HCV RNA levels between two time points, not the absolute levels. In the HCV mono-infected patients, IL28B genotype CC was associated with a steeper first phase decline during HCV treatment, 2.03 vs. 1.37 log₁₀ IU/mL, $p=0.01$ (Figure 3b). However, since patients with genotype CC had higher baseline HCV RNA viral loads (Figure 3a), the decline started from a higher HCV RNA level but later approached the absolute levels of non-CC genotype patients (data not shown). Others have reported this association of the IL28B genotype with first phase decline during HCV treatment in HCV genotypes 1-4 [79, 223, 224].

The early viral kinetics in the HIV/HCV co-infected patients are depicted in Figure 2, paper II. No significant difference in viral kinetics according to IL28B genotype was noted in this group, probably because of the small number of patients included.

7.1.5 HCV treatment outcome according to IL28B genotype (paper II)

The HCV treatment outcome according to IL28B genotype is depicted in Figure 3, paper II. Despite the effect on early viral kinetics in the HCV mono-infected patients, no significant effect of the IL28B genotype on RVR or SVR was found in either our HCV mono- or HIV/HCV co-infected patients. In total, 70% of mono-infected patients had RVR, and out of these more than 95% achieved SVR, regardless of IL28B genotype.

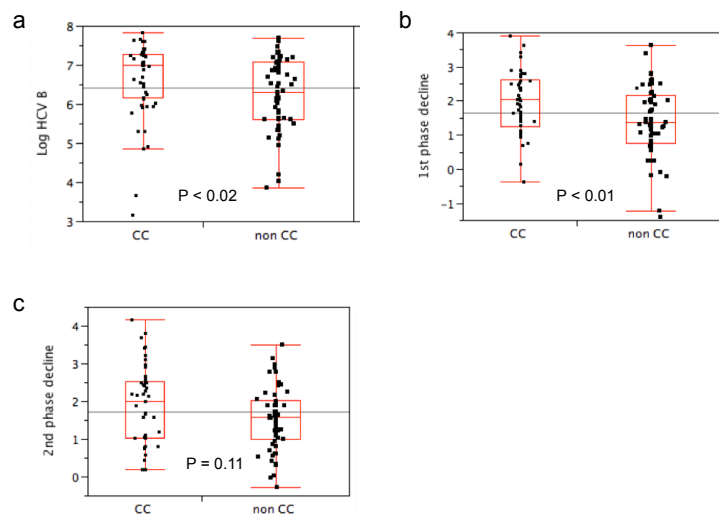
In univariate analyses, no baseline factor, including IL28B genotype, was significantly correlated with SVR and only disease duration with RVR ($P = 0.04$). Baseline HCV

RNA was nearly significantly associated with RVR ($P = 0.057$). Since HCV genotypes 2/3 have higher SVR rates, the sample size needed to show significant associations with SVR is larger than in HCV genotype 1.

There have been conflicting reports on the association between IL28B genotype and SVR in HCV genotype 2/3 patients. A recent meta-analysis reported a significant association between IL28B genotype and SVR in HCV genotype 2/3 but with lower strength than in HCV genotype 1 and 4 patients. They therefore agreed with our conclusion in paper II, that pre-treatment testing of IL28B genotype was of limited usefulness in patients with HCV genotype 2/3 [80].

The coming DAAs, which also are active against HCV genotype 2/3, are expected to make IFN-free HCV treatment possible for most of HCV infected patients in the near future. With the more potent antiviral activity of the DAAs, the role of host factors (including HIV co-infection and IL28B genotype) will probably be less important. Perhaps, HIV/HCV co-infected patients might achieve similar response to DAA treatment as HCV mono-infected patients. In a telaprevir-based triple therapy study, 33 HIV/HCV co-infected patients were compared with 116 HCV mono-infected patients. SVR12 did not differ significantly between groups but actually tended to be higher in co-infected patients, 61% vs. 42%, $p=0.06$ [225]. However, if this will be repeated in larger studies and valid for other DAAs remains to be seen.

Figure 3. HCV RNA levels at a) baseline b) 1st phase decline c) 2nd phase decline during peg-IFN+RBV treatment according to IL28B genotype CC versus non-CC in HCV genotype 2/3 mono-infected patients (n=99)



7.2 PAPER III+IV

7.2.1 HIV positive patients in Sweden (Paper III and IV)

In total, 5315 persons \geq 18 years were known to be HIV positive in September 2010. The transmission route for HIV was heterosexual in 50%, men who have sex with men (MSM) in 32%, and IDU in 8%. Transmission via blood transfusion, mother to child transmission, and via other or unknown routes accounted for less than 10%. The HIV treatment rate was 82% and varied significantly according to HIV transmission route. Among persons infected via blood transfusion ART was on-going in 90%, in MSM 85 %, in heterosexuals 83% and in PWID 75 % (data not shown).

7.2.2 Prevalence of anti-HCV (Paper III and IV)

In 4765 (90%) persons anti-HCV test results were available whereof 652 (14%) were anti-HCV positive (Figure 4). 550 patients (10%) lacked an anti-HCV test result. For a comparison of anti-HCV tested versus non-tested patients, see paper III.

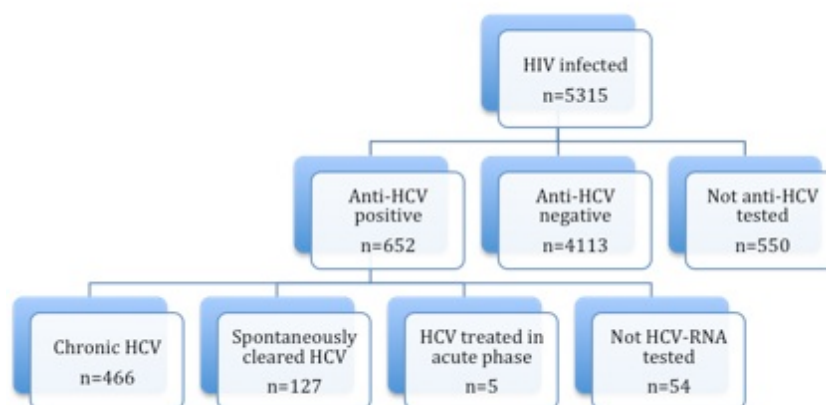
The prevalence of anti-HCV varied markedly according to HIV transmission route. Hence, 98% of PWID, 41% of persons who acquired HIV via blood transfusion, 5.5% of heterosexually HIV infected, and 3.7% of MSM were anti-HCV positive ($p < 0.01$) (Figure 2, paper IV). This is in line with other reports [116, 119, 126].

Among persons who had acquired HIV in Sweden the anti-HCV prevalence was 26% versus 6.2% in persons who were infected abroad, $p < 0.0001$. When stratifying for HIV transmission route, the differences were still significant in all transmission routes except for MSM (data not shown).

The anti-HCV prevalence was higher in males than in females, 15% versus 12%, $p = 0.01$. When analysing only persons with heterosexual HIV transmission route though, there was no significant difference in anti-HCV prevalence between males and females, 6.4% versus 4.8%, $p = 0.10$ (data not shown).

The 14% prevalence of anti-HCV in the Swedish HIV positive cohort is low by international comparison. It is also lower than previously reported from Sweden [119]. One possible explanation is the distribution of HIV transmission routes in the total Swedish cohort. PWID, who generally have the highest anti-HCV prevalence, only make up 8% of the Swedish cohort. Also, the near universal (90%) testing might result in a lower total prevalence. Since the 10% not tested were more likely to have a low anti-HCV prevalence we might even have overestimated the prevalence somewhat. On the other hand, the on-going HCV epidemic in the MSM group might not have been fully captured, since not all clinics had yet implemented yearly anti-HCV testing in this group.

Figure 4. HIV infected patients with chronic HCV in Sweden 2010



7.2.3 Chronic HCV in HIV infected individuals (Paper III and IV)

HCV RNA testing had been performed in 598/652 (92%) patients of whom 79% tested positive (Figure 4). These were defined as having a chronic HCV infection, corresponding to a chronic HCV prevalence of 11%. For a comparison of HCV-RNA tested versus non-tested patients, see paper III.

Demographics in the 466 HIV/HCV co-infected patients in Sweden are depicted in Table 1 (paper IV). Among heterosexuals, 33%, originated from countries with moderate (1.5-3.5%) to high (>3.5%) HCV prevalence (data not shown) [16]. In total, 79% patients had on-going ART of whom 76% had undetectable HIV RNA levels (Table 1, paper IV). The rate of undetectable HIV viral load did not differ significantly according to HIV transmission route (data not shown).

HCV genotyping was available in 76% of the HIV/HCV co-infected individuals. HCV genotype 1, 2, 3a and 4-6 was seen in 52%, 12 %, 31%, and 6.2% respectively. Genotype 1a constituted 75% of all genotype 1 cases. The domination of HCV genotype 1 stresses the need for new more effective DAAs to improve SVR rates. The genotype distribution is similar to the one reported in HCV mono-infected patients in Sweden and in the Euro-SIDA cohort [126, 226]. In Sweden, however, genotype 2 is more and genotype 4 less common.

Only eight persons (1.7%) also had a chronic hepatitis B virus infection. 68% had had a past HBV infection and 6.0% were isolated anti-HBs positive (data not shown). Hence, a previous HBV infection was very common, but only few were registered as HBV vaccinated. This probably reflects under registration, but also indicates that the HBV vaccination rate can be improved.

An alternative way to calculate the prevalence of chronic HCV in the Swedish cohort is to exclude patients who had a HCV treatment induced clearance of their HCV infection. Out of the 471 initially HCV RNA positive individuals, 61 persons had achieved SVR. Hence, there were 410 HIV infected persons with chronic HCV in Sweden in September 2010. This corresponds to a HIV/HCV co-infection prevalence of 9% ($410/4765=8.6\%$ of anti-HCV tested or $410/(4765-54)=8.7\%$ of HCV-RNA tested).

The HCV genotype distribution in these 410 HIV/HCV co-infected persons is slightly different from the one reported above. HCV genotype 1, 2, 3a and 4-6 was seen in 56%, 9.2%, 28%, and 6.9%, respectively. This is due to enrichment of HCV genotype 1 as a result of lower SVR rates in patients with this genotype.

7.2.4 Factors associated with spontaneous clearance of HCV (Paper III)

Spontaneous clearance of HCV was noted in 127/593 (21%) patients. This is within the expected range for co-infected persons. Also, many patients might have cleared their HCV before they became HIV positive, even though we do not have enough data on the consecutive order in which the HIV and HCV infections were acquired. PWID most likely acquired HCV before HIV, since the prevalence of HCV is high but HIV is still rare among Swedish PWIDs [24]. Furthermore, the risk of HIV transmission of by shared drug injection equipment is lower than with HCV transmission [227].

We were able to document seroconversion for HCV after the HIV diagnosis in 28% of the MSM group. Sexually transmitted HCV among HIV positive MSMs is an on-going epidemic [228].

In uni- and multi-variate analyses the only factor significantly correlated with spontaneous clearance of HCV in our 593 patients was HBsAg positivity. In total, 50% of HBsAg positive persons ($n=16$) cleared HCV vs. 21% in HBsAg negative persons. An adjusted odds ratio (aOR) of 17.3 (2.42-125.6), $p=0.003$ for spontaneous clearance of HCV was seen in HBsAg- positive versus -negative persons.

The association of chronic HBV infection and spontaneous clearance of HCV has been previously reported [30, 125, 229, 230]. The mechanism is not clear, but viral interference has been proposed [231].

Spontaneous HCV clearance was seen in 26% females and 19% males, $p=0.0563$, which is borderline significant. Female gender has previously been associated with higher spontaneous clearance rate of HCV [31, 125, 229].

7.2.5 IL28B genotype and spontaneous HCV clearance (Paper III)

IL28B genotyping was performed in 263 anti-HCV positive patients followed at the Karolinska University Hospital. Their demographics, and IL28B distribution are depicted in Table 3, paper III. For a comparison of IL28B genotype tested versus non-tested patients, see paper III. Differences in baseline demographics in IL28B genotype tested versus untested patients were noted but were unlikely to have had any major impact on the overall results.

A strong correlation between IL28B genotype and spontaneous HCV clearance was noted. Thus, 36% of individuals with CC versus only 13% with non-CC had spontaneously cleared their HCV infection, $p=0.0003$, aOR 5.45 (2.22-14.50), Table 3, paper III. Hence, the association of IL28B genotype with spontaneous HCV clearance in co-infected patients was confirmed in our study [32].

One limitation of this study is our definition of spontaneously cleared and chronic HCV, which might lead to misclassification. Acute HCV infection at the time of data extraction or the latest HCV RNA test would be misclassified as a chronic infection. HCV RNA sampling was performed according to the clinical routine and often there was only one sample available. However, the standard clinical definition of chronic HCV was applied. Since the majority of patients do not clear HCV spontaneously, this definition is mostly correct. Misclassification of acute HCV as chronic would lead to an underestimation of the rate of spontaneous clearance and a lower power to detect factors associated with clearance.

This might affect clearance rates according to transmission routes differentially, but is likely to be non-differential with regards to HBsAg positivity and IL2B genotype.

7.2.6 Three cases of spontaneous clearance of chronic HCV infection – association with IL28B genotype CC? (Paper III)

Among our 263 IL28B genotype tested patients, 3 had spontaneously cleared their *chronic* HCV infection after immune reconstitution induced by ART. These individuals became HCV-RNA negative without specific HCV treatment after previously being diagnosed to have a chronic HCV infection with at least 2 positive HCV-RNA tests 6 months apart. Their cases were briefly reported in paper III (Figure 1 a, b, c). All three patients had IL28B genotype CC.

Since IL28B genotype CC was found in 122/263 tested patients, of whom 106 were on ART, these 3 cases make up 3/106 (2.8%) of our patients on ART harbouring genotype CC. If only tested patients with IL28B genotype CC, ART and *chronic* HCV are considered in the denominator, they would make up 3/68 (4.4%). If the IL28B genotype results, the HCV chronicity rate and the ART uptake were extrapolated to our total co-infected cohort the cases would make up 3/151 (2.0%). Hence, our three cases represents only 2-4% of all our IL28B genotype CC patients. This means that the spontaneous clearance of a *chronic* HCV is indeed a rare event.

Our three cases were thoroughly verified, however, our methods do not rule out that there might be additional cases of spontaneous clearance of chronic HCV in our cohort. Our case 1c was found to have a low level of chronic T cell activation and a high level of T cell function as earlier published [232].

This possible association with spontaneous clearance of *chronic* HCV underlines the importance of the favourable IL28B genotype CC. In a subset of patients with this genotype, thus spontaneous clearance of HCV can occur beyond the acute stage in the setting of ART induced immune recovery.

In HIV/HCV co-infected patients with CD4+ T cell counts <350/microL, improvement of CD4+ T-cell counts using ART is recommended before HCV treatment is initiated to optimize the chance of SVR (EACS, guidelines 2013 v7.0, www.eacsociety.org).

ART has also been shown to slow down the rate of fibrosis progression. Taken together with our findings, we speculate that most, if not all, co-infected patients would benefit from early ART. Hence, ART should probably be started as soon as possible regardless of CD4+ T-cell count and precede the HCV treatment in HIV/HCV co-infected patients.

A more direct implication of our finding is that HCV RNA testing should be done immediately before the initiation of HCV treatment, since a subset of HIV/HCV co-infected patients may have cleared their chronic infection spontaneously.

7.2.7 HCV treatment uptake (paper IV)

The HCV treatment uptake (ever treated) in Sweden was 25% in 2010. This is in line with reports from the EuroSIDA cohort[218]. That cohort has the strengths of prospectively recorded data and huge size, but may actually overestimate the treatment uptake if the centres reporting to EuroSIDA are not representative of all centres in the respective countries.

It may also be in line with the treatment rate in HCV mono-infected patients in Sweden, although firm statistics are lacking [15, 71]. If similar, this would be in contrast with previous reports, where co-infected patients were considered less likely to have initiated HCV treatment than mono-infected patients. This is perhaps also the clinical notion from the perspective of a HCV mono-infection department, where the majority of patients probably will initiate HCV treatment. However, as already pointed out, these patients are already highly selected and often HCV treatment motivated. HIV/HCV co-infected patients attend the HIV outpatient department mainly to receive health care for their HIV infection. Their attendance is mandatory by the Communicable Diseases Act and does not depend on their knowledge of and/or treatment motivation for the HCV infection.

A major limitation of our study is that we do not have data on migrated or deceased HIV/HCV co-infected persons. Since HCV treated persons who achieve SVR have a lower risk of dying from HCV, this introduces a survival bias that may lead to an overestimation of the HCV treatment uptake if compared to prospective cohorts. The relative importance of liver related mortality in the Swedish HIV/HCV co-infected cohort has not been studied in detail.

In PWID, chronic compared with cleared HCV infection was not associated with mortality in a Danish nation-wide cohort study, probably due to competing causes of death in persons with on-going IDU. The mortality, however, was higher among persons with chronic HCV infection with other HIV transmission routes[233]. In the larger EuroSIDA material a difference in liver related mortality between persons with chronic versus cleared HCV was also found. No stratification by HIV transmission route was reported, but the cohort included 23% PWID [126]. Among PWIDs surviving long enough to develop long term complications, the liver related morbidity and mortality is high[234, 235]. In total, 64% of our patients with chronic HCV were infected with HIV via IDU. However, data on active versus former IDU are lacking in InfCare HIV.

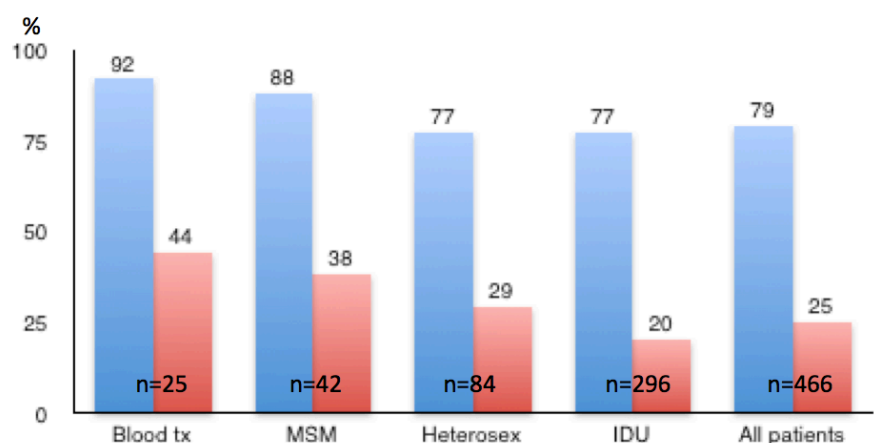
The HIV treatment rate, and rate of undetectable HIV viral load, indicate that many co-infected patients who had not yet initiated HCV treatment were adherent to their HIV treatment. A discrepancy between treatment initiation for HIV and HCV was obvious, since many more had initiated ART than HCV treatment. Certainly, ART is more urgent and all patients will need ART eventually, whereas HCV treatment might be postponed or not needed for all patients if the rate of fibrosis progression is slow.

7.2.8 Factors associated with initiation of HCV treatment (paper IV)

Three factors were found to be associated with a higher HCV treatment rate: HIV transmission route other than IDU, HCV genotype 2 or 3, and on-going ART. (Table 1 and 2, paper IV).

The HCV treatment rate varied significantly according to HIV transmission route. Hence, it was 20% in PWID, 29% in heterosexuals, 38% in MSM and 44% in persons infected via blood transfusion ($p < 0.01$) (Figure 5). MSM and persons who had acquired HIV infection via blood transfusion had increased odds of having initiated HCV treatment compared to PWID: aOR 2.60 (95% CI, 1.13-5.92), $p = 0.02$ and aOR 3.24 (95% CI 1.23-8.59), $p = 0.02$, respectively. When the analysis was repeated using anti-HCV positive persons as the denominator, instead of patients with confirmed chronic HCV, the results were similar (data not shown). The lower HCV treatment uptake among PWIDs compared to patients with other HIV transmission routes was most likely due to many factors, including on-going IDU, rendering the provider less inclined to offer treatment for this group. For comparison, the HIV treatment rate in HIV/HCV co-infected patients according to HIV transmission route is also depicted in Figure 5. There was no significant difference in HIV treatment rate according to transmission route in the HIV/HCV co-infected cohort. A trend, however, was noted for persons who acquired their HIV infection via blood transfusion to have a higher HIV treatment rate than PWID.

Figure 5. HIV (left bar) and HCV treatment uptake in % according to HIV transmission route



Patients with HCV genotype 2 or 3 had increased odds of having initiated HCV treatment when compared with patients with HCV genotype 1, 4, 5 or 6: aOR 2.19 (95% CI. 1.33-3.65), $p=0.0021$. This was probably because of higher SVR rates and the short treatment duration. Patients with on-going ART also had increased odds of having initiated HCV treatment compared with ART naive patients: aOR 3.40 (95% CI 1.42-9.56), $p=0.0045$. This could reflect their assumed better adherence to any treatment.

The recently published EuroSIDA study on HCV treatment uptake also reported higher treatment rates in MSM versus PWID. In their study, HIV-RNA<500 copies/mL and CD4+ T-cell count >350 were associated with higher HCV treatment rates, probably indicating good adherence to ART. A higher proportion of their treated versus non-treated patients had significant fibrosis (>F2). Unfortunately, no data on fibrosis stage are available in the InfCare HIV database. However, in contrast with our findings, they found no association between treatment rates and HCV genotype [218]. This is somewhat surprising, since the decision to initiate HCV treatment is usually based on weighing the individual's chance of SVR, which is higher in HCV genotype 2/3, against the risks of HCV treatment.

In our study, other demographic factors, including gender, ethnicity, university clinic or not were not significantly associated with HCV treatment initiation.

The SVR rate was 31% in HCV genotype 1, 87% in genotype 2, and 70% in genotype 3, which is comparable to those reported from large trials [186, 187, 192].

7.2.9 Barriers to HCV treatment (Paper IV)

The results of the questionnaires are depicted in Figure 6. Stated reasons for not having initiated HCV treatment by a) HIV/HCV co-infected patients (n=114), b) physicians (n=210), c) HIV/HCV co-infected PWIDs only (n=95) and d) physicians corresponding to 6c (n=95) are shown.

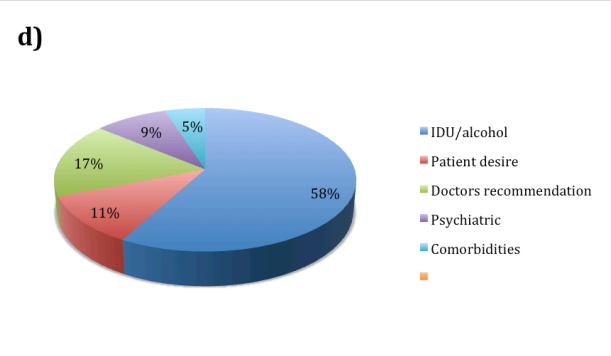
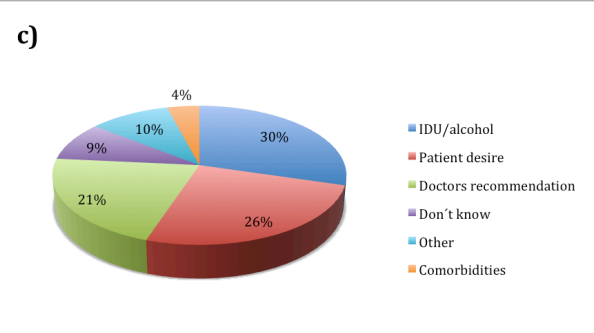
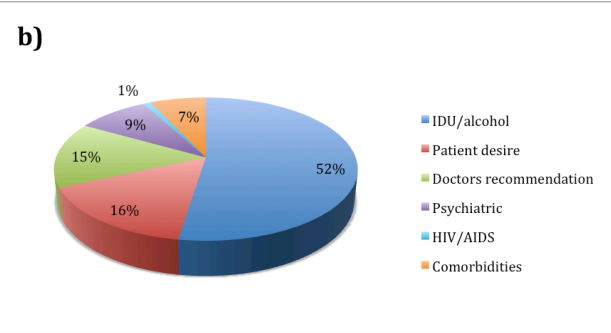
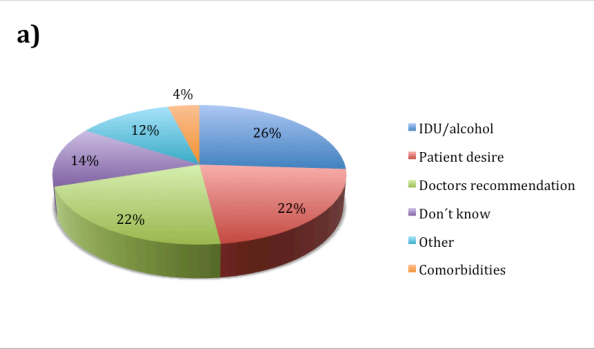
For details on the Stockholm cohort, response rates and a comparison of responders versus non-responders, see paper IV.

According to the questionnaires, the main barrier to HCV treatment in HIV/HCV co-infected patients was IDU/alcohol abuse. Very few had somatic contraindications, and only one patient could not be treated because of AIDS.

In contrast, the results from the HCV mono-infected patients highlighted the importance of the attending doctor's recommendation, stated by 52% of patients and 62% of physicians. Since HCV mono-infected patients are already selected when attending the outpatient department of an infectious diseases clinic, these two populations cannot readily be compared. For example, only one (1.3%) of the mono-infected patients reported recent IDU, vs. 46% of the co-infected patients. We therefore decided not to include the results of HCV mono-infected patients and their physicians in paper IV.

In this well-defined cohort of non-treated patients the response rate in HIV/HCV co-infected patients was low (55%). This is a major limitation of the questionnaire-based sub-study. Non-responders were more likely to be born outside of Sweden, thus poor Swedish might have hindered participation. Also, non-responders were less likely to be on ART, which could indicate poor adherence or a recent HIV diagnosis.

Figure 6. Reasons stated for not having initiated HCV treatment by a) co-infected patients b) physicians c) PWIDs d) physicians corresponding to 6c



The response rate of the physicians was high (88%). Other studies may include only the opinion of the attending physician, or are based on retrospective charts review[67]. According to our results, patients and physician stated reasons are not always in agreement (Figure 6, c-d). Other qualitative research methods, such as focus group discussions, might be considered in order to explore the causes of non-treatment from the patient's perspective.

In a recent publication, system-, practitioner- and patient-level barriers to HCV care in HIV/HCV co-infected patients were reviewed [236]. The dominating cause of non-treatment in our study, on-going or recent drug abuse, can be interpreted as mainly a practitioner-level barrier but also a patient-level barrier, since both physicians and patients seemed to regard this as an absolute contra-indication for HCV treatment. Interestingly, many patients with on-going IDU stated that they would accept HCV treatment if offered. However, other patient-level barriers such as low perceived need for HCV treatment, fear of side effects and unstable housing were also important.

With the new DAAs, the treatment duration will be shorter and side effects much less pronounced. Hence, there may be increased possibilities to treat PWID even though their risk of reinfection must be taken into account. Mathematical modelling suggests that increasing HCV treatment uptake of this group could have an impact on the HCV epidemic and even be cost-effective [65, 66]. The cost of HCV treatment will, however, remain an obstacle for a broader treatment uptake.

8 CONCLUSIONS

The IL28B genotype CC was associated with a higher baseline HCV RNA viral load and a steeper first phase decline during peg-IFN+RBV treatment in HCV genotype 2/3 mono-infected patients. However, this did not translate into higher rates of SVR. The importance of pre-treatment IL28B can thus be questioned in patients with HCV genotype 2/3 planning interferon-based HCV treatment. The small number of HIV/HCV co-infected patients included in the treatment study prevents firm conclusions to be drawn. (Paper II)

IL28B genotype CC was strongly associated with spontaneous clearance of acute HCV in HIV/HCV co-infected patients. Also, three cases of spontaneous clearance of *chronic* HCV after immune reconstitution induced by ART were noted, all with genotype CC. This finding, although rare (about 2-4% of the cohort), underlines the importance of IL28B genotype CC and lends some support to the initiation of ART before HCV treatment in co-infected patients. It also indicates that HCV-RNA testing should be done prior to initiating HCV treatment in HIV/HCV co-infected patients. Among other investigated baseline factors, only a chronic HBV infection was associated with spontaneous clearance of HCV. (Paper III)

The prevalence of anti-HCV in the total HIV infected Swedish cohort was 14% in 2010. This corresponds to a 9-11% prevalence of chronic HCV. This is low by international comparison and lower than previously reported[119]. A probable reason is the distribution of HIV transmission routes in the Swedish HIV infected cohort, where IDU is relatively rare (8%). HCV genotype 1, mostly 1a, dominates. (Paper III and IV)

The cumulative HCV treatment uptake in the HIV/HCV co-infected cohort in Sweden 2010 was 25%. This is in line with treatment uptake reported from Europe, and may also be in line with published data on treatment uptake in HCV mono-infected patients in Sweden[15, 71, 218]. If so, this would contradict previous reports of HIV/HCV co-infected persons having lower access to HCV treatment than HCV mono-infected patients. (Paper IV)

The HCV treatment rate was higher in patients with HCV genotype 2/3, HIV transmission route other than IDU and on-going ART. Interestingly, no differences in HCV treatment rate based on gender, ethnicity or treatment facility were found. A major reason for not having initiated HCV treatment was on-going or recent IDU. (Paper IV)

The higher HIV treatment rate, and rate of undetectable HIV viral load, indicate that many patients who had not initiated HCV treatment were adherent to their HIV treatment. When interferon-free DAA combinations soon will become available, HCV treatment should expand to include more HIV/HCV co-infected patients in order to prevent morbidity and mortality from HCV in this population. (Paper IV)

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Paper I

ORIGINAL ARTICLE

Effect of control selection on sustained viral response rates in genotype 2/3 HCV mono-infected versus HIV/HCV co-infected patients

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Abstract

Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) co-infected patients have lower rates of sustained viral response (SVR) to treatment than HCV mono-infected patients. A rapid viral response (RVR) with negative HCV-RNA at week 4 predicts SVR in most patients. We evaluated the RVR for the prediction of SVR in mono- and co-infected patients, and the effect caused by the selection of mono-infected controls on SVR rates. Co-infected ($n=13$) and mono-infected naïve patients ($n=100$) with HCV genotype 2/3 were treated with 135 µg pegylated interferon α -2a weekly and weight-based ribavirin daily for 24 weeks. For each co-infected patient, 2 mono-infected controls matched for genotype, baseline viral load, and age, were chosen. RVR was achieved in 6/13 (46%) co-infected, 16/26 (62%) matched controls, and 69/98 (70%) mono-infected patients. All co-infected, 14/16 (88%) matched controls, and 66/69 (96%) mono-infected patients with RVR achieved SVR. In total SVR was reached by 10/13 (77%) co-infected patients and 20/26 (77%) matched controls, somewhat lower than the 86/100 (86%) mono-infected patients (not significant). The ability of RVR to predict SVR was high both in co-infected and mono-infected patients with genotypes 2 and 3 chronic HCV, and the results indicate that co-infected patients with well controlled HIV (with CD4 T-cell counts above 300/ μ l) can be offered the same treatment as mono-infected patients.

Introduction

Co-infection with hepatitis C virus (HCV) remains an important risk for morbidity and mortality in human immunodeficiency virus (HIV)-infected individuals [1], and progression to cirrhosis has been found to be faster in co-infected than in mono-infected patients in the pre-highly active antiretroviral therapy (HAART) period [2]. HIV/HCV co-infected patients have lower sustained viral response (SVR) rates to HCV treatment with pegylated interferon (peg-IFN) and ribavirin (RBV) than HCV mono-infected patients [3–8]. The reason for this is not fully understood but is probably multi-factorial, including baseline risk factors such as advanced fibrosis, HCV viral load, and immunosuppression [9]. During treatment, suboptimal dosing, dose reductions, and treatment withdrawals are more frequent in co-infected versus mono-infected patients [7,8,10].

The HCV-RNA decay has been reported to be slower in co-infected than mono-infected patients, although our own recent study contradicts this finding [11,12]. Furthermore, relapses after treatment in co-infected patients have been more frequent than in mono-infected patients, in part due to suboptimal RBV dosing due to intolerance [10].

The optimal dose of peg-IFN and RBV, and the optimal duration of HCV treatment in co-infected patients have not been firmly established [13].

A weight-based dose of RBV (1000/1200 mg daily) has been associated with a higher SVR rate than a flat low dose (800 mg daily) for genotypes 2 and 3, and is recommended in the international HCV treatment guidelines for co-infected patients regardless of genotype [14]. In Sweden a RBV dose of 11 mg/kg body weight combined with peg-IFN α -2a 135 µg or peg-IFN α -2b 1 µg/kg body weight

weekly is recommended according to current HCV treatment guidelines from the Swedish Medical Products Agency and the Swedish Reference Group for Antiviral Therapy, and a treatment duration of 24 weeks for genotypes 2 and 3 [15]. The current Swedish guidelines recommend these lower doses of peg-IFN to mono-infected patients with genotype 2/3 HCV infection [16].

In the pivotal IFN mono-therapy trials in mono-infected patients, these lower Swedish doses of peg-IFN have yielded similar SVR rates as the internationally used doses [17,18]. In agreement with this, the SVR rates were the same in the IDEAL study with a 1 µg/kg and 1.5 µg/kg dose of peg-IFN α -2b when used in combination with RBV for genotype 1 infections in mono-infected patients [19].

The international recommended duration of treatment for co-infected patients is longer for some subgroups of co-infected patients than in the mono-infected. Hence, 48 weeks has been considered standard of care for all genotypes. However, more recent data suggest that 24 weeks of treatment is sufficient in co-infected patients with genotypes 2 and 3 with a rapid viral response (RVR), provided that they lack unfavourable baseline factors for response [14].

In this study, 135 µg peg-IFN α -2a weekly was given for 24 weeks in 100 HCV mono-infected and 13 HIV/HCV co-infected patients. Furthermore, the effect caused by the selection of mono-infected controls on SVR rates and the ability of RVR to predict SVR in mono- and co-infected patients were assessed.

Materials and methods

This study was performed at a single centre, the Karolinska University Hospital Huddinge, Stockholm, Sweden, between September 2003 and October 2006. In total 100 consecutive HCV mono-infected and 13 HIV/HCV co-infected naïve patients with genotype 2 or 3 chronic hepatitis C were studied. Patients with chronic hepatitis B, other concomitant liver diseases, ongoing substance abuse, or a severe psychiatric disease were excluded.

The 13 co-infected patients were well controlled regarding HIV, with stable undetectable HIV RNA viral loads for a minimum of 6 months in the 10 patients on antiretroviral treatment (ART). Three patients had not yet initiated ART, all with well preserved immune status and CD4 T-cell counts above 350/µl. Patients on didanosine or zidovudine were switched to other drugs prior to inclusion.

All patients were monitored by experienced nurses. The patients were encouraged to report side

effects and efforts were made to treat these urgently to maximize adherence to treatment. Referral to a psychiatrist, dermatologist or nutritionist was made when necessary.

In accordance with the Swedish consensus, which allows genotypes 2 and 3 to be treated without histological evaluation, liver biopsies were not performed [16].

The Göteborg University cirrhosis index (GUCI) and aspartate aminotransferase to platelet ratio index (APRI) scores were calculated to approximate the stage of fibrosis [20,21].

Treatment

All patients were treated with peg-IFN α -2a (Pegasys) 135 µg/week and RBV (Copegus) 11 mg/kg/day for 24 weeks. No growth factors were used.

Virological methods

HCV-RNA levels were measured at baseline, on day 2, and at weeks 1, 2, 4, 12, 24 and 48, and analysed using the Roche Taqman Real-Time PCR (detection limit of 15 IU/ml) [22].

HCV genotyping was performed with a line probe assay (Inno-LiPA HCV II, Innogenetics NV, Gent, Belgium) or an in-house method [23,24].

Definition of response

RVR was defined as negative HCV-RNA at week 4. Complete early viral response (cEVR) was defined as negative HCV-RNA at week 12. Partial EVR (pEVR) was defined as at least a 2 log₁₀ drop in HCV-RNA viral load at week 12. In patients who failed to achieve pEVR, treatment was stopped. End of treatment response (ETR) was defined as negative HCV-RNA at the end of treatment (week 24) and SVR as negative HCV-RNA at 6 months after treatment had stopped.

Statistics

The primary end point was virological response in mono-infected, mono-infected matched controls, and in co-infected patients according to whether or not RVR was achieved. HCV-RNA levels of <15 IU/ml were set to 14 IU/ml for statistical analysis.

The Chi-square test or Fisher's exact 2-tailed test was used to test categorical variables. The Wilcoxon rank sum test was used for continuous variables. A *p*-value of <0.05 was considered statistically significant.

For the sub-analysis, each of the 13 HIV/HCV co-infected patients was matched to 2 HCV mono-infected control patients. Matching for genotype, baseline viral load, and age (± 5 y), was done in that order by an investigator blinded to all other baseline demographic factors and treatment outcomes.

In the analysis, the HIV/HCV co-infected patients, their 26 HCV mono-infected controls, and the total 100 HCV mono-infected patients were compared.

Ethics

The study was performed in accordance with the Helsinki declaration and was approved by the local ethics committee. The study subjects provided written informed consent for participation in the study.

Results

In total, 100 consecutive HCV mono- and 13 HIV/HCV co-infected patients were included. Twenty-six of the HCV mono-infected patients were chosen as controls matched for genotype, baseline viral load, and age with the co-infected patients. The baseline demographics are outlined in Table I.

The median CD4 T-cell count of the HIV-positive patients was 433 (range 301–858). The median nadir CD4 T-cell count was 200 (range 80–350)/ μ l prior

to initiation of ART. Among the 10 patients on anti-retroviral therapy, 3 were on a regimen including abacavir.

Baseline risk factors associated with SVR

Individual factors. There were 11 males among the 13 co-infected patients vs 48 among the 100 mono-infected patients and 13 among the 26 matched control-group patients (85% vs 48% and 50%, respectively, $p < 0.05$). The median age in co-infected patients was 51 y vs 45 y in mono-infected patients; the median age was 50 y in the matched controls (matching variable). The body mass index (BMI) was similar in all groups. Almost all patients were Caucasians.

Fibrosis stage. Since liver biopsies were not performed, fibrosis stage was estimated by taking into account the duration of HCV infection and by using the APRI and GUCI fibrosis scores [20,21].

The co-infected patients had a median duration of HCV infection of 31 y vs 23 y in the mono-infected patients (p =not significant (NS)). The matched controls, however, had a similar duration of their chronic hepatitis C infection as the co-infected patients (30 vs 31 y).

The co-infected patients had a median GUCI score of 0.71 vs 0.74 in their matched controls. The mono-infected patients had a median GUCI

Table I. Baseline demographics in all HCV mono-infected, HCV mono-infected matched controls, and HIV/HCV co-infected patients.

	HCV mono-infected		
	All ($n=100$)	Matched controls ($n=26$)	HIV/HCV co-infected ($n=13$)
Age, median (range)	45 (20–69)	50 (30–61)	51 (38–62)
Gender M/F	48/52	13/13	11/2
Race, White/Asian/Other	94/2/4	25/0/1	11/1/1
Weight kg, median (range)	75 (48–109)	77 (50–103)	72 (57–96)
BMI kg/m ² , median (range)	25 (18–36)	25 (19–35)	24 (18–29)
Transmission			
IV drug abuse	68	21	12
transfusion	8	2	1
sporadic	24	3	0
Duration of HCV infection y, median (range)	23 (2–52)	30 (2–43)	31 (19–36)
HCV-RNA IU/ml, ^a median (range)	4.7 M (0.0015–69 M)	2.7 M (0.43–29.5 M)	3.1 M (0.89–22.7 M)
>800,000 IU/ml %	73 (73/100)	88 (23/26)	100 (13/13)
HCV genotype 2/3	41/59	8/18	4/9
ALT μ kat/l, median (range) (upper limit of normal: 0.70)	1.3 (0.3–6.2)	2.0 (0.5–5.9)	1.3 (0.5–4.3)
APRI score, median (range)	0.61 (0.19–5.05)	0.65 (0.21–4.68)	0.57 (0.3–5.39)
GUCI score, median (range)	0.58 (0.18–6.08)	0.74 (0.18–6.08)	0.71 (0.33–6.48)
CD4+ T-cells/ μ l blood, median (range)	–	–	433 (301–858)
On antiretroviral therapy (including abacavir)	–	–	10/13 (3/13)

HCV, hepatitis C virus; HIV, human immunodeficiency virus; M, male; F, female; BMI, body mass index; IV, intravenous; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; GUCI, Göteborg University cirrhosis index.

^aAssay: Roche Taqman Real-Time PCR.

score of 0.58, not significantly different from the co-infected. All groups had similar APRI scores.

HCV viral factors. Genotype 3 was seen in 69% of the co-infected patients and the control group (matching variable) vs in 59% of the mono-infected patients ($p=NS$).

The baseline median HCV-RNA was 3,100,000 IU/ml in the co-infected, 2,700,000 IU/ml in the control group (matching variable), and 4,700,000 IU/ml in the mono-infected group. The co-infected group thus had a lower median HCV-RNA level than the mono-infected group, but the difference was not statistically significant.

The proportion of co-infected patients with a baseline high viral load (HVL, defined as $>800,000$ IU/ml), however, was 100% vs 73% in the co- vs mono-infected group ($p < 0.05$). In the matched control group, 88% had an HVL at baseline vs 100% in the co-infected patients ($p=NS$).

Virological response

The early hepatitis C viral kinetics (day 1–week 12) in the co-infected patients and their matched controls has been reported previously and did not differ significantly between the groups [11]. The overall treatment outcome in the 100 mono-infected patients has also been reported previously [25].

The treatment outcome (percentage of patients achieving RVR, cEVR, and SVR) is outlined in Table II.

Table II Treatment outcome in all HCV mono-infected, HCV mono-infected matched controls, and HIV/HCV co-infected patients (Taqman PCR, detection limit=15 IU/ml).

	HCV mono-infected		
	All ($n=100$)	Matched controls ($n=26$)	HIV/HCV co-infected ($n=13$)
Week 4, RVR n (%)	69/98 (70) ^a	16/26 (62)	6/13 (46) ^a
Week 12, cEVR (%)	96/100 (96)	26/26 (100)	12/13 (92)
Week 12, pEVR (%)	0/100 (0)	0/26 (0)	1/13 (8) ^c
Week 24, ETR (%)	96/100 (96)	25/26 (96) ^b	13/13 (100) ^c
Week 48, SVR (%)	86/100 (86)	20/26 (77)	10/13 (77) ^c
Relapse (%)	10/96 (10)	5/25 (20) ^b	3/13 (23) ^c

HCV, hepatitis C virus; HIV, human immunodeficiency virus; RVR, rapid viral response; cEVR, complete early viral response; pEVR, partial early viral response; ETR, end of treatment response; SVR, sustained viral response.

^a p -Value RVR co-infected vs mono-infected 0.09; 2 patients missing due to lack of sera.

^bOne mono-infected patient discontinued therapy at week 14 due to adverse events and did not achieve either ETR or SVR.

^cOne patient with pEVR and ETR had prolonged treatment to 48 weeks due to violation of protocol. He finally achieved SVR and is included in the SVR rate given.

RVR. RVR was achieved by 6/13 (46%) co-infected vs 69/98 (70%) mono-infected patients ($p=0.09$). In total 16/26 (62%) of the mono-infected matched controls achieved RVR vs 6/13 (46%) co-infected patients ($p=NS$).

EVR. In total 96/100 (96%) of all mono-infected and 12/13 (92%) of the co-infected patients achieved cEVR, with no significant difference between the groups. Among the 4 mono-infected patients with only pEVR, 1 reached SVR. The only co-infected patient with pEVR was given a prolonged treatment (48 weeks) due to violation of the protocol, and finally achieved SVR.

SVR. The SVR rate among co-infected patients was 10/13 (77%) vs 86/100 (86%) in the total mono-infected group, a non-significant difference. The SVR rate in the co-infected patients was identical to that in the matched controls (20/26; 77%).

In patients who reached RVR, SVR rates were 88–100%, regardless of HIV status. Four out of 7 co-infected patients and 19/29 (66%) mono-infected patients lacking RVR still achieved SVR (Figure 1).

In mono-infected patients with genotype 2, 35/41 (85%) achieved SVR vs 51/59 (86%) in genotype 3 infected patients (data not shown). All 4 co-infected patients with genotype 2 achieved SVR; however, 1 was treated for 48 weeks due to violation of protocol. Six out of 9 co-infected patients with genotype 3 reached SVR (data not shown).

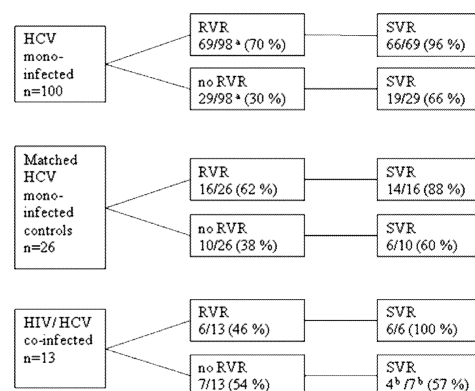


Figure 1. Sustained viral response (SVR) depending on the achievement of rapid viral response (RVR) or not in all HCV mono-infected, HCV mono-infected matched controls, and HIV/HCV co-infected. ^aMissing week 4 data in 2 patients due to lack of sera. ^bOne HIV/HCV co-infected patient with pEVR, ETR and SVR was treated 48 weeks due to violation of the protocol. He is included in the SVR calculation.

Relapse was seen in 3/13 (23%) co-infected (all 3 failed to achieve RVR) and 10/96 (10%) mono-infected patients.

All 3 co-infected patients with relapse had low nadir CD4 T-cell counts prior to initiation of ART (80, 84 and 105/ μ l, respectively). The corresponding figures for co-infected patients who achieved SVR were in the range of 87–350/ μ l, and only 3/10 had counts below 200/ μ l.

Ribavirin dosing. Co-infected patients, their matched controls, and the total mono-infected patient group were given a similar weight-adjusted RBV dose at baseline (median 11.1 vs 11.4 and 11.4 mg/kg/day, respectively; p =NS).

Dose reductions of peg-IFN and/or RBV were done in 8/13 (62%) of co-infected patients vs in 32/100 (32%) of the mono-infected patients and 8/26 (31%) of the matched controls ($p < 0.05$).

Adverse events. All patients except 3 completed a full 24-week treatment course. One mono-infected patient in the control group stopped therapy at week 14 due to a severe generalized toxicodermatitis and did not achieve SVR. In 2 mono-infected patients (non-controls) who failed to achieve cEVR or pEVR, treatment was stopped week 12. A detailed adverse report in the mono-infected patients has been reported previously [25].

Discussion

HIV/HCV co-infected versus all HCV mono-infected controls

SVR rates in our study did not differ significantly between the co-infected (10/13; 77%) and mono-infected patients (86/100; 86%). However, due to the small sample size of co-infected patients, our power to detect such a difference was low.

The co-infected group had significantly more males and more frequent dose reductions than the mono-infected group, both factors known to be associated with a lower SVR [10]. They also had a tendency towards a higher number of genotype 3 infections, higher age, longer duration of the HCV infection, and a higher GUCI score (indicating a more advanced fibrosis stage), although not statistically different. The aforementioned factors all generally result in lower SVR rates [26]. A higher baseline viral load is generally correlated to a lower SVR rate than a lower load [4]. In our study, the median baseline HCV-RNA viral load was actually lower in the co-infected than in the total mono-infected group. Usually co-infected

patients tend to have higher baseline viral loads than mono-infected patients [9]. The proportion of patients with HVL, however, was significantly higher among the co-infected patients, affording the co-infected an unfavourable baseline factor. Hence, the proportion of co-infected patients achieving RVR was slightly lower than in the total mono-infected group (6/13, 46% vs 69/98, 70%); however this difference was not significant.

HIV/HCV co-infected versus matched HCV mono-infected controls

The differences in baseline factors were mainly eliminated by matching with mono-infected control patients. The co-infected patients, however, included more males and experienced more dose reductions than the matched control group. The median HCV-RNA viral load in the matched controls was similar to that in the co-infected group. The proportion of HVL in the co-infected patients, however, was 100% vs 88% in the mono-infected controls. Despite this the SVR rate achieved in the co-infected group was identical to that in the matched controls (77%). Furthermore, the early viral kinetics did not differ between co-infected patients and their matched controls, as reported earlier [11].

Our findings thus suggest that well controlled HIV patients do not have substantially lower SVR rates than mono-infected patients when treated according to the operating Swedish guidelines for mono-infected patients.

The 77% SVR rate noted in the co-infected patients in this study was well in line with that of co-infected genotype 2/3 patients treated for 24 weeks ($n=96$) in the PRESCO trial (67%), although a lower peg-IFN dose (135 vs 180 μ g/week) and a lower RBV dose (median 11.1 vs 14.9 mg/kg/day) was used in the present study [7].

Tolerability of treatment was high in the present study, thus only 1 of the 100 mono-infected patients withdrew prematurely from therapy due to adverse events and all co-infected patients completed treatment.

The majority of patients who achieved RVR finally accomplished SVR regardless of HIV status. This is in accordance with the findings of others [9]. On the other hand, in patients lacking RVR, co-infection was associated with a slightly but not significantly lower SVR rate than in mono-infection (57% vs 66%, p =NS).

This study supports previous reports showing that co-infected patients with genotype 2 or 3 achieving RVR can safely be treated for 24 weeks only. This has already been suggested in the European AIDS Clinical Society (EACS) guidelines of 2008, pro-

vided that patients do not have advanced fibrosis. A further prerequisite is a baseline viral load <400,000 IU/ml [14].

For patients with genotype 2 or 3 who do not achieve RVR, the optimal duration of treatment is not known. In the co-infected, 48 weeks of treatment is recommended in the EACS guidelines vs 24 weeks in the Swedish guidelines.

To conclude, the 135 µg peg-IFN α-2a dose used according to Swedish guidelines combined with weight-based RBV dosing offered a high SVR and adherence rate. This, however, needs to be confirmed in a prospective larger randomized trial. In mono-infected patients, SVR rates did not change substantially by the selection of matched controls compared to that in the total group. Finally, the results of this small study indicate that co-infected patients with well controlled HIV, with CD4 T-cell counts above 300/µl, can be offered the same treatment as mono-infected patients.

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Paper II

HCV RNA decline in chronic HCV genotype 2 and 3 during standard of care treatment according to IL28B polymorphism

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SUMMARY. The IL28 gene is highly associated with sustained viral response (SVR) in patients infected with genotype 1 after standard of care (SOC) treatment with peg-IFN and ribavirin. It is also associated with a steeper first phase HCV RNA decline during treatment. In genotype 2 and 3 infections, these correlations are less obvious. We studied the IL28B association to rapid viral response (RVR), SVR, first and second phase HCV RNA decline during treatment in 100 HCV mono-infected and 13 HCV/HIV co-infected patients. We found a significantly higher mean baseline HCV RNA level in IL28B SNP CC than non-CC mono-infected patients, 6.99 vs 6.30 log₁₀ IU/mL ($P = 0.02$), and a significantly larger median 1st phase decline in patients with CC than non-CC genotype, 2.03 vs 1.37 log₁₀ IU/mL, respectively. The overall SVR rate in HCV mono-infected patients was 87% vs 77% in HCV/HIV

co-infected patients, with no correlation to IL28B SNP. In mono-infected patients with RVR, the SVR rate was high and independent of IL28B genotype. In mono-infected patients who failed to achieve RVR who had IL28B CC and non-CC genotype, 64% and 67% achieved SVR, respectively. In genotype 2 and 3 infected patients, the 1st phase HCV RNA decline was steeper in patients with IL28B CC vs non-CC genotype during SOC treatment. This did not translate into a higher frequency of RVR or SVR. Hence, the clinical relevance of pretreatment analysis of IL28B polymorphisms in genotype 2 and 3 infected patients can be questioned in patients with expected high SVR rate.

Keywords: HCV RNA, Hepatitis C virus, IL28B, peg-IFN, ribavirin.

INTRODUCTION

Hepatitis C (HCV) is a major cause of morbidity and mortality in HCV mono-infected and HCV/HIV co-infected patients [1,2]. In those who achieve sustained viral response (SVR) with standard of care treatment (SOC) with peg-interferon (peg-IFN) and ribavirin (RBV), the long-term outcome is improved [3]. The current HCV treatment, however, is associated with frequent side effects, a high cost, and the access to treatment is variable, and only a minority of HCV/HIV co-infected patients are offered SOC treatment [4]. To promote treatment further, identifi-

cation of the patient who is most likely to respond is important.

Several important factors predictive of SOC treatment response such as age, fibrosis stage, genotype and viral load are well known [5,6].

Recently, the single-nucleotide polymorphism (SNP), rs12979860, near the IL28B gene (coding for IFN-lambda-3) has shown a strong association with SVR after SOC treatment both in HCV mono-infected and HCV/HIV co-infected genotype 1 patients [7–9]. The IL28B SNP also correlates with a faster 1st and 2nd phase decline of the viral load during SOC treatment in genotype 1 infections and a more frequent rapid viral response (RVR) rate [10]. In genotype 2 and 3 infected patients, this association seems to be less pronounced [11–14]. Hence, IL28B correlation to RVR and non-RVR has yielded divergent results [11–13,15]. In this study, the correlation of the IL28B SNP and HCV RNA decline during the 1st and 2nd phase of SOC treatment in genotypes 2 and 3 infected HCV patients was studied, in both HCV mono- and HCV/HIV co-infected patients. We also correlated the IL28B gene polymorphism to RVR and SVR in these patients.

Abbreviations: APRI, ASAT to platelet ratio; ART, antiretroviral treatment; cEVR, Complete early viral response; ETR, end of treatment response; EVR, early viral response; GUCI, Gothenburg University Cirrhosis Index; HCV, hepatitis C virus; peg-IFN, peg-interferon; RBV, ribavirin; RVR, rapid viral response; SNP, single nucleotide polymorphism; SOC, standard of care; SVR, sustained viral response.

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MATERIAL AND METHODS

Patients were recruited at the Karolinska University Hospital Huddinge, Stockholm, Sweden, as described earlier [16–18]. In total, 100 consecutive HCV mono-infected and 13 HCV/HIV co-infected naive genotype 2 or 3 patients were studied. Patients with chronic hepatitis B, other concomitant liver diseases, ongoing substance abuse or severe psychiatric disease were excluded.

Ten of 13 co-infected patients had had undetectable HIV RNA levels on antiretroviral treatment (ART) for a minimum of 6 months prior to inclusion. Three co-infected patients had not yet initiated ART, all with CD4 T-cell counts above 350.

Liver biopsies are not performed in genotype 2 and 3 infected patients according to the Swedish consensus guidelines, which allows treatment without histological evaluation.

Gothenburg University Cirrhosis Index (GUCI) and ASAT to platelet ratio (APRI) scores were used to have an estimate if advanced or nonadvanced stage of fibrosis was at hand.

Hepatitis C treatment

All patients were treated with SOC consisting of peg-IFN alpha-2a (Pegasys, Welwyn Garden City, UK) 135 µg/week and RBV (Copegus, Roche AB, Stockholm, Sweden) 11 mg/kg/day during 24 weeks according to Swedish consensus guidelines [19].

Ninety-seven percentage of mono-infected and all co-infected patients completed a full 24 weeks treatment course.

Virological methods

Hepatitis C virus-RNA levels were measured at baseline, day 2, week 1, 2, 4, 12, 24 and week 48 and analysed by Roche Taqman Real-Time PCR (detection limit of 15 IU/mL) [20].

Hepatitis C virus genotyping was performed with a line probe assay (Inno-LiPA HCV II, Innogenetics NV, Gent, Belgium) or an in-house method [21].

rs12979860 SNP genotyping

Genotyping for the IL28B rs12979860 SNP was performed with an in-house Taqman-based allele-specific PCR method on DNA extracted from frozen EDTA plasma. The SNP was defined as rs12979860 genotype CC, CT or TT.

The following primers and probes were used: rs12979860 forward, GCCTGTCTGTAAGTGAACCA; rs12979860 reverse, GCGCGAGTGAATTCAAC; probe C, FAM-CTGGTTACGCCCTTC-MGB; probe T, VIC-TGGTTCGCGCCTTC-MGB.

HCV RNA decline

The viral decline was analysed during the 1st phase, defined as the decline in HCV RNA levels from baseline to treatment

day 2, and 2nd phase as the decline in HCV RNA levels from HCV RNA day 2 to treatment week 2 or 4. RVR was defined as negative (<15 IU/mL) HCV RNA at week 4. Complete early viral response (cEVR) was defined as negative HCV RNA week 12. Partial EVR (pEVR) was defined as at least a 2 log₁₀ drop in HCV-RNA levels from baseline to treatment week 12. Patients who failed to achieve pEVR stopped treatment. End of treatment response (ETR) was defined as negative HCV RNA at treatment cessation (week 24) and SVR also at 6-month follow-up after treatment.

Statistics

The primary end point was to study whether the 1st and 2nd phase HCV RNA decline in mono- and in co-infected patients varied according to IL28B genotype CC or CT/TT (non-CC).

Furthermore, the absolute HCV-RNA values at baseline, day 2, week 1, 2 and 4 were analysed according to CC or CT/TT (non-CC) genotype.

Hepatitis C virus RNA levels of < 15 IU/mL were set to 14 IU/mL for statistical analysis.

The chi-square test or Fisher's exact two-tailed test was used to test categorical variables.

The Wilcoxon rank sum test was used for continuous variables. A *P*-value <0.05 was judged to be statistically significant.

Ethics

The study was performed in accordance with the Helsinki declaration and was approved by The Local Ethics committee. The study persons gave their informed written consent.

RESULTS

Demographics

Mono-infected: A total of 100 consecutive HCV mono-infected patients treated with SOC were included. A majority (94%) of our patients were Caucasians. There were 48 males (48%) with a median age of 45 years. HCV genotype 3 was seen in 59%. Overall the baseline median HCV RNA was 6.67 log₁₀ IU/mL.

An estimate of the fibrosis stage (advanced vs nonadvanced) was made based on the duration of the HCV infection, and by the APRI and GUCI fibrosis scores [22,23].

The median disease duration was 23 years. The median GUCI score was 0.58 in mono-infected patients. The APRI scores were not significantly different in any patient group.

Co-infected patients: A total of 13 HCV/HIV co-infected with a mean age of 51 years were treated and included, whereof 85% were Caucasians. Of these 11 (85%) were males. HCV genotype 3 was found in 69%. Overall the

baseline median HCV RNA was 6.49 log₁₀ IU/mL. The median disease duration was 31 years, and not different from that in mono-infected patients ($P = ns$). The median GUCI score was 0.71.

The baseline demographics according to IL28B genotype CC vs non-CC in HCV mono- and HCV/HIV co-infected patients are given in Table 1.

The baseline median HCV RNA in HCV mono-infected patients with the IL28B CC genotype was significantly higher than in patients with the non-CC group: 9 750 000 IU/mL (6.99 log₁₀ IU/mL) vs 2 000 000 IU/mL (6.33 log₁₀ IU/mL), respectively, $P = 0.02$.

Prevalence of rs12979860 genotypes in the study population according to HCV genotype

In the 100 mono-infected patients, the overall prevalence of the rs12979860 genotype CC, CT and TT was 44%, 52% and 4%, respectively. The corresponding figures in genotype 2 infected patients were 43%, 55% and 3%, and 46%, 49% and 5% in genotype 3 infected, respectively. In the co-infected patients, the corresponding figures were 46%, 46% and 8%, respectively.

Overall HCV RNA 1st and 2nd phase decline according to rs12979860 genotype

The overall HCV RNA decline during the 1st and 2nd phase decline according to the rs12979860 IL28B genotype in HCV mono- and HCV/HIV co-infected patients is given in Table 2 and Fig. 1. This difference was not seen in the co-infected patients because of the small number of patients included (Table 2).

The absolute median HCV-RNA levels (log₁₀ IU/mL) at different time points during the first 4 weeks of SOC treatment according to IL28B genotype are depicted for HCV mono-infected patients in Fig. 2a, and for the 13 HCV/HIV co-infected patients, the individual HCV-RNA levels according to IL28B genotype CC and non-CC patients are given in Fig. 2b.

1st phase HCV RNA decline during SOC treatment

In the HCV mono-infected patients, the median decline in HCV RNA level from baseline to day 2 (1st phase decline) was significantly higher for patients with the CC than non-CC genotype, 2.03 vs 1.37 log₁₀ IU/mL, respectively ($P = 0.01$, Figs 1 & 2a).

Table 1 Baseline demographics in HCV genotype 2 or 3 mono- and HCV/HIV co-infected patients according to IL28B CC or non-CC genotype

	HCV mono-infected		HCV/HIV co-infected	
	CC ($n = 44$)	Non-CC ($n = 55$)	CC ($n = 6$)	Non-CC ($n = 7$)
Age, median (range)	44 (22–63)	45 (20–69)	50 (38–56)	51 (40–62)
Gender M/F	19/25	28/27	5/1	6/1
Race White/Asian/Other	43/0/1	51/0/4	5/1/0	6/0/1
Weight kg, median (range)	75 (48–105)	75 (50–109)	72 (57–90)	75 (65–96)
BMI kg/m ² , median (range)	26 (18–36)	24 (30–35)	22 (18–26)	25 (21–29)
Transmission				
i.v. drug abuse	30	37	6	6
Transfusion	4	4	0	1
Sporadic	10	14	0	0
Duration of HCV infection years, median (range)	24 (4–52)	22 (2–43)	29 (19–36)	32 (29–35)
HCV RNA IU/mL ^a , median (range)	9.75 M (0.0015–69 M)	2.0 M (0.0071–50 M)	1.55 M (0.89–22.7 M)	6.2 M (0.90–10.2 M)
HCV genotype 2/3	17/27	23/32	2/4	2/5
ALT ukat/L, median (range) (Upper limit of normal: 0.70)	1.6 (0.3–6.2)	1.1 (0.3–3.8)	1.5 (0.7–4.3)	1.0 (0.4–1.7)
APRI score, median (range)	0.73 (0.26–3.29)	0.58 (0.19–5.05)	1.17 (0.46–5.40)	0.48 (0.30–2.24)
GUCI score, median (range)	0.78 (0.26–3.95)	0.49 (0.18–6.08)	2.04 (0.50–6.48)	0.63 (0.33–2.46)
CD4 ⁺ T-cells/ μ L blood, median (range)	–	–	367 (301–498)	743 (310–858)
On antiretroviral therapy (including abacavir)	–	–	4/6 (1/6)	6/7 (2/7)

^aAssay: Roche Taqman Real-Time PCR.

Table 2 Mean baseline HCV RNA and HCV RNA decline (\log_{10} IU/mL) during the 1st and 2nd phase decline in mono- and HCV/HIV co-infected patients with genotype 2 and 3 according to IL28B CC or non-CC genotype

	Mono-infected, <i>n</i> = 99	<i>P</i> -value	Co-infected, <i>n</i> = 13	<i>P</i> -value
HCV RNA baseline				
CC	6.99	0.02	6.20	0.95
Non-CC	6.30		6.79	
1st phase decline				
CC	2.03	0.01	1.49	0.78
Non-CC	1.37		1.30	
2nd phase (d2-w2)				
CC	2.00	0.11	2.50	0.20
Non-CC	1.58		0.94	
2nd phase (d2-w4)				
CC	3.60	0.14	3.10	0.15
Non-CC	3.22		2.62	

The corresponding figures in the co-infected patients were 1.49 and 1.30 \log_{10} IU/mL, respectively, for patients with the CC and non-CC ($P = 0.78$, ns, Figs 1 & 2b).

2nd phase HCV RNA decline during SOC treatment

In the HCV mono-infected patients, the median decline in HCV RNA level from day 2 to week 2 reflecting the 2nd phase decline was 2.00 and 1.58 \log_{10} IU/mL in patients

with the CC and CT/TT genotype, respectively ($P = 0.11$, ns, Figs 1 & 2a).

In the co-infected patients, the corresponding figures were 2.50 vs 0.94 \log_{10} IU/mL ($P = 0.20$, ns, Figs 1 & 2b).

In the HCV mono-infected patients, the median decline in HCV RNA level from day 2 to week 4, also reflecting the 2nd phase decline, was 3.60 and 3.22 \log_{10} IU/mL, respectively, in patients with the IL28B CC and non-CC genotype ($P = 0.14$, ns).

In the HCV/HIV co-infected patients, the corresponding figures were 3.10 vs 2.62 \log_{10} IU/mL ($P = 0.15$, ns). The individual HCV RNA levels during the first 4 weeks treatment in the HCV/HIV co-infected patients are given in Fig. 2 panel c and d according to IL28B genotype CC vs non-CC.

RVR and SVR during SOC treatment according to IL28B genotype

RVR and SVR in mono-infected patients according to IL28B genotype are shown in Fig. 3.

The proportion of patients with RVR in mono-infected patients with CC was 74% vs 67% in patients with non-CC SNP ($P = 0.41$, ns). In HCV/HIV co-infected patients, the corresponding figures were 67% vs 29% ($P = 0.17$, ns).

In mono-infected patients achieving RVR, the proportion of patients with SVR was high (97%) and independent of rs12979860 genotype.

In mono-infected patients failing to achieve RVR, 64% and 67% with IL28B CC and CT/TT genotype, respectively, achieved SVR (data not shown).

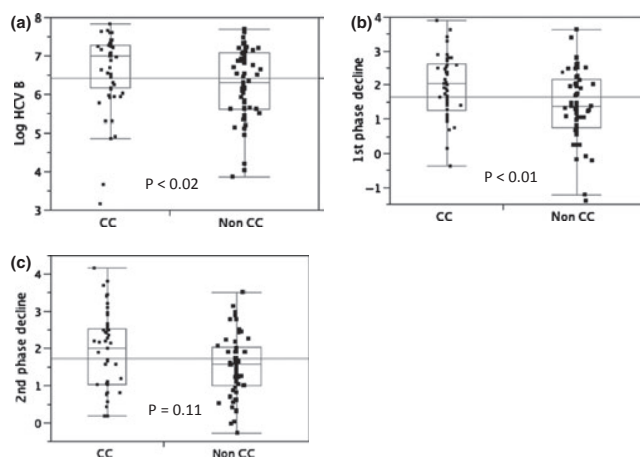


Fig. 1 Boxplot of HCV RNA levels at baseline and during the 1st and 2nd phase viral decline during SOC treatment according to IL28B SNP CC or non-CC in HCV genotype 2 and 3 mono-infected patients ($n = 99$). The lower and upper bar in the box depicts the 25th and 75th percentile, respectively. SOC, standard of care treatment consisting of peg-IFN and ribavirin. Panel (a) baseline HCV RNA levels (\log_{10}). Panel (b) \log_{10} HCV RNA decline from baseline to day 2 (reflecting the 1st phase decline). Panel (c) \log_{10} HCV RNA decline from day 2 to week 2 (reflecting the 2nd phase decline).

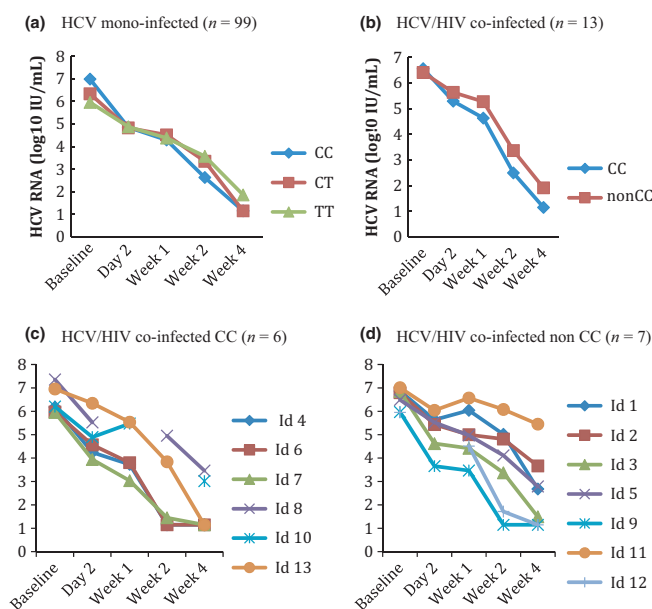


Fig. 2 Median HCV RNA (\log_{10} IU/mL) levels at baseline, day 2, week 1, 2 and 4 during SOC treatment according to IL28B genotype in HCV genotype 2 and 3 mono-infected and HCV/HIV co-infected patients. SOC, standard of care treatment consisting of peg-IFN and ribavirin. Panel (a) Mean HCV RNA (\log_{10} IU/mL) levels in HCV mono-infected patients. Panel (b) Mean HCV RNA (\log_{10} IU/mL) levels in HCV/HIV co-infected patients. Panel (c) Individual HCV RNA (\log_{10} IU/mL) levels in HCV/HIV co-infected patients with IL28B CC genotype. Panel (d) Individual HCV RNA (\log_{10} IU/mL) levels in HCV/HIV co-infected patients with IL28B non-CC genotype.

The overall SVR rate in HCV mono-infected patients was 86/99 (87%) vs 10/13 (77%) in HCV/HIV co-infected patients and did not differ according to IL28B SNP. Furthermore, separate analyses of the RVR and SVR rates in genotype 2 and 3 infected patients did not reveal any significant difference according to IL28B genotype (Fig. 3).

The influence of baseline factors such as disease duration, gender, weight, baseline HCV RNA viral load, APRI score as surrogate marker for fibrosis, genotype 2 vs 3 and IL28B SNP CC vs non-CC on RVR and SVR was analysed. In the univariate analyses, no baseline factor was significantly correlated to SVR and only disease duration to RVR ($P = 0.04$). Baseline HCV RNA was nearly significantly associated with RVR ($P = 0.057$). As no baseline factor was significantly associated with SVR, no multivariate analysis was performed.

DISCUSSION

In this study, we found a significantly steeper 1st phase HCV RNA decline during standard of care treatment in genotype 2 and 3 mono-infected HCV patients with CC vs

CT/TT rs12979860 genotype as recently noted by others in genotype 3 infected [14] and genotype 2 and 3 infected patients [11]. A trend towards a more steep 2nd phase decline was also seen in patients with the CC rs12979860 vs non-CC genotype; however, this was not significant. Concerning RVR, however, we found no significant association with the rs12979860 genotype. This possibly reflects the high response rate seen with SOC treatment in genotype 2 and 3 infected patients. Findings in other studies with larger number of genotype 2 or 3 infected patients have yielded conflicting results on the influence of IL28B genotype on RVR rate [12–15,24]. RVR, however, had a high probability of achieving SVR [18].

In our co-infected group, there was no significant difference in HCV RNA kinetics or in the frequency of RVR and SVR. The power to detect such differences, however, was low because of the small number of co-infected persons included.

In an Italian study of mainly genotype 2 infected patients, Mangia *et al.* found a significant association with SVR and the IL28B genotype CC in patients lacking RVR but not in patients with RVR [12]. In this study, a trend

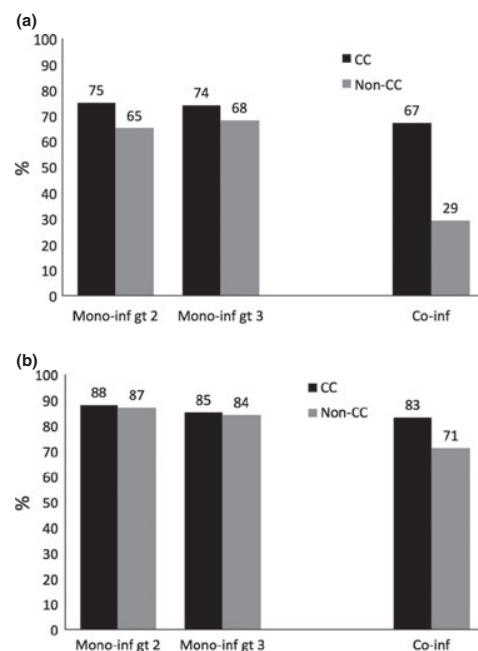


Fig. 3 Rapid viral response and SVR during SOC treatment according to IL28B CC and non-CC genotype in HCV mono- and HCV/HIV co-infected patients with genotype 2 or 3. CC columns in black and non-CC columns in grey. RVR, rapid viral response; SVR, sustained viral response; SOC, standard of care treatment consisting of peg-IFN and ribavirin. Panel (a) RVR according to IL28B CC and non-CC genotype in HCV mono- and HCV/HIV co-infected patients with genotype 2 or 3. Panel (b) SVR according to IL28B CC and non-CC genotype in HCV mono- and HCV/HIV co-infected patients with genotype 2 or 3.

towards higher baseline HCV RNA viral load was reported in patients with the CC genotype. In the study of Mangia *et al.*, however, few patients with HCV infection caused by

genotype 3 were included. In the study by Moghaddam *et al.* on the contrary, the majority of patients included were infected with genotype 3 and these authors found a significant association with RVR but not SVR in patients with the CC genotype [15].

In a German study, Sarrazin *et al.* reported an association with SVR but not with RVR in IL28B CC genotype patients with both genotype 2 and 3 [13]. In this study, a significant association with higher baseline HCV-RNA viral load was seen in CC patients with genotype 2 and 3 as has previously been described in genotype 1 infected patients [7,10,11].

In our study, we found a significant steeper 1st phase HCV RNA decline in genotype 2 and 3 infected patients with the CC genotype than in patients with the non-CC genotypes in line with what has recently been shown in another Swedish study [11]. We did, however, not find a higher RVR or SVR rate. Our findings were probably caused by the high response rate seen in our genotype 2 and 3 patients, meaning that small differences cannot be discerned. For this, larger patient numbers are needed.

In another recent Scandinavian study mainly consisting of genotype 3 patients, however, the IL28B CC genotype was associated with RVR but not with SVR [15]. We found that the SVR rate among our patients was similar in genotype 2 and 3 infected patients as was the RVR rate [18]. Thus, RVR was seen in 70% of our patients and SVR in more than 95% of those achieving RVR, irrespective of whether they were infected with genotype 2 or 3 [18].

To conclude, we found that the 1st phase HCV RNA decline was steeper in patients with IL28B CC vs in non-CC genotype during standard of care treatment in genotype 2 and 3 infected patients, but that this did not translate into a higher frequency of RVR or SVR. Small differences might be seen in studies including large patient numbers, but the clinical relevance of pretreatment analysis of IL28B genotypes in genotype 2 and 3 infected patients can be questioned because these patients are expected to have high SVR rates anyway.

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Paper III

Spontaneous clearance of chronic HCV in HIV/HCV co-infected patients with ART induced immune reconstitution

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List of abbreviations:

HCV: hepatitis C virus

HCC: hepatocellular carcinoma

PWID: people who inject drugs

peg-IFN : pegylated interferon

ART: antiretroviral treatment

OR: odds ratio

aOR: adjusted odds ratio

CI: confidence intervals

MSM: men who have sex with men

Abstract

Background and aims

The IL28B genotype correlates with spontaneous clearance of acute hepatitis C virus (HCV), and with sustained viral response to peg-IFN plus ribavirin treatment.

Here, we report on three cases of spontaneous resolution of a *chronic* HCV infection in HIV/HCV co-infected patients after ART induced immune reconstitution. Furthermore, the correlation of IL28B genotype and baseline demographical factors with spontaneous HCV clearance was analyzed in the total Swedish HIV/HCV co-infected cohort, from which the three cases were derived.

Methods

Among all 5315 Swedish HIV infected patients in September 2010, demographical data from 652 HIV/HCV co-infected patients were extracted. Spontaneous clearance of HCV was defined as a positive anti-HCV test but a negative HCV-RNA test in the absence of HCV treatment. A subset of 263 anti-HCV positive patients was tested for IL28B genotype.

Results

Three patients with *chronic* HCV had spontaneously cleared their HCV infection after ART, all with IL28B genotype CC. These cases make up 2-4% of all our HIV/HCV co-infected patients on ART with genotype CC.

The IL28B genotype CC was also found to have a strong correlation with a spontaneous HCV clearance. Thus, 36% of patients with the CC versus 13% with the non-CC genotype had cleared their acute HCV infection, $p=0.0004$, OR 5.02 (2.11-12.92). Among other baseline factors, only a chronic hepatitis B infection was associated with spontaneous clearance of HCV.

In total 14% of Swedish HIV positive patients were anti-HCV positive whereof 79% also were HCV RNA positive.

Conclusions

We found that immune reconstitution with ART occasionally can induce HCV clearance in HIV/HCV co-infected patients with established *chronic* HCV in patients with IL28B genotype CC. Spontaneous clearance of a HCV infection is strongly correlated with IL28B genotype CC.

Key words

HIV/HCV co-infection, spontaneous HCV clearance, IL28B genotype, immune reconstitution, ART

Introduction

Chronic hepatitis C virus (HCV) infection affects more than 170 million persons worldwide and put them at risk of developing end stage liver disease and hepatocellular carcinoma (HCC) [1]. Acute HCV in HCV mono-infected individuals is spontaneously cleared in 15-40%, but the majority will go on to a chronic infection [2]. A lower rate of spontaneous HCV clearance of an acute infection is generally seen in patients with HIV infection than in HCV mono-infected individuals [3].

HIV/ HCV co-infection is common in many individuals due to shared routes of transmission. Hence, a total anti-HCV prevalence of 15-30% has been noted in HIV positive persons [4]. The prevalence, however, is dependent on the mode of HIV acquisition, and thus varies in different cohorts, countries, and epidemiological settings. People who inject drugs (PWID) are generally noted to have the highest HCV prevalence[4-9]

The IL28B genotype has been found to have a strong influence on the sustained viral response rate to pegylated interferon (peg-IFN) plus ribavirin treatment, and to spontaneous clearance of HCV both in HCV mono- and HIV/HCV co-infected patients [10-16].

Demographical factors, such as gender and hepatitis B infection, have also been associated with spontaneous clearance of HCV both in HCV mono- and HIV/HCV co-infected persons [17-21]

In Sweden, all known HIV/HCV co-infected patients have been included in a database (InfCare HIV) in which demographical, virological and treatment data are recorded. In this database studies largely devoid of selection bias can be done since it includes the total Swedish cohort.

Here, we analyzed the spontaneous clearance rate of HCV in HIV/HCV co-infected patients in this cohort. We also determined the IL28B genotype in a subgroup and correlated it with spontaneous clearance of HCV.

Spontaneous clearance of *chronic* HCV is a rare event but case reports in co-infected patients without HCV therapy have been published [22-32]). We report on three such cases.

Patients and methods

Inclusion of patients

On the 28th of September 2010, 5315 adult living persons were known to be HIV infected in Sweden. According to the Swedish law of communicable diseases all HIV infected patients are required to keep a regular contact with their health care provider (an Infectious Diseases or Dermatology/Venereology specialist). All known HIV positive patients in Stockholm and Gothenburg have been prospectively included in the InfCare HIV research database since 2003, and all patients living in Sweden since 2009. Retrospective data from the beginning of the 1990-s have also been included on patients from Stockholm and Gothenburg.

Demographical data of all 652 living anti-HCV and anti-HIV positive adults, 18 years of age or older, were extracted. Age, sex, ethnicity, HIV transmission route, clinical data, prior AIDS diagnosis, CD4+ T-cell count, virological data (anti-HCV serology, HCV-RNA levels, HCV genotype, HIV-RNA levels, HBV serology), and HCV and/or HIV treatment data were collected. Five patients who had received HCV treatment for acute hepatitis C were excluded from the analysis of factors associated with spontaneous clearance of HCV.

Spontaneous clearance of HCV was defined as a positive anti-HCV test but a negative HCV-RNA test in a HCV treatment naïve patient. Chronic hepatitis C was defined as a positive HCV-RNA test. Hepatitis B infection was defined as a positive HBsAg test.

Analysis of markers for HIV, HCV and HBV

Analyses of HIV-RNA, anti-HCV antibodies, and HBV serology were performed utilizing routine techniques at the local virological laboratories.

HCV RNA was analyzed in anti-HCV positive individuals with the Roche TaqMan Test (detection limit of 15 IU/mL). HCV genotyping was performed with a line probe assay or an in-house method.

IL28B genotyping

Anti-HCV positive patients from the Stockholm area (n=263) were tested for IL28B rs 12979860 SNP with a Taqman-based allele-specific PCR method (Applied Biosystems Inc, Foster City, CA, USA), using the ABI 7500 Fast equipment. Human DNA was extracted from plasma obtained from EDTA blood kept at -70°C. Human genomic DNA was purified from 500 µL of plasma and performed according to manufacturer's instructions using the QIAamp DNA Mini kit (Qiagen, Tokyo, Japan), except for one change in the elution step; elution was done using 25 µL elution-buffer.

All TaqMan probes and primers were designed and synthesised by Applied Biosystems Inc. Automated allele calling was performed using SDS software from Applied Biosystems. The primers and probes used were: rs12979860 Forward primer: 5'GCCTGTCGTGACTGAACCA3', Reverse primer: 5'GCGCGGAGTGCAATTCAAC3', Vic probe: 5'TGGTTC~~C~~CGCCTTC3', Fam probe: 5'CTGGTTC~~A~~CGCCTTC3'.

The SNP was defined as rs 12979860 genotype CC, CT or TT genotype.

Statistics

Differences between groups were compared with chi-square test for categorical variables and the Wilcoxon Rank Sum test for continuous variables. In the univariate model we included age, sex, ethnicity, HIV transmission route, prior AIDS diagnosis, CD4+ T-cell cell count, present ART, plasma HIV-RNA level, HBsAg status, and IL28B genotype. Factors with a p-value <0.10 were included in a multivariate model. Logistic regression was used to identify variables associated with spontaneous HCV clearance. The results are presented as odds ratio (OR) and adjusted OR (aOR) with 95% confidence intervals (CI). A p-value of < 0.05 was considered statistically significant. All data were analyzed using JMP software version 9.0.0.

Ethics

The study was performed in accordance with the Declaration of Helsinki and was approved by the Regional Ethics committee (Dnr 2010/1782-31).

Results

Anti-HCV and HCV-RNA prevalence in the total Swedish HIV population

An anti-HCV result was available in 4765/5315 (90%) known HIV infected persons. In total 14% were anti-HCV positive whereof 92% had been tested for HCV RNA. A chronic hepatitis C infection was diagnosed in 79% whereas 21% spontaneously had cleared their HCV infection (Table 1).

No anti-HCV test result was available in 550 (10%) patients. In this group, 46% were females versus 36% in the anti-HCV tested group. The corresponding figures for PWID were 2.2% versus 8.6%, MSM (men who have sex with men) 28% versus 34%, and for patients with prior AIDS diagnosis 9.6% versus 16%, ($p < 0.05$ for all comparisons). There was no significant difference in age or ethnicity between anti-HCV tested and non-tested (data not shown).

Anti-HCV positive patients who had not been tested for HCV RNA ($n=54$) were more likely to be ART naive than tested, 30% versus 15%, $p < 0.01$. No significant difference in sex, age, risk group or ethnicity was noted between tested and not tested individuals (data not shown).

Factors associated with spontaneous clearance of HCV

Demographical data according to spontaneous clearance of HCV or not in the 593 Swedish HIV/HCV positive individuals tested for HCV-RNA are depicted in Table 2. In univariate analysis, the only factor significantly correlated with spontaneous clearance of HCV was HBsAg positivity. Eight of 16 patients (50%) with a positive HBsAg test versus 118/559 (21%) with a negative test

had spontaneously cleared HCV, odds ratio (OR) 14.7 (2.47-98.1), $p=0.006$, Table 2.

In the multivariate analysis adjusted for sex and route of transmission an adjusted odds ratio (aOR) of 17.3 (2.42-125.6), $p=0.003$ for spontaneous clearance of HCV was seen in HBsAg- positive versus -negative persons (data not shown). Five out of the 16 HBsAg positive patients were born in HBV intermediate to high endemic countries (Somalia, Burundi, Russia, Iran, Thailand) [33].

Spontaneous HCV clearance was seen in 26% females and 19% males, $p=0.0563$. The HCV clearance rate in persons who had been HIV infected via blood transfusions was 29%, via heterosexual contacts 28%, via IDUs 20%, and via homosexual contacts 16%. A difference was noted in clearance rate in heterosexuals versus IDUs approaching significance, $p=0.0546$ (Table 2). No significant difference was seen in the rate of spontaneous HCV clearance in persons with ongoing ART (22%) versus in ART naïve persons (14%), $p=0.10$. In total 79% of the anti-HCV positive patients had ongoing ART at the time of data extraction (Table 2).

IL28B correlation with spontaneous HCV clearance

IL28B genotyping was performed in 263 anti-HCV positive patients followed at the Karolinska University Hospital. Their demographics and IL28B distribution are depicted in Table 3.

The following IL28B genotype distribution was found: 46% CC, 43% CT and 11% TT. A strong correlation between IL28B genotype and spontaneous HCV clearance was noted. Thus, 36% of individuals with CC versus only 13% with non-CC had spontaneously cleared their HCV infection, $p=0.0004$, OR 5.02 (2.11-12.92), Table 3.

In the multivariate analysis adjusted for sex, HIV transmission route, and HBsAg status the only significant baseline factor predicting spontaneous HCV clearance was the IL28B genotype CC with an aOR of 5.45 (2.22-14.50),

p=0.0003 (data not shown). No other baseline demographic factor was significantly correlated with HCV clearance in the subgroup tested for IL28B genotype, Table 3.

Compared to patients who had not been tested for IL28B the tested subgroup was significantly older than the non-tested group (50 versus 47 years), and more likely to be PWID (70% versus 56%), and less likely to be MSM (4% versus 12%), and also less likely to be of Asian ethnicity (3% versus 7%), p<0.05. There was no significant difference in sex, AIDS diagnosis, ART, undetectable HIV-RNA, CD4+ T-cell count or HBV status between the IL28B tested and non-tested individuals (data not shown).

Spontaneous clearance of HCV in HIV infected individuals with a chronic HCV infection

Three patients in the IL28B tested anti-HCV positive subgroup spontaneously cleared their *chronic* hepatitis C infection after introduction of ART.

Since IL28B genotype CC was found in 122/263 tested patients, of whom 106 were on ART, these three cases make up 3/106 (2.8%) of our patients on ART with genotype CC. If only IL28B genotype tested patients with genotype CC on ART with chronic HCV are considered in the denominator, they would make up 3/68 (4.4%). If the IL28B genotype distribution, the HCV chronicity rate and ART uptake were extrapolated to our total co-infected cohort, our cases would make up 3/151 (2.0%).

In all three cases the ART caused immune reconstitution, which probably in turn caused the spontaneous HCV clearance. These individuals became HCV-RNA negative without any specific HCV therapy after previously being diagnosed having a chronic HCV infection with at least 2 positive HCV-RNA tests 6 months apart. All three had IL28B genotype CC. Their cases are briefly reported here.

1. A 58-year-old homosexual male seroconverted simultaneously for both HIV and HCV genotype 4 in July 2008. He had fluctuating HCV-RNA levels between 847 000, 268 000 and 3 700 000 IU/ml repeatedly during 2008 - 2010. He had markedly elevated aminotransferases in

August 2009 when a liver elasticity measurement with Fibroscan showed a mean elasticity of 16.9 kPa indicating cirrhosis and/or active inflammation. ART (raltegravir, tenofovir and emtricitabine) was started in February 2010 when his CD4+ T-cell count was 384, but was interrupted after one month due to an ALT flare. At this time his bilirubin value was 83-193 (ULN <26), ALT 3.39-6.43 (ULN < 1.20), AST 6.03-22.82 (ULN < 0.76), and the HCV RNA level 225 000 IU/ml. The CD4+ T-cell count was 390. The flare subsided after 2 months (22 months after the HCV diagnosis) and HCV-RNA became negative. He was restarted with the same ART regimen and had no flare reaction. Hereafter, HCV-RNA has repeatedly been tested negative during 2 years. (Figure 1A)

2. A 62-year-old woman with IDU 15 years earlier. HIV was diagnosed in 1985 and HCV genotype 2b in 1993. HCV-RNA was repeatedly positive between 2003 and 2007 and the HCV RNA levels fluctuated between 2 600 000 and 13 600 000 IU/ml. She had been on highly active ART since 1997. Her nadir CD4+ T-cell count was 240. She had an aminotransferase flare in December 2007. In 2008, when HCV therapy was planned to start HCV-RNA had spontaneously become negative. After this she has been continuously negative for HCV-RNA during 4 years. Her ART regimen at the time of HCV-RNA clearance was atazanavir/ritonavir, abacavir and lamivudine and the CD4+ T-cell count was 438. (Figure 1B)
3. A 48-year-old woman with HIV diagnosed 1985 and HCV genotype 2b in 1997. HCV-RNA was repeatedly positive between 2001 and 2004 with fluctuating HCV RNA between 4290 and 171 000 IU/ml. She had been on ART since 1996 with a nadir CD4+ T-cell count of 170. At the time of spontaneous HCV-RNA clearance in 2005 she was on lopinavir/ritonavir, stavudine and tenofovir, and the CD4+ T-cell count was 880. After this HCV-RNA has been repeatedly negative in serum during 18 months[24]. (Figure 1C).

All these three patients were repeatedly tested negative for HCV-RNA three times after their first negative HCV RNA test.

Discussion

We found that three of our HIV/HCV co-infected patients spontaneously cleared their *chronic* HCV infection once they had achieved immune reconstitution induced by ART, all with IL28B genotype CC. Clearance of *chronic* HCV infection without specific HCV treatment is rarely seen, but several case-reports in HIV/HCV co-infected patients have previously been published [22-32]. Most of these cases, if not all, have been associated with immune reconstitution after initiation of ART. No information, however, has been provided on the IL28B genotypes in these reports. Our cases, although indeed rare, might support that ART should be initiated prior to HCV treatment, in particular in patients with the IL28B genotype CC. Another more direct implication is that HCV RNA testing should be done immediately before the initiation of HCV treatment, since a subset of HIV/HCV co-infected patients with IL28B CC might have cleared their chronic infection spontaneously.

Furthermore, the IL28B genotype CC was strongly correlated with spontaneous clearance of acute HCV in our HIV/HCV co-infected patients. Thus, 36% of individuals with CC versus only 13% with non-CC spontaneously eradicated their HCV infection. These findings are in line with previous findings both in HCV mono- and HIV/HCV co-infected patients [11, 12, 14, 15, 34-38]. Differences in baseline demographics in IL28B genotype tested versus untested patients were noted but these differences were unlikely to have had any major impact on the overall results and conclusions.

The mechanism, through which the IL28B genotype and spontaneous HCV clearance is associated, is not well understood. The IL28B gene encodes interferon lambda 3, which induces antiviral activity by itself and induces interferon-stimulating genes (ISGs), which also have an antiviral activity. Favorable IL28B genotypes are associated with lower levels of pre-activated ISGs, but are induced more strongly after IFN-alfa treatment[15].

The only investigated baseline demographic factor associated with spontaneous HCV clearance beside the IL28B genotype in our Swedish HIV/HCV co-infected cohort was chronic hepatitis B. The prevalence of chronic HBV, however, was only 2.7%. Persons born in HBV intermediate to high endemic countries most likely had acquired their HBV infection vertically as children, before they had acquired HCV or HIV. We do not have any information on the order in which the patients acquired the individual viral infections.

The association between a chronic HBV infection and a higher rate of spontaneous HCV clearance has been reported earlier both in HCV mono- and HIV/HCV co-infected patients [17-21, 39-41]. The mechanism causing this is not known, but viral interference has been proposed [18, 42]. Spontaneous HCV clearance was seen in 26% females and 19% males, $p=0.0563$. Female gender has previously been associated with higher spontaneous clearance rate of HCV [17, 18, 21].

A limitation of our study is that our definition of acute and chronic HCV does not rule out that there may be further cases of spontaneous clearance of *chronic* HCV among our patients. Also, a few cases of acute HCV may have been misclassified as chronic. HCV RNA sampling was performed in the clinical routine, and sometimes only one test was available.

Another weakness in our study is the missing information of the consecutive order in which the HIV and HCV infections were acquired. However, we were able to note seroconversion for HCV in 28% in the MSM group. Regarding PWID, most patients in this group had probably acquired their HCV infection before the HIV infection as reported earlier [43]. The HIV transmission route indicated does not necessarily imply that this actually was the route by which the HCV infection was acquired. Hence, an overestimation of heterosexual HCV transmission was probably at hand, since heterosexual transmission of HCV is uncommon [44, 45].

In our study a total anti-HCV prevalence of 14% was found corresponding to a chronic HCV prevalence of 11%. Somewhat higher figures have earlier been reported from other European centers [8].

To conclude, we found that immune reconstitution after the introduction of ART can induce spontaneous clearance of a *chronic* HCV infection in HIV/HCV co-infected patients with IL28B genotype CC. Furthermore, in line with previous reports, genotype IL28B CC was strongly correlated with the spontaneous clearance of acute HCV in HIV/HCV co-infected patients. These findings may argue for introducing ART treatment prior to commencement of HCV treatment in HIV/HCV co-infected patients.

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Table 1 Results of HCV testing in HIV positive patients in Sweden

	n	%
Anti-HCV tested	4765 /5315	90
Anti-HCV positive	652/ 4765	14
HCV RNA tested	598/ 652	92
HCV RNA positive	466/593*	79

*5 patients (all MSM) were excluded due to treatment of HCV in the acute phase

Table 2: Demographics in 593 HIV infected anti-HCV positive patients in Sweden according to if they had chronic or spontaneously cleared HCV.

	Total, n (%)	Chronic HCV, n (%)	Spontaneously cleared HCV, n (%)	p-value
Patients	593 (100)	466 (79)	127 (21)	NA
Age (years, median, IQR)	48 (42-54)	48 (42-54)	49 (41-54)	NA
Sex				0.056
Males	410 (69)	331 (81)	79 (19)	
Females	183 (31)	135 (74)	48 (26)	
Ethnicity				0.12
Caucasian	488 (82)	385 (79)	103(21)	
Other	105 (18)	81 (77)	24 (23)	
Risk group				0.17
PWID	369 (62)	296 (80)	73 (20)	
Heterosexual	117 (20)	84 (72)	33 (28)	
MSM	50 (8.4)	42 (84)	8 (16)	
Blood-tx	35 (5.9)	25 (71)	10 (29)	
Other/unknown	22 (3.7)	19 (86)	3 (14)	
HBV status				<0.01
HBsAg pos	16 (2.7)	8 (50)	8 (50)	
HBsAg neg	559 (94)	441(79)	118 (21)	
Not available	18 (3.0)	17 (94)	1 (6)	
HIV treatment				0.10
Ongoing	470 (79)	366 (78)	104 (22)	
Naive	90 (15)	77 (86)	13 (14)	
Pause	33 (5.6)	23 (70)	10 (30)	

Table 3: Demographics and IL28B genotype in 263 IL28B genotype tested HIV/HCV positive patients according to if they had chronic or spontaneously cleared HCV infection.

	Total, n (%)	Chronic HCV, n (%)	Spontaneously cleared HCV, n (%)	p-value
Patients	263 (100)	201 (76)	62 (24)	NA
Age (years, median, IQR)	50(44-54)	50 (44-54)	52 (43-55)	NA
Sex				0.08
Males	181 (69)	144 (80)	37 (20)	
Females	82 (31)	57 (70)	25 (30)	
Ethnicity				0.37
Caucasian	228 (87)	176 (74)	52(26)	
Other	35 (13)	25 (71)	10 (29)	
Risk group				0.47
PWID	183 (70)	140 (77)	43 (23)	
Heterosexual	52 (20)	38 (73)	14 (27)	
Blood-tx	12 (4.6)	10(83)	2 (17)	
MSM	10 (3.8)	7 (70)	3 (30)	
Other	6 (2.3)	6 (100)	0 (0)	
HBV status				0.41
HBsAg pos	5 (1.9)	3 (60)	2 (40)	
HBsAg neg	258 (98)	198 (77)	60 (23)	
IL28B gt				<0.001
CC	122 (46)	78 (64)	44 (36)	
Non-CC	141 (54)	123 (87)	18 (13)	

Fig. 1(A), (B), (C). Three HIV/HCV co-infected patients with spontaneous clearance of a *chronic* HCV infection. The arrow indicates the time of HCV-RNA disappearance. Graph adapted from InfCare HIV.

Fig. 1A.

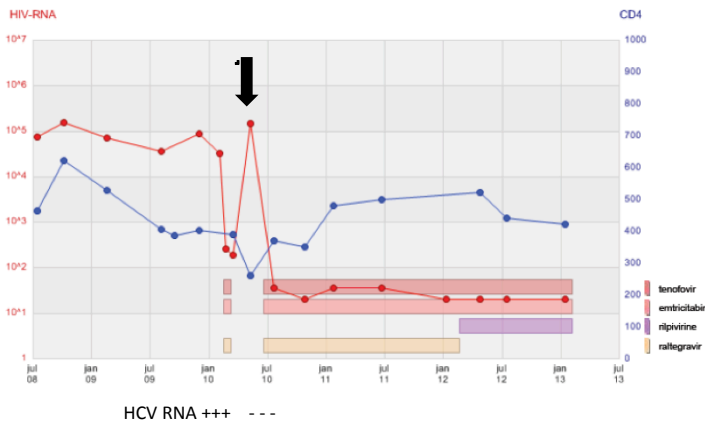


Fig. 1B.

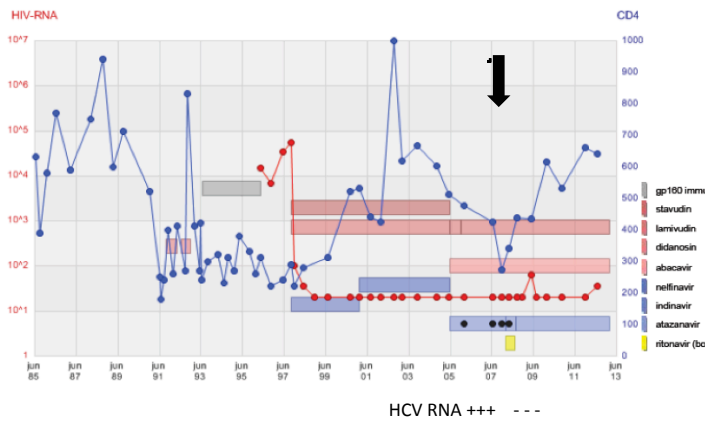
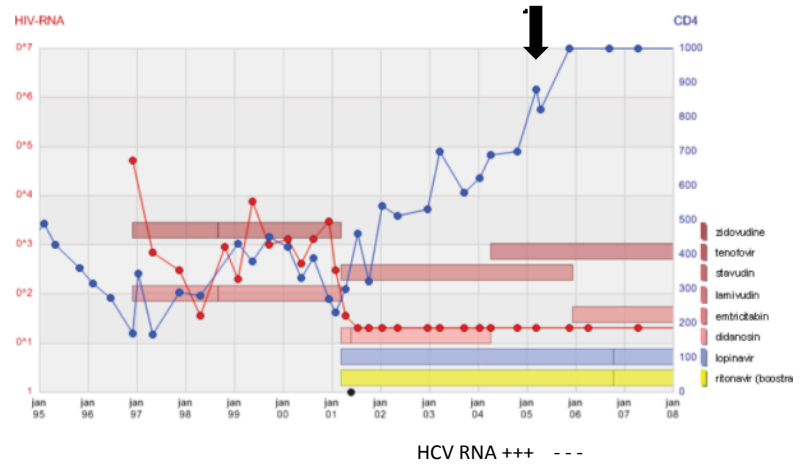


Fig. 1C.



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Paper IV

Lower uptake of HCV treatment than HIV treatment in HIV/HCV co-infected patients

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Abstract

Background

HCV co-infection is a leading cause of death in HIV positive patients. Despite a strong indication to treat HCV, treatment uptake in co-infected patients is generally lower than in HCV mono-infected patients.

Our aim was to determine the HCV and HIV treatment rates and to define factors associated with initiation or deferral of HCV treatment in Swedish HIV/HCV co-infected patients.

Methods

All 5315 adult HIV positive patients in Sweden have been included in the InfCare HIV database. From this database demographic, virologic and treatment data from 652 HIV/HCV co-infected patients were extracted in September 2010.

Factors associated with initiation of interferon-based HCV treatment were analysed. In a subgroup, patient- and physician-reported reasons for not having initiated HCV treatment were investigated.

Results

The anti-HCV prevalence was 14% and the chronic HCV infection rate 11%. In total, 25% of HIV/HCV co-infected patients had initiated HCV treatment. HCV genotype 2 or 3, HIV transmission route other than IDU, and on-going HIV treatment were factors associated with a higher HCV treatment rate. The main reason for not having initiated HCV treatment was IDU or alcohol abuse, stated by both patients and their attending physicians.

Conclusions

The 14% prevalence of anti-HCV noted in Swedish HIV-infected patients was low by international comparisons. This reflects that the MSM and heterosexual HIV transmission routes were dominating whereas IDU was relatively uncommon in our study. The 25% HCV treatment rate noted in our HIV/HCV co-infected patients was high and in the same magnitude of that published in HCV mono-infected patients in Sweden. The HIV treatment rate, however, was considerably higher than the HCV treatment rate. This needs to be improved in the future.

Background

HIV (human immunodeficiency virus) and hepatitis C virus (HCV) co-infections are common due to shared routes of transmission. Hence, a total anti-HCV prevalence of 15-30% is noted in HIV positive persons[1]. However, the prevalence is dependent on the mode of HIV acquisition and varies in different cohorts, countries, and epidemiological settings. People who inject drugs (PWID) generally have the highest HCV prevalence rates[2-6].

Liver diseases, mainly attributable to HCV, have become a leading cause of death in HIV positive patients with access to antiretroviral therapy (ART) [7-14]. End stage liver disease (ESLD) in the HIV/HCV co-infected population has thus increased over the last 15 years in the USA, and is presently an important clinical problem[15].

To predict the future impact of ESLD in HIV/HCV co-infected patients knowledge of the prevalence of chronic hepatitis C in this population is important.

HIV/HCV co-infected patients have faster progression of fibrosis than HCV mono-infected, which should translate into a strong HCV treatment indication [16-18]. Sustained viral response (SVR) after HCV treatment is associated with a lower incidence of ESLD and death [19, 20]. The number of HIV/HCV co-infected patients who has initiated anti-HCV treatment is generally low, and lower than in HCV mono-infected patients[21-28].

In Sweden all known HIV-infected patients have been included in a database (InfCare HIV) in which demographical, virological, and treatment data are recorded. This database was used to estimate the prevalence of HIV/HCV co-infection in Sweden, and to examine to what extent such patients had initiated HCV and HIV treatment. Furthermore, factors associated with commencement of HCV treatment were collected. Reasons for not having initiated HCV treatment were investigated in a subgroup residing in Stockholm.

Patients and methods

Inclusion of patients

On the 28th of September 2010, 5315 adult living persons were known to be HIV infected in Sweden. According to the Swedish Communicable Diseases Act all HIV infected patients are required to keep a regular contact with their health care provider.

HIV positive patients in Sweden have been prospectively included in the InfCare HIV research database since 2009. Retrospective data from the beginning of the 1990s have also been included from patients in Stockholm and Gothenburg.

Demographical data of all living anti-HCV and anti-HIV positive adults above 18 years (n=652) were extracted including age, sex, ethnicity, HIV transmission route, clinical data (prior AIDS diagnosis, CD4+ T-cell count), virological data (anti-HCV serology, HCV-RNA levels, HCV genotype, HIV-RNA levels, HBV serology), HCV treatment data and antiretroviral therapy, (ART).

Hepatitis C and B outcome definitions

Chronic hepatitis C was defined as a positive HCV-RNA test in an anti-HCV positive individual. Spontaneous clearance of HCV was defined as a treatment naïve patient with a positive anti-HCV test but a negative HCV-RNA test.

A patient was defined as having a chronic hepatitis B virus (HBV) infection if HBsAg was positive, and to have had a previous HBV infection if anti-HBc (+/- anti-HBs) were positive with a negative HBsAg test, and to be immune to HBV from vaccination when anti-HBs was positive as an isolated test.

Analysis of markers for HIV, HCV and HBV

Analyses of HIV-RNA, anti-HCV antibodies, and HBV serology were performed at the local virological laboratories by routine techniques. HCV RNA was analysed in anti-HCV positive individuals with the Roche TaqMan Test (detection limit of 15 IU/ml). HCV genotyping was performed with a line probe assay or an in-house method.

Hepatitis C and HIV treatment outcome

Hepatitis C treatment had been given with interferon (IFN) or pegylated interferon (peg-IFN) alone or in combination with ribavirin. SVR was defined as an undetectable HCV RNA 6 months after HCV treatment stop. Non-response (NR) was defined as a detectable HCV RNA test 6 months after end of treatment.

Persons who had received HCV treatment for an acute hepatitis C (n=5) were excluded from the analyses of factors associated with treatment of chronic HCV.

An undetectable HIV RNA viral load was defined as <20 HIV RNA copies/ml.

Questionnaires

A questionnaire was given to HIV/HCV co-infected patients in the Stockholm area who had not yet initiated HCV treatment investigating the underlying reasons. The attending physician was given a separate questionnaire for every patient he/she cared for whom had not yet initiated HCV treatment.

Reasons for not having initiated HCV treatment

Reasons for not having initiated treatment were categorized as intravenous drug use (IDU)/alcohol (on-going or recent abuse), patient desire not to start (including not motivated to initiate HCV treatment, fear of side effects, loss of income in case of sick leave and unstable social situation), doctors recommendation (including no indication due to mild fibrosis or postpone treatment until better HCV treatments becomes available), psychiatric disease, comorbidities (somatic contraindication to HCV treatment, including

ESLD, excluding HIV-related contraindications), HIV/AIDS (uncontrolled HIV infection, on-going opportunistic infection), don't know or other.

The questionnaires from every patient-doctor pair were assessed for agreement or discrepancies concerning the main reason stated for not having initiated HCV treatment.

Patients were also asked if any intravenous drug had been used during the previous 6 months, and to state their current alcohol consumption.

Statistics

Differences between groups were compared with chi-square test for categorical variables and the Wilcoxon Rank Sum test for continuous variables.

When analysing the importance of baseline demographic factors for having initiated HCV treatment or not we included age, sex, ethnicity, born in Sweden or not, HIV transmission route, prior AIDS diagnosis, CD4+ T-cell count, present ART, plasma HIV-RNA level, HBsAg status, HCV genotype and department (university clinic or not) in the univariate model. Factors with a p-value <0.10 were included in a multivariate model. Logistic regression was used to identify variables associated with having started HCV treatment. The results are presented as odds ratio (OR) and adjusted OR (aOR) with 95% confidence intervals (CI).

A p-value of < 0.05 was considered statistically significant.

All data were analysed using JMP software version 9.0.0.

Ethics

The study was performed in accordance with the Declaration of Helsinki and was approved by the Regional Ethics committee (Dnr 2010/1782-31 and 2011/514-31/1).

Results

Prevalence of anti-HCV

In total, 5315 persons ≥ 18 years were HIV positive in Sweden in September 2010. The transmission route for HIV was heterosexual in 50%, men who have sex with men (MSM) in 32%, and IDU in 8%. Transmission via blood transfusion, mother to child transmission, and via other or unknown routes accounted for less than 10% (data not shown).

In 4765 (90%) persons anti-HCV test results were available whereof 652 (14%) were anti-HCV positive (Figure 1).

550 patients (10%) lacked an anti-HCV test result. In this group 46% were females vs. 36% in the anti-HCV tested group. The corresponding figures for PWID were 2.2% versus 8.6%, for MSM 28% versus 34%, and for those with a prior AIDS diagnosis 9.6% versus 16%, ($p < 0.05$ for all comparisons). There was no significant difference in age or ethnicity between anti-HCV tested and non-tested (data not shown).

The prevalence of anti-HCV varied markedly according to the HIV transmission route. Hence, 98% of PWID, 41% of persons who acquired HIV via blood transfusion, 5.5 % of those heterosexually HIV infected, and 3.7% of MSM were anti-HCV positive ($p < 0.01$) (Figure 2).

Among persons who had acquired HIV in Sweden the anti-HCV prevalence was 26% versus 6.2% in persons who were infected abroad, $p < 0.0001$. The anti-HCV prevalence was higher in males than in females, 15% versus 12%, $p = 0.01$ (data not shown).

Chronic HCV in HIV infected individuals

HCV RNA testing had been performed in 598/652 (92%) patients of whom 79% tested positive (Figure 1). These were defined as having a chronic hepatitis C infection, corresponding to a chronic HCV prevalence of 11%. Spontaneous clearance of HCV was noted in 127/593 (21%). The IL28B

genotype CC and HBsAg positivity were the only factors significantly associated with spontaneous clearance of HCV [29].

Patients lacking a HCV RNA test (n=54) were more likely to be ART naive than those tested, 30% versus 15%, $p < 0.01$. No significant difference regarding gender, age, HIV transmission route or ethnicity was noted between individuals tested or not tested (data not shown).

In total, 471 individuals tested HCV RNA positive. Five MSM patients had received HCV treatment for an acute HCV infection, of whom four had achieved SVR. These persons were therefore excluded from further analysis. Demographics in the 466 HIV/HCV co-infected patients in Sweden are depicted in Table 1.

In total, 366/466 (79%) patients had on-going ART of whom 285 (76%) had undetectable HIV RNA levels (< 20 copies/ml)(Table 1).

The HCV genotype was available in 354 (76%) of the HIV/HCV co-infected individuals. HCV genotype 1, 2, 3a and 4-6 was seen in 52%, 12 %, 31%, and 6.2% respectively (Figure 3). Genotype 1a constituted 75% of all genotype 1 cases.

Eight persons (1.7%) also had a chronic hepatitis B virus infection, 68% had had a past HBV infection and 6.0% were isolated anti-HBs positive (data not shown).

In total, 118/466 (25%) patients had received HCV treatment.

Among those who had completed their treatment 57/103 (55%) had achieved SVR. Fifteen patients had on-going HCV treatment or had not completed follow-up. The SVR rate varied according to HCV genotype and was 31% in genotype 1, 87% in genotype 2, and 70% in genotype 3.

Factors associated with initiation of HCV treatment

Demographical data in the 466 patients with chronic HCV infection are depicted in Table 1 according to having initiated HCV treatment or not. The univariate and multivariate odds ratios (ORs) for having initiated HCV treatment are given in Table 2.

The HCV treatment rate varied significantly according to the HIV transmission route. Hence, it was 20% in PWID, 29% in heterosexuals, 38% in MSM and 44% in persons infected via blood transfusion ($p < 0.01$) (Table 1 and Figure 3). MSM and persons who had acquired HIV infection via blood transfusion had increased odds of having initiated HCV treatment compared to PWID: aOR 2.60 (95% CI, 1.13-5.92), $p = 0.02$ and aOR 3.24 (95% CI 1.23-8.59), $p = 0.02$, respectively (Table 2).

For comparison, the HIV treatment rate in HIV/HCV co-infected patients according to HIV transmission route is depicted in Figure 3. There was no significant difference in HIV treatment rate according to transmission route. A trend, however, was noted for persons who acquired their HIV infection via blood transfusion to have a higher HIV treatment rate than PWID, 23/25 (92%) vs. 227/296 (77%), $p = 0.0501$ (data not shown).

Patients with HCV genotype 2 or 3 had increased odds of having initiated HCV treatment when compared with patients with HCV genotype 1, 4, 5 or 6: aOR 2.19 (95% CI. 1.33-3.65), $p = 0.0021$ (Table 2).

Patients with on-going ART had increased odds of having initiated HCV treatment compared with ART naive patients: aOR 3.40 (95% CI 1.42-9.56), $p = 0.0045$ (Table 2). Patients with undetectable HIV-RNA had a higher HCV treatment rate than patients with detectable HIV-RNA during on-going ART in the univariate analysis. This difference, however, was not significant in the multivariate analysis (Table 2).

There were no significant difference in HCV treatment rate according to gender, age, ethnicity (Caucasian vs. non-Caucasian), country of birth (Sweden or not), prior AIDS diagnosis or not, CD4+ T-cell count or having a chronic hepatitis B or not (Table 1, data not shown).

Reasons for not having initiated HCV treatment

The HIV/HCV co-infected cohort in Stockholm

In total 322 patients in Stockholm were HIV/HCV co-infected. The Stockholm cohort constituted 322/466 (69%) of the total HIV/HCV co-infected cohort in Sweden. Of these persons, 82 (25%) had received HCV treatment whereas 240 had not.

The HIV/HCV co-infected cohort in Stockholm included more Caucasians (88% versus 71%), PWID (67% versus 55%), and MSMs (11% versus 5%), than the rest of the Swedish cohort ($p < 0.05$). Other demographics and baseline characteristics were similar.

Questionnaires in patients and attending physicians

Among the 240 patients eligible for inclusion, four patients had received HCV treatment, 12 (5%) had died and 18 moved from the catchment area. Hence 206 could be asked to participate whereof 114 (55%) answered the questionnaire. Very few patients ($n=3$) declined participation, however, some did not attend the HIV outpatient department regularly within the study period. The responders were more often born in Sweden (83% vs. 67%), and more likely to have on-going ART (81 vs. 63%) than the non-responders ($p < 0.05$). Among responders 80% were PWID, vs. 67% of non-responders. There was no significant difference regarding age, gender, CD4+ T-cell count, AIDS diagnosis, or HCV genotype between responding and non-responding co-infected patients.

Among the 16 attending physicians who cared for the HIV/HCV co-infected patients (3-26 co-infected patients/physician) the response rate was 210/240 (88%).

Patient questionnaire results

The results of the questionnaires answered by HIV/HCV co-infected patients are depicted in Figure 4a. The main reason for not having initiated HCV treatment was on-going/recent IDU or alcohol abuse (stated by 26%), followed by patient desire/social situation (22%) and doctor's recommendation (22%). Among patients 68/114 (60%) stated that they had been offered HCV treatment by their caregiver. Today, 68/114 (60%) would accept such an offer. 52/114 (46%) had injected drugs intravenously during the last 6 months.

Physician questionnaire results

Answers given by the attending physicians are depicted in Figure 4b. Reasons for not having initiated HCV treatment as stated by the attending physician were on-going/recent IDU or alcohol abuse in 53 % (9% had concomitant psychiatric disease), patient's desire in 16%, doctor's recommendation in 15%, psychiatric disease in 9%, and somatic co-morbidities in 7%. In one patient (0.5%) HIV/AIDS was the main reason for not having initiated HCV treatment. In 22/210 (10%) of all patients, the attending physicians deemed it possible to start interferon including HCV treatment soon. In 71/210 (34%) HCV treatment including interferon was considered to be intolerable.

The agreement between patient and physician stated main reason for each patient-doctor pair was 59% (data not shown).

Discussion

Prevalence of anti-HCV

Among Swedish HIV infected patients, a total anti-HCV prevalence of 14% was found. Higher figures have earlier been reported from a European study [3]. The MSM and heterosexual transmission routes were dominating whereas PWID was relatively uncommon in our patients but not in the European cohort. This might explain the lower prevalence found in our study. The nearly universal (90%) anti-HCV testing in the Swedish cohort minimizes the risk of selection bias. However, since female sex and HIV transmission routes other than IDU and MSM were overrepresented among our patients untested for HCV markers, the 14% prevalence found in our study might be an overestimation.

HCV prevalence according to HIV transmission route

The varying anti-HCV prevalence found according to the HIV transmission route is expected [1-6]. Thus, a very high anti-HCV prevalence was found among our PWIDs (98%). HIV is still a rare disease among PWIDs in Sweden, with an estimated prevalence of <5% [30]. HCV on the other hand is very common [30]. Hence, HCV is probably acquired earlier than HIV in most HIV infected PWID. A needle exchange program is available in a few cities in Sweden and has only very recently started in Stockholm. Subsequently, this program has not yet had any major impact on the HCV prevalence.

In the MSM group an anti-HCV prevalence of only 3.7% was found. However, not all clinics had yet implemented anti-HCV testing on a yearly basis in MSM persons. Hence, this figure might be an underestimation. Several cases of acute HCV infection were noted in our MSM cohort as reported elsewhere[31-33].

Chronic HCV in HIV infected persons

A chronic HCV prevalence of 11% was noted in the Swedish HIV infected cohort. The HCV genotype distribution was similar to that found in HCV mono-infected patients in Sweden [34], and in HIV/HCV co-infected patients reported in the Euro-SIDA cohort [35, 36].

HCV treatment uptake

The cumulative HCV treatment uptake of 25% noted in our study in 2010 is similar to that noted in the Euro-SIDA cohort in the same year [36]. HCV treatment uptakes between 2% and 60% has been reported in HIV/HCV co-infected patients, partly depending on the selection of patients included [21-28].

In 2005 the national cumulative HCV treatment uptake in Sweden was 12% [27]. This estimate was based on sales of peg-IFN and an estimated national HCV prevalence of 0.5%.

In 2006 a 14% HCV treatment uptake was reported in Sweden, based on 1000 peg-IFN prescriptions per year between 2001 and 2006 and 43 000 notified HCV infected individuals [37].

The 25% HCV treatment uptake in our study is thus comparatively high indicating a treatment uptake in HIV/HCV co-infected patients in the same order as that published in HCV mono-infected patients in Sweden.

Factors associated with initiation of HCV treatment

A higher HCV treatment rate was seen in HCV genotype 2 or 3 infected patients, in patients with HIV transmission routes other than IDU, and in patients with on-going ART.

Being infected with HCV genotype 2 or 3 was independently associated with initiation of HCV treatment, probably because of higher SVR rates and the short treatment duration. The lower HCV treatment uptake among PWIDs compared to patients with other HIV transmission routes was most likely due to many factors (including on-going IDU) rendering the provider less inclined to offer treatment for this group. Indeed, in HIV/HCV co-infected patients in

Stockholm, the main reason for not having initiated HCV treatment as stated by both patients and their physician, was on-going IDU/alcohol abuse.

Somatic comorbidities and HIV/AIDS were uncommon.

Patients with on-going ART also had a higher HCV treatment uptake. This could reflect their assumed better adherence to any treatment.

The HIV treatment rate and rate of undetectable HIV viral load, indicate that many patients who had not initiated HCV treatment were adherent to their HIV treatment. A discrepancy between treatment initiation for HIV and HCV was obvious, since many more patients had initiated ART than HCV treatment. Certainly, ART is more urgent and all patients will need ART eventually whereas HCV treatment might be postponed if the rate of fibrosis progression is slow.

However, when interferon-free DAA combinations soon will become available, HCV treatment should expand to include more HIV/HCV co-infected patients in order to prevent morbidity and mortality from HCV in this population.

Conclusion

We found an anti-HCV prevalence of 14% and a chronic HCV infection rate of 11% in the total HIV infected cohort in Sweden. Of these, 25% had received HCV treatment, a figure in the same range as in published data on HCV mono-infected patients. A higher HCV treatment rate was seen in HCV genotype 2 or 3 infected patients, persons with HIV transmission route other than IDU, and in patients with on-going ART. The main reason for not having initiated HCV treatment among our HIV/HCV co-infected patients in Stockholm was IDU or alcohol abuse.

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Figure 1 HIV-infected patients with chronic hepatitis C in Sweden 2010

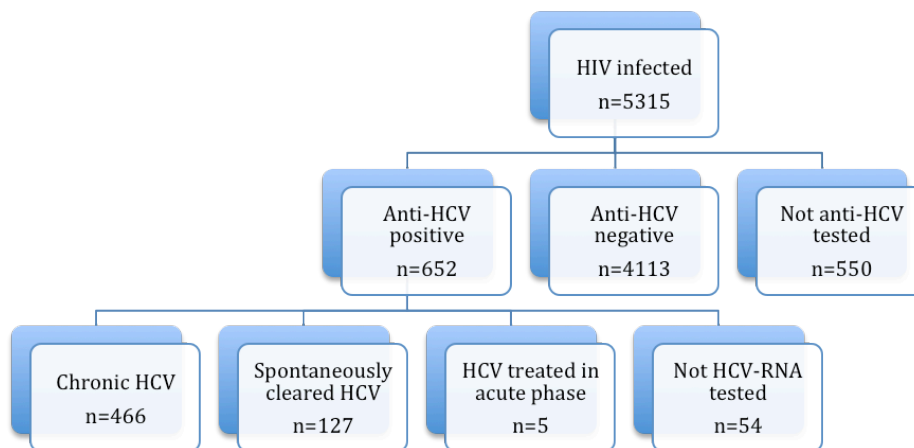


Figure 2: The anti-HCV prevalence in percentage according to HIV transmission group in HIV infected Swedish patients

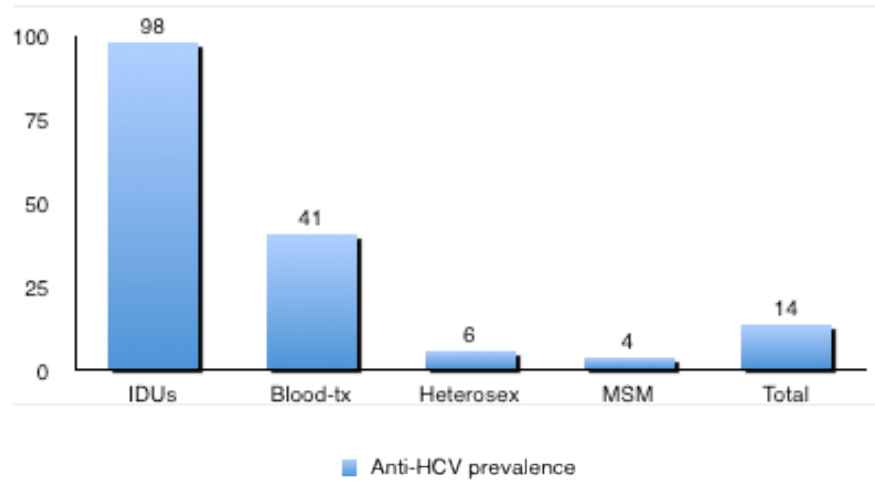


Table 1: Baseline and clinical characteristics in HIV/HCV co-infected patients in Sweden according to having initiated HCV treatment or not

	Total, n(%)	HCV treated, n(%)	Not HCV treated, n(%)	p-value
Patients	466 (100)	118 (25)	348 (74)	
Age (median, IQR)	48 (42-54)	49 (42-55)	48 (42-53)	0.44
Gender				0.67
Males	331 (71)	82 (25)	249 (75)	
Females	135(29)	36 (27)	99 (73)	
Ethnicity				0.67
Caucasian	385 (83)	96(25)	289 (75)	
Other	49 (11)	20 (41)	29 (59)	
Missing	33 (7.1)	2 (6.1)	31 (94)	
Transmission route				<0.01
IDU	296(64)	58 (20)	238 (80)	
Heterosexual	84 (18)	24 (29)	60 (71)	
MSM	42 (9)	16 (38)	26 (62)	
Blood tx	25 (5.4)	11 (44)	14 (56)	
Other	19 (4.1)	9 (47)	10 (53)	
HCV genotype				0.01
1	183 (39)	45(25)	138 (75)	
2	41 (8.8)	15 (37)	26 (63)	
3	108 (23)	39 (36)	69 (64)	
4-6	22 (4.7)	4 (18)	18 (82)	
missing	112 (24)	15 (13)	97 (87)	
HIV treatment				<0.01
Ongoing	366 (79)	104 (28)	262 (72)	
Naive	77 (17)	9 (12)	68 (88)	
Pause	23 (4.9)	5 (22)	18 (88)	
HIV RNA undet				<0.01
Yes	285 (61)	89 (31)	196 (69)	
No	91 (20)	16 (18)	75 (82)	
Not on HIV tx	90 (19)	13 (14)	77 (86)	

Table 2: Variables associated with having started HCV treatment in HIV/HCV co-infected patients in Sweden, n=466

Data are presented as Odds Ratio (OR) and adjusted OR (aOR) with 95% confidence interval (CI)

	Univariate		Multivariate	
Variable (n)	OR (95% CI)	p-value	aOR (95% CI)	p-value
Transmission route				
IDU (296)	1 (reference)		1 (reference)	
Heterosexual (84)	1.64 (0.93-2.83)	0.085 (ns)	1.33 (0.69-2.48)	0.38 (ns)
MSM (42)	2.53 (1.25-4.98)	0.010	2.60 (1.13-5.92)	0.025
Blood tx (25)	3.22 (1.37-7.46)	0.0084	3.24 (1.23-8.59)	0.018
Other (19)	5.47 (1.83-17.20)	0.0027	6.59 (1.90-25.07)	0.0033
HCV genotype				
1,4-6 (205)	1 (reference)		1 (reference)	
2,3 (149)	1.81 (1.14-2.88)	0.019	2.19 (1.33-3.65)	0.0021
HIV treatment				
Naive (77)	1 (reference)		1 (reference)	
Ongoing (366)	3.00 (1.51-6.65)	0.0011	3.40 (1.42-9.56)	0.0045
HIV RNA undet				
No (91)	1 (reference)		1 (reference)	
Yes (285)	2.13 (1.20-3.97)	0.0090	1.84 (0.93-3.83)	0.078 (ns)

Figure 3: HIV and HCV treatment uptake in percentage in HIV/HCV co-infected patients (n=466) according to HIV transmission route

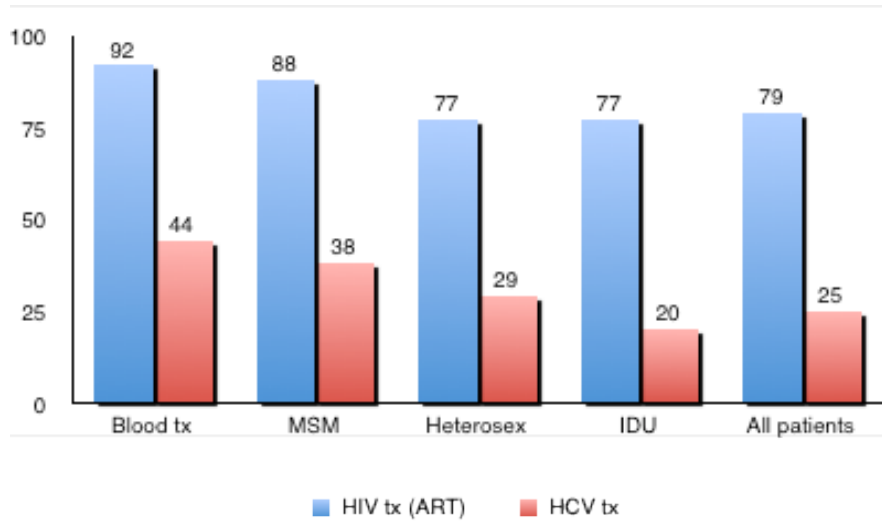


Figure 4: Reasons stated for not having initiated HCV treatment in HIV/HCV co-infected patients in Stockholm

- a. In patients (n=114)**
- b. In physicians (n=210)**

Figure 4a

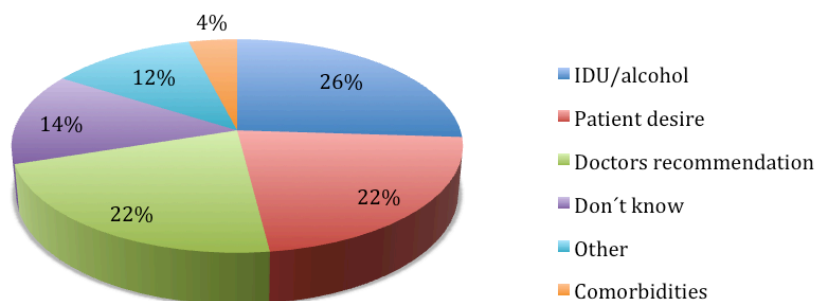
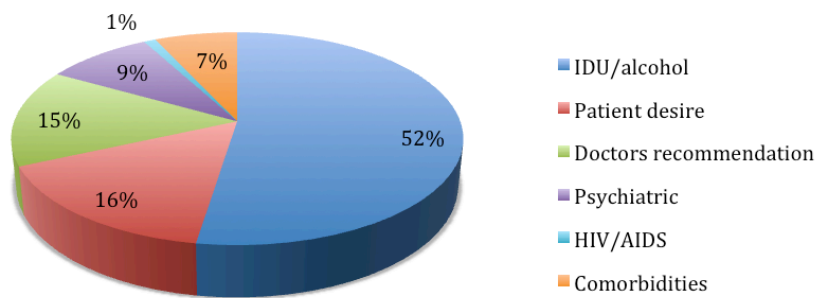


Figure 4b



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