Blood Borne Viruses (HIV, HBV and HCV) among Participants of a Swedish Needle Exchange Program

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Blood Borne Viruses (HIV, HBV and HCV) among Participants of a Swedish Needle Exchange Program

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Abstract

People who inject drugs (PWID) are at high risk of infection with pathogenic blood borne viruses, most importantly HIV, hepatitis B (HBV) and hepatitis C (HCV). Transmission is mostly parenteral within the PWID population; however, this group is a reservoir for further spread in the community. Harm reduction as a concept encloses several interventions aiming at reducing risks and harmful consequences within high risk groups, such as PWID. Needle exchange programs (NEPs) are included in such interventions. In Sweden, access to NEPs was limited to only two locations for two decades; a NEP was opened in Lund in 1986 and in the neighbouring city of Malmö in 1987. New legislation in 2006 allowed for NEPs to be opened nationwide in Sweden.

The aim of this thesis was to evaluate the prevalence and incidence of HIV, HBV and HCV among participants of the Malmö NEP, identifiable by the national identity number during 1997-2005 (n=1183) (paper I). We found HIV prevalence and incidence to be minimal. A NEP providing access to sterile injection equipment and regular HIV screening to PWID may thus maintain a low incidence of HIV, if introduced before HIV has disseminated in this group. HBV incidence had decreased after the introduction of HBV vaccination in 1994. Most countries have implemented HBV vaccination in their childhood vaccination programs, but this has only recently been done in Sweden. Thus only a few of the NEP participants had been vaccinated before NEP enrolment, while approximately one third had been exposed to HBV. Those susceptible to HBV were offered vaccination at NEP enrolment and 60% of susceptible participants 1994-2013 completed the basic vaccination schedule (three doses). We then studied the response to the basic vaccination schedule and up to three booster doses among NEP participants vaccinated 1994-2013 and found that while the response rate to the basic vaccination schedule was lower than expected among NEP participants (75%), it could be improved by subsequent booster doses (to 85%) (paper IV). For HCV, incidence was high among NEP participants enrolling 1997-2005 (38/100 person years at risk, or 31/100 pyr when adjusted for baseline viremia). Through follow-up of viremia in 150 individuals with documented HCV seroconversion we could characterize several patterns of viremia during the first year after infection, with an overall clearance rate of 32% (paper II). Spontaneous viral clearance was associated with female gender and younger age. Through phylogenetic analysis of viral strains among individuals with incident infection and with infection prevalent already at NEP enrolment we found that 32% of the study participants were part of totally 32 transmission clusters. Belonging to a cluster was associated with incident infection and use of heroin as the main injection drug (paper III). The majority of HCV infections become chronic, with progressive liver fibrosis resulting in liver cirrhosis and end-stage liver disease, (including hepatocellular carcinoma) in a large proportion over time. Recently, new treatment possibilities have been introduced for HCV, raising the idea of providing antiviral treatment as prevention (TasP) of HCV transmission. In order to provide TasP efficiently it is critical to understand how HCV is transmitted among PWID, which we have studied within the Malmö NEP. A NEP can provide access to diagnostic testing, vaccination against HBV and linkage to care (both for infectious diseases and for drug dependency). Combination of prospective studies with real-time data, contact tracing and further assessment and linkage to HCV treatment, all provided through the NEP, could have a significant health impact for PWID both on the individual level and an effect on HCV transmission on a group level.

Key words Hepatitis C, hepatitis B, HIV, people who inject drugs, needle exchange programs

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I Malmö rispas dimman av färjornas sirener. Och på andra sidan sundet börjar världen.

Ur ”Vintersaga” av Ted Ström

Contents

List of papers

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

I. Blomé MA, Björkman P, Flamholc L, Jacobsson H, Molnegren V, Widell A. Minimal transmission of HIV despite persistently high transmission of hepatitis C virus in a Swedish needle exchange program. J Viral Hepat. 2011 Dec;18(12):831-9.

II. Alanko Blomé M, Björkman P, Molnegren V, Höglund P, Widell A. Hepatitis C viremia patterns in incident hepatitis C infection and one year later in 150 prospectively tested persons who inject drugs. PLoS One. 2014 May 15;9(5).

III. Alanko Blomé M, Josephson M, Jacobsson H, Molnegren V, Björkman P, Widell A and Medstrand P. Hepatitis C virus transmission among participants of a Swedish needle exchange program – a phylogenetic cluster analysis. Manuscript.

IV. Alanko Blomé M, Björkman P, Flamholc L, Jacobsson H and Widell A. Vaccination against hepatitis B virus among people who inject drugs – a 20 year experience from a Swedish needle exchange program. Under revision.

Abbreviations

AIDS	acquired immunodeficiency syndrome
Anti-HBc	anti-hepatitis B core antibody
Anti-HBs	anti-hepatits B surface antibody
Anti-HCV	anti-hepatitis C antibody
Anti-HDV	anti-hepatitis D antibody
ART	anti-retroviral therapy
CDC	Centers for Disease Control and Prevention (Atlanta, GA, USA)
DAA	Direct-acting antivirals
DALY	disability adjusted life-years
DAM	diacetylmorphine treatment
EMCDDA	European Monitoring Centre of Drugs and Drug Addiction
HBsAg	hepatitis B surface antigen
HBeAg	hepatitis B e-antigen
HBV	hepatitis B virus
HBV-DNA	hepatitis B virus deoxy-ribonucleic acid
HCV	hepatitis C virus
HCV RNA	hepatitis C ribonucleic acid
HIV	human immunodeficiency virus
IQR	interquartile range
MMT	methadone maintenance treatment
MSM	men who have sex with men
NEP	needle exchange program
NGO	non-governmental organization
NIN	national identity number
OST	opiate (opioid) substitution therapy
PWID	people who inject drugs
PCR	polymerase chain reaction
SDUU	Swedish Drug Users' Union
SIS	supervised injection site
SVR	sustained viral response
TasP	Treatment as prevention

Introduction

People who inject drugs (PWID) are exposed to various health hazards; adverse drug effects including fatal overdoses, infections with both viral and bacterial agents and intentional and accidental trauma, all resulting in high morbidity and mortality rates within this population (1-5). Psychiatric comorbidity, primary and secondary, is substantial, with serious health and socio-behavioural consequences (6, 7). Through crumbling social relations and collapse of the personal economy, injection drug use may lead to increasingly vulnerable living conditions including homelessness and an increased exposure to different pathogens (8). In settings where drug policies are solely based on law enforcement, without public health and human rights considerations, the health risks of PWID (including transmission of HIV and hepatitis) may markedly increase (9, 10). Furthermore, several obstacles to health care access exist for PWID. The illicit status of injection drug use generates social stigmata and many PWID might fall through the safety nets of society. Even in Sweden, with a tightly knit net of social services and a climate making living outdoors very difficult, unstable living conditions and various co-morbidities are a reality for many PWID.

The number of PWID worldwide has been estimated to 15.9 million, with approximately 3.0 million HIV positive, 6.4 million exposed to hepatitis B virus (1.2 million with chronic infection) and 10 million exposed to hepatitis C virus (7 million with chronic infection) (11, 12). In the Global Burden of Disease Study in 2010, illicit drug dependency was estimated to directly account for 20.0 million disability-adjusted life years (DALYs) (13). With opioid and amphetamine dependency dominating over other drugs, opioid dependency accounted for the largest contribution to the burden of DALYs (9.2 million). Injection drug use as a risk factor for HIV accounted for 2.1 million DALYs and as a risk factor for HCV for 502 000 DALYs.

The dawn of the HIV epidemic in the 1980s illuminated the exposed situation of PWID. Although the initial cases of HIV were observed among men who have sex with men (MSM) and patients having received blood products (e.g. haemophiliac patients), PWID were soon identified as one of the main groups for HIV transmission both within PWID networks and beyond these by sexual transmission routes (14, 15).

The concept of Harm Reduction - ways to reduce and limit damage among already vulnerable high risk groups - has for decades been strongly supported and promoted by the World Health Organization (WHO). For PWID, harm reduction has enclosed access to clean needles, syringes and other drug paraphernalia through needle exchange programs (NEPs), opioid substitution therapy (OST) and risk reduction counselling. In some regions, harm reduction interventions include also supervised injection sites (SIS), diacetylmorphine treatment (DAM), overdose prevention, social support and peer education.

In Sweden, however, many of these harm reduction measures remain still controversial with restrictions and limited access. During the time when data for studies I-III in this thesis were collected (1997-2005), only two NEPs were allowed to operate in Sweden (in the neighbouring cities Lund and Malmö, 20 km apart in the very south of the country, in Skåne county). Furthermore, syringes and needles cannot be purchased legally without a prescription in Sweden. New legislation in 2006 allowed for nationwide NEP introduction. Today (2016), six additional NEPs are operating (five in the southern half and the sixth and northernmost in the capital Stockholm – leaving the less densely populated northern half of the 1500 km long country without NEPs). The Swedish Drug Users' Union (SDUU) has run an informal NEP in Stockholm since 2008 (the official NEP in Stockholm was opened in 2013). SDUU and other Non-Governmental Organizations (NGOs) are also working with peer education and information. In 2015 the Swedish Public Health Agency published their guidelines for improved harm reduction measures for PWID, including increased access to NEPs (16).

The high prevalence of HIV, hepatitis C (HCV) and hepatitis B (HBV) among PWID has a significant impact on morbidity and mortality, both as mono- and coinfections. HIV infection can with the aid of modern anti-retroviral treatment be a manageable, stable chronic disease. Chronic infection with HBV and HCV may progress to liver fibrosis and cirrhosis, with further evolution into end stage liver disease and hepatocellular carcinoma (HCC) in a proportion of individuals over time. Currently, the mortality burden of chronic viral hepatitis is estimated to be similar to that caused by HIV/AIDS. In reaction to this, the WHO recently launched a global health sector strategy for 2016-2021: Towards Ending Viral hepatitis (17). The objectives are set high:

- A world where viral hepatitis transmission is halted and everyone living with viral hepatitis has access to safe, affordable and effective care and treatment.
- Elimination of viral hepatitis as a major public health threat by 2030.
- Reduction of the incidence of chronic hepatitis infection and reduction of the annual deaths from chronic hepatitis.

Since safe and effective vaccines are available against HBV (and do not exist against HIV and HCV), the WHO strategy calls for an increase in routine childhood HBV vaccination coverage. Most countries have already implemented HBV vaccination in their childhood vaccination schedules and it is also now being gradually introduced in Sweden. Through NEPs vaccination against HBV can be offered to PWID. One of our aims was to study the coverage and efficiency of HBV vaccination in this setting.

For HCV among PWID, the new hepatitis strategy promotes a major increase in provision of sterile needles and syringes, the current coverage deemed too low to have a significant impact on hepatitis epidemics. Recently new treatment options for HCV have become available. With these new direct-acting antivirals (DAAs) oral treatment can be given safely, during relatively short time periods and with very high rates of sustained viral response. Thus, implementing "treatment as prevention", as a means of eliminating HCV by 2030 has also been suggested.

To achieve these ambitious goals, it is however important to know where to aim. A NEP can provide a platform to reach the target population at risk for HCV infection and to understand the routes of HCV transmission among PWID. In this thesis, I have studied the prevalence and incidence of HIV, HBV and HCV, with specific focus on viral kinetics and transmission patterns for HCV among participants of the Malmö NEP.

Aim of the thesis

The overall aim of this thesis was to investigate the prevalence and incidence of HIV, HBV and HCV among participants of the Needle Exchange Program at the Department of Infectious Diseases at the Skåne University Hospital in Malmö, southern Sweden. Specific aims were:

I. To assess the prevalence and incidence of HIV, HBV and HCV among PWID with access to the NEP.

II. To study the natural course of HCV kinetics around seroconversion to anti-HCV within this group.

III. To investigate the transmission patterns of HCV among the NEP participants through phylogenetic analysis.

IV. To assess the coverage and efficiency of HBV vaccination among NEP participants.

Infectious diseases and injection drug use

General aspects

Background

Documentation of early forms of intravenous injection dates back to the 1650s, when a syringe made of animal bladder fixed to a goose quill was used to inject wine and opium into the veins of dogs by Sir Christopher Wren (18). Soon thereafter, trials of administering intravenous injections in humans were performed in Germany, but were apparently not successful and further attempts were postponed until the 1800s. Several persons subsequently described subcutaneous administration, but Alexander Wood from Edinburgh and Charles-Gabriel Pravaz from Lyon are generally credited for inventing the syringe for subcutaneous injection (19). Hygienic measures were improved by the syringe of Luer with a sharp needle fitted for aseptic heating, with sterilization by heating in an autoclave further developed by Pasteur, Chamberland and Koch (18).

Intravenous access is used in all medical fields as the fastest way of administering drugs - antibiotics, anaesthetics and fluids - in order to achieve high, efficient concentrations and rapid effects. Recreational injection drug use occurs when using the intravenous access to inject (illicit) substances in a non-medical setting to achieve the maximal effects of the substance. Many of these substances, such as heroin and other opioids, are very powerful with narrow safety margins, capable of causing respiratory depression or other harmful adverse effects (20, 21).

In addition, if the substance injected has been contaminated with other, exogenous harmful substances or pathogens (bacteria, viruses and parasites) they are concomitantly injected into the blood stream and might cause further adverse effects or infectious diseases (3). In injection settings where sharing of syringes, needles and other drug paraphernalia (filters, cups, cookers and solutions) occur, transmission of infectious agents from one individual to another might be affected by the sharing methods and syringe construction themselves, by behavioural factors including social network patterns and by factors involving the host´s immune system and characteristics of the infectious agent (22-25).

If hygienic measures while injecting are insufficient, endogenic infections with bacteria from the skin or oral cavity (through licking the needle before injecting, which was reported from 30% of participants in an interview study) might occur, leading to bacteraemia and other severe complications such as endocarditis, septic arthritis or brain abscess (26-30).

Bacterial infections

Bacterial infections can progress rapidly with high mortality rates if left untreated, but can, with early access to adequate care, be expected to have a good prognosis (31). Bacterial infections pose a substantial threat to PWID, displayed by the full panorama from fulminant sepsis to slowly progressing subclinical disease (3). Injection drug use may also lead to malnourishment, sexual risk behaviour and homelessness, creating an environment where exposure to and infection with sexually transmitted diseases (STDs) or tuberculosis (TB) could occur (32-34). Injecting-related injuries and diseases (IRIDs) include cutaneous conditions such as abscesses and cellulitis together with sequelae such as septicaemia, bacterial endocarditis, septic arthritis and osteomyelitis. Aspiration of stomach contents in an unconscious state might in itself pave way for pneumonia and respiratory distress. Below, skin and soft tissue infections, endocarditis and tuberculosis will be discussed in brief.

Skin and soft tissue infections (SSTI)

SSTIs among PWID are caused by *Staphylococcus aureus* in the majority of cases and PWID are often colonized with *S. aureus* (35, 36). Spread of methicillin-resistant *S. aureus* has also been observed among PWID, complicating treatment and constituting a reservoir for further transmission beyond PWID populations (37). Difficulties accessing veins damaged after long periods of injecting, (especially for women) may lead to increasingly harmful injection practices. This includes selection of more vulnerable sites for intravenous injection (the neck or groin), skin-popping (accidental or intentional extravasal injecting in skin or muscle) or excess use of citric acid, thus causing tissue damage predisposing to wound infections with anaerobic bacteria (38, 39).

Skin infections with spore-forming bacteria (*clostridium botulinum, clostridium tetani, clostridium novyi* and *bacillus anthracis*) have been associated with injection drug use, with severe consequences. In 2000, a case of cutaneous anthrax leading to disseminated infection with septic shock, meningitis and lethal outcome, was diagnosed in a person injecting heroin by skin-popping in Norway (40). Later similar cases occurred in an outbreak 2009-2010 in the United

Kingdom and in 2012-2013 in several European countries (Germany, France, UK and Denmark) (41). The source was suggested to be contaminated heroin batches and in fact, studies by comparative molecular typing (multilocus variable-number tandem repeat analysis, MLVA, and a broad single nucleotide polymorphism, SNP, analysis) indicate that all anthrax outbreaks among PWID in Europe from the Norwegian case forward might originate from the very same strain, circulating for more than a decade, possibly from one single source of contaminated heroin (42).

Endocarditis

Right sided endocarditis (mainly affecting the tricuspid valve) accounts for 10% of all cases of infective endocarditis (IE) in population based surveys and for a higher proportion of IE in PWID (43). Concurrent involvement of both left and right sided valves may occur (44). The majority of cases are caused by *Staphylococcus aureus* (70%), while infections with streptococcal species or Gram negative organisms, fungi or diphtheroids also occur. Polymicrobial infections and unusual organisms are probably associated with injections with contaminated diluent. An algorithm has estimated that approximately 10% of PWID presenting with fever will have echocardiographic evidence of IE and with bacteraemia this number will rise to around 40% (45). The triad of injection drug use, staphylococcal septicaemia and septic pulmonary embolism is suggested to be pathognomonic for tricuspid valve endocarditis (46). In a study from inner city Vancouver the majority (60%) of hospitalizations between 1994 and 2000 for IE were in PWID (43). The Swedish national register of endocarditis estimated that almost 80% of the right sided cases in 2008-2015 were among PWID (347). The majority of these (80%) were due to *S. aureus*, while streptococcal species, enterococci, coagulase negative staphylocci and Gram negative bacteria were the agents behind 15% of the infections. A few percent were polymicrobial.

The pathogenic mechanism behind the increased prevalence of right sided IE in PWID is suggested to be due to injected particulate matter together with injected bacterial loads (43). Immune function may also be involved, as HIV infection has been suggested as a risk factor for IE, especially as uncontrolled infections with CD4 counts <200/mm3) have been associated with a higher mortality rate in right sided IE (47).

The clinical presentation may be varied and the diagnosis is based on Duke's criteria including examination preferably with transesophageal echocardiography (48). Treatment may involve both surgery and bacteriocidal antibiotics, which usually are given parenterally over several weeks, depending on the organism. This may pose some adherence difficulties for PWID, but as the course of the disease may be extended even in patients receiving adequate antibiotic therapy,

with development of further septic pulmonary embolism and abscesses, in-patient care is recommended at least over the first two weeks (347).

Tuberculosis

Tuberculosis (TB) is a disease associated with poor, crowded living conditions since ancient times, caused by an acid-fast rod-shaped bacterium (*mycobacterium tuberculosis)*, first identified by Robert Koch in 1882. Transmission occurs through air borne aerosol. Thus, PWID living under poor socio-economic conditions with unstable housing including shelters or other congregate settings such as prisons may be at risk for infection. Most infected persons develop latent TB infection (LTBI), with a lifetime risk of active disease of 5-10%. Active disease often affects the lungs, but may engage other organs or multiple sites simultaneously. By tradition, the tuberculin test has been used to detect LTBI, but now the IGRA-test (interferon-gamma release assay) has become widely used.

According to the WHO, there were 9.6 million new TB cases in 2014, the large majority of cases occurring in low and middle income countries, especially in sub-Saharan Africa and Asia. Globally, active TB prevalence among PWID shows wide variations from 0.5% to 66% depending on setting, study populations, case detection methods and TB definition (33). Also in Europe, regional variations were obvious; rates of LTBI infection ranged from 17% to 52%, highest among PWID prisoners in Spain in the early 1990s and lowest among OST centre clients in Estonia in 2007. In certain geographic regions drug-resistant strains are common. TB is also an AIDS-defining condition in HIV-infected PWID. The risk of LTBI progressing to active disease is accelerated by coinfection with HIV (351). As there still is no certain way of knowing who will progress from latent to active infection, it is important to establish methods to efficiently target screening and therapy for LTBI (49). Here, NEPs may be useful by facilitating access to testing for LTBI and case-finding for active TB among PWID. This has taken place in New York as well as in the Baltimore area in the 1990s, when a sharp increase in TB incidence was observed among local PWID (50, 51).

Viral infections

Blood borne viral pathogenic infections, most importantly HIV, HBV and HCV, are often asymptomatic in acute stages, but pose major threats on the long term health for the affected individual and may be further transmitted within the drug using population and beyond. Infections with two other hepatitis viruses, hepatitis A (HAV) and hepatitis E (HEV), have also been observed among PWID. They are often associated with symptoms in the acute phase, but do not develop into chronic infection and they are mainly transmitted by fecal-oral routes. In Malmö, cyclic outbreaks of HAV were noticed among PWID in the 1970s and 1980s and in 1994-95, but they have since then not circulated among PWID in this region. Vaccination against HAV was introduced in the Malmö NEP in 1999. In Denmark, anti-HEV was prevalent in 17% of tested prisoners and PWID in a study published in 2002, but the authors concluded that this was not related to risk factors for blood borne or sexual transmission (52).

Following outbreaks of jaundice after vaccination campaigns in the 1940s and 1960s among army servicemen, it became apparent that unsafe injections could lead to transmission of blood borne infections and guidelines were issued for sterile equipment for each injection (53). Jaundice epidemics occurring among PWID were also acknowledged in the same period, as a warning signal for how fast and efficiently blood borne viruses were transmitted in high risk groups and a sort of a prequel of things yet to come. Nobody could, however, anticipate the magnitude and consequences of HIV infection in general and among high risk groups in particular. However, after the discovery of HCV it was observed that HCV prevalence and incidence exceeded those of both HIV and HBV, making HCV the most common pathogen with blood borne transmission among PWID. The viral structures, pathogenesis, epidemiology, prevention and treatment of HIV, HBV and HCV will be discussed below.

HIV

Viral structure and pathogenesis

After the observation of the first cases of acquired immune deficiency syndrome (AIDS), several converging lines of research linked a human T-cell lymphotropic retrovirus, human T-lymphotropic virus type III (HTLV-III), to this clinical syndrome (54). The causative agent was identified in 1983 and later renamed human immunodeficiency virus, HIV (55). The complete genome was described in 1985 as a 9.7 kilo base, plus stranded, enveloped RNA virus with long terminal repeat structures, long open reading frames encoding gag (group-specific antigen, coding for the viral capsid proteins), pol (polymerase, coding for reverse

transcriptase), and env (envelope-associated proteins) genes (56, 57). The RNA genome of HIV is reversely transcribed into DNA, which is integrated with cellular DNA. It is then transcribed into viral RNAs from the incorporated DNA and further translated into protein from the viral RNA. The viral genome contains major genes (gag, pol, and env) and also accessory genes (tat, rev, nef, vpr, vif, and vpu/vpx) promoting virus infectivity and modulating host cell functions (58). Particularly nef and tat (transactivator of transcription) are involved in the cytopathic changes following infection, leading to either rapid cell death, persistent viral replication or latency (59). The gag encoded capsid protein p24, the reverse transcriptase and the env encoded large glycoprotein gp160 (cleaved into two smaller ones, gp120 and gp41), are used for diagnostic antibody testing. Detection of HIV RNA is used to monitor outcome of antiretroviral treatment. The virus primarily binds to CD4 molecules on T helper cells, but also enters other cell types (macrophages, dendritic cells, Langerhans cells, B cells and granulocytes) (60). Due to genetic drift of neutral mutations and natural selection, the virus evolves and changes its preferred target cell subtype during the course of the infection (61). After binding to the CD4 receptor, the virus binds to chemokine receptors on the target cell surface to allow cellular entry (CCR5 and CXCR4).

HIV has been classified into two distinct virus types (HIV-1 and HIV-2) and the most important, HIV-1, further into subtypes or clades (with clade C causing approximately half of all infections globally) (59). Of the two HIV genotypes, HIV-1 is by far (95%) more prevalent globally and expanding, while HIV-2 is endemic and most common in West-Africa. HIV-2 is associated with a slower disease progression (only 20-30% of those infected proceed to AIDS) and dual infection with both viruses has also been shown to progress more slowly than infection with only HIV-1 (62).

HIV pathogenesis is multifactorial and complex. Persistent immune activation through different pathways is a major mechanism of disease progression, increasing the turnover and eventual exhaustion of uninfected T cells, altering the function of these cells and other important components of the immune system. The subsequent loss of CD4+ cells finally disarms the host's immune defense system, leaving the host susceptible to a range of opportunistic infections (such as pneumocystis pneumonia, Kaposi sarcoma and tuberculosis). The current treatment guidelines recommend treatment initiation long before critical levels are reached (63).

HCV and HIV coinfection is common, with a prevalence of 45-90% in some PWID cohorts (64). HIV influences the progression of HCV disease through several mechanisms; increased HCV replication, a decreased rate of HCV clearance during acute infection, and accelerated fibrogenesis (the latter likely to be partly mitigated by HIV ART) (64). Interestingly, the increased hepatic

inflammation by combined exposure to HCV and HIV has been shown to be further exacerbated by morphine (the bioactive product of heroin) (65). Altered T cell subset distribution has been observed among PWID with triple-infections with HIV, HBV and HCV, which, however, was suggested to be mainly attributed to HIV infection and/or injection drug use (and not HBV and/or HCV) (66).

Also, there is growing evidence for accelerated neuropathogenesis in HIV infected opioid users, due to selective increases in microgliosis and opioid enhanced HIV-mediated neuronal death and delayed recovery of injured neurites (67). HIV-1 proteins, such as gp120 and tat, released by infected glia have been implicated as possible mediators of neurotoxicity, but differences have been observed between opioids in their neurotoxic and neuroinflammatory interactions with tat. Methadone has been found to interact with tat to increase production of chemokines (CCL5/RANTES), while buprenorphine was partially neuroprotective at a low concentration, possibly due to its unique pharmacological profile at multiple opioid receptors (68). It has also been suggested that methamphetamine may influence HIV-related pathology by converging with HIV proteins (gp120, gp41, tat, vpr and nef) on various pathways causing neuronal apoptosis. (69, 70).

Diagnostics

Soon after the discovery of HIV in 1983, the first serum antibody ELISA and Western blot tests were developed for diagnostic measures (71). HIV-1 antigen tests aimed at p24 proved thereafter especially valuable in diagnosis of primary infections (72-74). Measuring viral load by PCR is widely used since 1996 to monitor disease progression in untreated patients (the highest levels found at infection, usually followed by a decline and thereafter again increasing) and in patients on treatment to follow treatment efficiency and identify suspected drug resistance (75, 76).

Undiagnosed HIV infections are still a major problem globally, with a heavy impact on morbidity, mortality and onward transmission especially in high risk groups (PWID, MSM). Late presenters, defined as <350 CD4+ T-cells/mm3 or AIDS at diagnosis, constituted approximately half of the new HIV cases in Europe in 2010-2013, with an increase in late presentation in both male and female PWID (77).

A prerequisite for testing to take place is easily accessible testing facilities. To increase testing, especially among high risk groups, several methods have been applied. These include opting out-strategies from tests offered at STD clinics, trigger-based testing for hospitalized patients, HIV rapid tests based on HIV-antibody detection in saliva or in blood as well as self-testing in high risk settings (78-81). Failure to return for results and false perception of one's serostatus still

remains a considerable problem, associated with conditions such as unstable housing and unemployment (79).

Epidemiology

By the time of the identification of HIV, pandemic spread of HIV-1 had already been established and a subepidemic dominated by subtype B had found its way from Africa via Haiti to the USA (82). From the American continent and the Caribbean subsequent spread to the rest of the world occurred, with multiple introductions in Western Europe among both MSM and PWID, while Central and Eastern Europe remained isolated for the most part of the early epidemic, probably due to the low population mobility for political reasons (83).

The global HIV incidence reached its peak in 1997, at 3.3 million new infections, but showed a declining tendency between 1997 and 2005. This coincided with coordinated interventions; such as the creation of the Joint United Nations Programme on HIV/AIDS (UNAIDS) in 1996, the Global Fund to Fight AIDS, Tuberculosis and Malaria in 2002 and the US President's Emergency Plan for AIDS Relief (PEPFAR) in 2003. Large amounts of global funding have thus been aimed at HIV preventive measures and treatment, resulting in an overall decline in HIV mortality in low and middle income countries since 2004, from a peak of 1.8 million deaths in 2005 to 1.2 million deaths in 2015 and a stable annual incidence since 2005 at about 2.6 million per year (84).

Of the estimated 15.9 million PWID worldwide, 3.0 million are thought to be HIV positive (thus accounting for approximately 10% of the 33 million people living with HIV worldwide) (11). Large regional variations have been observed, with the highest numbers of PWID found in China (mid-estimate of HIV prevalence 12%), the USA (HIV 16%) and Russia (HIV 37%). Overall, HIV prevalence among PWID was estimated to 20–40% in five countries (in Europe, Asia and South America) and over 40% in nine (in Europe, Asia, Africa and South America). Country-level midpoint HIV prevalence ranged from less than 0.01% to 72.1% (Estonia). The percentage of HIV among PWID in Sweden was estimated at 5.4% (11).

Approximately 12 000 cases of HIV have been reported in Sweden since 1983. By 1986, 1200 HIV positive cases had been diagnosed, two thirds in the Stockholm region, 60% of whom were estimated to be infected through male-to male sex and 30% through injection drug use (85). Aiming at avoiding further HIV transmission among PWID in Sweden, the first NEP was opened in Lund 1986, followed by one in Malmö 1987. During the time of studies I-III of this thesis (1997-2005), in 2001, an increase in HIV infections among PWID in the Swedish capital region was observed. Although this increase could be related to improved contact tracing, some of the HIV non-subtype B cases were linked to the outbreaks in the Baltic

Sea Region (86). Furthermore, the common origin of a new wave of HIV transmission among PWID in Stockholm in 2006, was a recombinant virus (CRF01_AE) imported from Finland (87).

Around 7 000 persons are living with HIV in Sweden today, resulting in a low overall HIV prevalence at 0.07 %. During the past 5 years approximately 450 new cases have been diagnosed annually, many of non-Swedish origin and already diagnosed prior to arrival in Sweden (88).

Prevention

There is no available HIV vaccine. Several strategies to prevent the acquisition or onward transmission of HIV have been launched; a wide array of biomedical approaches using agents or devices that block virus spread either physically (male and female condoms) or chemically (pre-exposure prophylaxis, PrEP, applied systemically or topically) combined with behavioural support strategies based on information, motivation and skills (89). A key issue is the correct knowledge of one's own HIV status. The concept of expanded testing, direct linkage to effective care and adherence to antiretroviral therapy is known as "Treatment as Prevention" (TasP). Initially aimed at preventing mother to child transmission, implementing TasP in other high risk settings such as among PWID also have considerable clinical benefits and reduce further transmission.

Post-exposure prophylaxis (PEP) has long been used in HIV management, while the concept of pre-exposure prophylaxis (PrEP) was more recently introduced. PrEP typically consists of a once-daily oral tenofovir or tenofovir-emtricitabine regimen for HIV-negative persons with expected high risk behaviour (mainly MSM). This concept might perhaps be adjusted for implementation also for PWID in the future (90, 91).

Treatment

The introduction of highly active antiretroviral therapy (HAART, now known as ART) in 1996 turned HIV from a death sentence into a manageable, chronic disease. Still, lifelong treatment with continuous adherence is necessary and can be difficult to achieve among high risk groups such as PWID. Antiretroviral drugs (ARVs) are classified by the viral life-cycle step they inhibit or by their chemical structure; (non)nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs) and inhibitors of integrase, protease, CCR5 and fusion, respectively. ARVs have to be given as combination therapy in order to reduce the risk of selection of drug resistance.

It has been estimated that approximately 40% of people living with HIV receive ART worldwide, with large regional variations. The number of HIV positive PWID estimated to receive ART varied from less than one recipient per 100 HIV

positive PWID (Chile, Kenya, Pakistan, Russia, and Uzbekistan) to 100 recipients per 100 HIV-positive PWID in six European countries (Finland, Germany, Greece, Slovakia, Spain, and the Netherlands (92). Sweden has recently been declared to be the first country to reach the UNAIDS/WHO 90-90-90 goal of HIV care targets (90% of all people living with HIV should know their HIV status, 90% of those diagnosed should receive ART and 90% of those should have durable viral suppression, meaning that 73% of all HIV-infected individuals should have a suppressed viral load) (93, 94). Vaccine trials and curative treatment has so far been unsuccessful, due to viral immune escape mechanisms in addition to the multitude of entry mechanisms as well as the persistence of long-lived, latently infected, resting memory CD4+ T cells (60, 95).

HBV

Viral structure and pathogenesis

HBV belongs to the family *hepadnaviridae* and is an enveloped, partially double stranded DNA virus that replicates via an RNA intermediate. The first lead to this virus was detection of an antigen in blood samples from Australian aborigines by Baruch Blumberg (later Professor of both Medicine and Anthropology and receiver of the Nobel Prize in 1963 for this breakthrough finding). This "Australia antigen" was initially linked erroneously with leukemia and trisomy 21, but quite soon the true connection to serum hepatitis was revealed by transfusion specialist Harvey Alter (96, 97). It was soon detected that the Australia antigen was a protein produced in large amounts as small, rounded or filamentous, uninfectious particles (Hepatitis B surface Antigen, HBsAg).

In 1970 the HBV infectious viral particle (named the Dane particle after its discoverer) was identified as a 42 nm double shelled particle with HBsAg on its surface. Dane particles represented only a minority of the HBsAg particles in blood (98).

The Dane particle (HBV virion) consists of the outer envelope, with lipids and HBsAg, and the inner nucleocapsid (core), containing the hepatitis B core antigen (HBcAg), enclosing the double stranded virus DNA and a DNA polymerase (which, interestingly, does not finish synthesizing one strand before the viral capsid is closed). In the hepatocyte nucleus, the DNA polymerase carried with the virion converts the partially double stranded gapped circular genomic DNA into a relaxed covalently closed circular fully double stranded molecule (HBV cccDNA), which then remains in the cell nucleus and serves as the transcriptional template for HBV RNA production. The virus is transferred to the liver cell membrane and released into the bloodstream, leaving a single-stranded gap in its viral DNA) (96). Another antigenic determinant, HBeAg (discovered in 1972 by Swedish virologist

Magnius) is a partially prematurely terminated form of the HBcAg gene. HBeAg circulates in the blood as a soluble protein and is associated with greater infectivity (99). Seroconversion to its corresponding antibody, anti-HBe, is associated with a lesser risk for transmission.

The nucleotide sequence of the HBV genome consists of four large open reading frames (ORFs), all coded on the minus strand; the core, pol and surface genes encoding the HBcAg, DNA polymerase/reverse transcriptase and HBsAg, respectively. A fourth gene, the X gene, is thought to regulate the level of transcription of viral genes and is a potential factor in viral hepatocarcinogenesis (100).

Eight HBV genotypes (A–H) have been defined based on nucleotide sequence divergence, with varied geographic distribution (genotypes A-D being the most prevalent globally), as well as reported variations concerning disease progression and response to interferon (101-103).

Acute infection is asymptomatic in approximately half of the adult cases and the other half is associated with symptoms as jaundice, nausea, pruritus, flu-like symptoms (hepatosplenomegaly might be present, but is more often seen in reactivated infections, which otherwise might be difficult to distinguish clinically from an acute infection) (104). Many of those infected vertically (>90%) or in childhood (20-30%) become chronically infected, compared to only around 5% of those infected as adults (105). Conversely, in children symptomatic acute infection is much less common than in adults.

The pathogenesis of chronic HBV infection is complex and also influenced by several factors (in particular immune response, but also age, gender, concomitant infection with HCV and/or HIV and alcohol consumption). Chronic HBV infection has traditionally been divided into four phases based on the virus–host interaction: immune tolerance (recently renamed non-inflammatory phase), immune clearance (renamed inflammatory phase), low or non-replication, and reactivation (106, 107). The first phase is characterized by the presence of HBeAg and high levels of HBV DNA, but with no or little elevation of alanine aminotransferase (ALT) or histological findings. This HBeAg-positive phase might last 10-30 years in perinatally infected patients, while it can pass quickly or be absent in persons infected in adulthood. In the immune clearance phase the markers of liver inflammation are elevated, with fluctuating and then decreasing levels of HBV DNA and subsequent seroconversion to anti-HBe. The low or non-replication phase after HBeAg seroconversion is characterized by low or undetectable levels of HBV DNA and liver disease remission (inactive carrier state). Due to HBV variants not expressing HBeAg, disease progression may still occur at a rate of 1–3 per 100 person years following HBeAg seroconversion (105).

In patients with HBeAg negative chronic hepatitis, cirrhosis incidence rates have been estimated to 2.8 and 9.7 per 100 person years in East Asian and European countries, respectively. Beyond that, the 5-year cumulative risk of developing HCC in the cirrhotic patient has shown some regional variations (estimated to be 17% in East Asia and 10% in the Western Europe and the United States), while the 5-year liver related death rate is estimated to 15% in Europe and 14% in East Asia (105).

Even in some persons with anti-HBc but no detectable HBsAg left, low levels of HBV-DNA may still be found in serum and liver tissue. Termed occult HBV infection (O-HBV), this state has been associated with HBV reactivation, advanced liver fibrosis and cirrhosis and the development of hepatocellular carcinoma. Importantly, immunosuppression in such patients (due to chemotherapy, organ transplantation, corticosteroids and uncontrolled HIV infection), can lead to reactivation of hepatitis B. Highly divergent findings on the prevalence of O-HBV among PWID have been reported, ranging from 0-45% (108, 109).

Furthermore, a new antigen-antibody system associated with HBV was reported in 1977 by Rizzetto et al (110). This new antigen, first named delta, was an unrelated defective RNA-virus – hepatitis D (HDV). It is dependent on helper functions from a simultaneous HBV infection to establish and maintain infection. Such a superimposed hepatitis D (HDV) infection may lead to a more rapid progression to cirrhosis despite low replication of HBV (111). Several outbreaks of HDV have been observed among PWID globally, the first case in Sweden in a chronic HBsAg-carrier in Malmö in 1973. A subsequent analysis found that this introduction of HDV among the Malmö PWID led to an increase in anti-delta prevalence to 72% among the chronic HBsAg carriers in this population in 1979-1981 (112). More recently, high and increasing prevalence rates of HDV infection have been observed among PWID in other world regions (113).

Diagnostics

Specific serologic assays aimed at the viral antigens (HBsAg and HBeAg) and antibodies (anti-HBs, anti-HBc, and anti-HBe) are used to determine the presence and phase of HBV infection. The presence of HBsAg and anti-HBc IgM indicate acute infection, while persisting HBsAg for longer than 6 months (with anti-HBc IgG) defines chronic infection. The presence of anti-HBc without the antigens may often indicate resolved infection, but anti-HBs is an accepted marker for resolved HBV infection. The exclusive presence of anti-HBs (>10 mIU/ml) indicates immunity from vaccination.

In recent years standardized assays for the detection and quantification of HBV DNA have been widely used in assessing the relative risk of developing liver

disease and when to start treatment as well as monitoring treatment response (114).

Quantitative analysis of levels of HBsAg and HBeAg may also be used to monitor the natural course of HBV infection and the treatment response, while the correlation with HBsAg levels and intrahepatic total HBV DNA might not be completely consistent (115).

Epidemiology

An "icterus epidemic" had been recognized by Lürmann in 1885, in the aftermath of a small pox vaccination campaign (96). Subsequent post-vaccination outbreaks of hepatitis were observed in recipients of yellow fever vaccine; the largest in 1942 among U.S. American Army personnel with 50,000 clinical cases and probably 280,000 additional subclinical HBV infections (116, 117).

HBV is highly infectious and more easily transmitted than HIV and HCV through parenteral, sexual and perinatal transmission routes (12). The mode of transmission is of importance and especially relevant with transmission among PWID, ten or less virus particles are sufficient to start a HBV infection if they are injected intravenously (levels too low for detection by the most sensitive screening techniques), while transmission from small wounds and intimate mucocutaneous contact may occur from a highly viremic person ($>10^7$ viruses/ml plasma) but is rarer at viral levels lower than 10^5/ml (96).

HBV infection is a serious global public health problem, accounting for 500,000-1.2 million deaths per year and is annotated as the tenth leading cause of death worldwide (118). The absolute number of HBsAg-positive individuals worldwide was estimated to 240 million in 2005. The area with the highest HBsAg prevalence (>8%) is Western sub-Saharan Africa, followed by regions with high intermediate prevalence of 5–7% (Eastern sub-Saharan Africa, Asia, Oceania) and low intermediate prevalence of 2–4% (Latin America, Eastern Europe, North Africa, the Middle East) and low prevalence settings of <2% (North and Central America and Western Europe), with some country-specific exceptions. The prevalence of chronic HBV infection decreased in most regions during 1990-2005 (mainly in Central sub-Saharan Africa, Tropical and Central Latin America, Southeast Asia and Central Europe) (118). As most chronic HBsAg carriers are infected from HBeAg positive mothers or other family members in childhood and the strongest HBsAg decline has been observed among children in South East Asia and elsewhere, the prevalence decline is attributed to the expanded immunization program (119-121).

Approximately 14 million people in Europe are chronically infected with HBV, resulting in around 36 000 related deaths annually (122). Worldwide, 6.4 million PWID of the total number of 16 million are estimated to be anti-HBc positive and

1.2 million HBsAg positive (12). HBV exposure levels among PWID varied widely across countries, from 4.2% (Slovenia) to 85% (Mexico) and HBsAg ranged from 5–10% in 21 countries and over 10% in 10 (the highest HBsAg prevalence observed in endemic, often Asian, countries) (12).

Prevention

An ethically much criticized vaccination approach in institutionalized children by Krugman in the early 1970s showed that the concept of HBV vaccination was possible. This was followed by several trials, including early studies with plasma-derived vaccines (123, 124). That source of HBsAg was however discontinued, when some of the HBsAg carrier plasma donors used for vaccine production developed AIDS, which stirred up concern. As a safe method, recombinant HBsAg produced in yeast or mammal (CHO) cells soon replaced the plasma derived vaccine (125). Vaccination against HBV was implemented in Taiwan in 1984. Here, vaccination of newborns together with HBV immunoglobulin to infants of high risk (HBsAg and HBeAg positive) mothers have been shown to reduce the number of HBV carriers in the juvenile population as well as the incidence of HCC among children aged 6–14 years (105, 126). In 1986 the yeast-derived HBsAg (not completely identical to the natural HBsAg, but immunogenic) became the standard vaccine against HBV and proved to be inexpensive, highly protective and well tolerated (96).

In 1992 the WHO recommended implementation of universal childhood vaccination and it is estimated that 90% of countries routinely vaccinate newborns against HBV and approximately 70% are now delivering 3 immunization doses (127). Until recently, HBV vaccination in Sweden has only been offered to health care workers and high risk populations, such as children born to mothers with chronic HBV infection, MSM, partners to HBV-positive persons and PWID. Now HBV vaccination is gradually being introduced in the childhood vaccination program also in Sweden. For PWID, the WHO promotes incentives to increase uptake and completion of HBV vaccination, for example through NEPs (128). Promising results have been observed for HBV vaccination through NEPs in the USA and for PWID attending drug treatment centres in Denmark (129, 130).

Treatment

All HBV viral nucleic acid originates from cccDNA in the infected hepatocyte, but cccDNA can at present not be specifically targeted for treatment or elimination. The efficient common approach is viral suppression by nucleotide/nucleoside analogues (NAs), however, such treatment has to be continued for extended periods of time (several years or even lifelong), since the risk of recurrence after discontinuation is high. Current guidelines recommend therapy in HBeAg negative patients until HBsAg seroclearance, which only takes place rarely and even in

those cases cccDNA will remain (131). The risk for resistance induction was most pronounced against first-generation drugs (such as lamivudine and adefovir). More recently added nucleoside analogues entecavir and tenofovir (with low risk of resistance) are now recommended as the first-line treatment of chronic HBV infection (132, 133). Access to the necessary baseline and follow-up health care contacts might however be subjected to regional variations and group-specific limitations (as for PWID) (134, 135).

There is a risk for HBV rebound and reactivation after discontinuation of NA therapy, which might be higher among PWID with sudden inability to adhere to treatment (136). Since reactivation can be serious and sometimes more fatal than the primary infection (around 10%), antiviral treatment should be considered also for this reason (137).

As many of the antiretroviral agents used to treat HIV infection also have activity against HBV, co-infected patients may reactivate their HBV infection when HIV treatment is adjusted (especially when drugs with activity against HBV such as lamivudine, tenofovir and emtricitabine are discontinued) (137). Patients with HIV infection should thus be tested for HBV markers and patients with HBsAg and /or anti-HBc should not be switched away from agents with anti-HBV activity.

HCV

Historical background

The existence of a parenterally transmitted type of viral hepatitis that was neither hepatitis A nor B was suggested by Prince and Alter in the 1970s (138, 139). The agent, provisionally named hepatitis non-A, non-B, withstood identification for a decade and a half until it was identified in 1989 by Houghton and co-workers (140, 141). The discovery went over a chimpanzee, which had been experimentally infected with a contaminated serum derived clotting factor preparation, from which a molecular combinatorial expression library was built (142). Out of many thousands of clones a single one expressed a protein that correctly identified coded samples from identified persons, who had developed post transfusion non-A, non-B hepatitis. From this first lead, the whole viral RNA was determined from overlapping cDNA clones (141). Also, the relevant viral proteins suitable as antigens were identified (143, 144). The virus was named hepatitis C virus (HCV).

Viral genome organization and structure

The HCV genome consisting of about 9400 nucleotides is a single stranded, positive sense RNA molecule, classified as a *Hepacivirus* in the family *Flaviviridae* (145). The single open reading frame is translated to a single polypeptide and then cleaved by host peptidases or viral proteases into structural (core and envelope 1 and 2) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Figure 1). The NS2 and NS3 proteins serve as proteases, the NS3 also has a helicase function and NS5B is an RNA polymerase. NS4A and NS4B correctly orientate the replication complex to a vesicular structure, the membraneous web where HCV particles are made. They leave from the infected hepatocyte via metabolic pathways, that are involved in lipid metabolism and the HCV virions circulate in blood partly hidden in a so called lipoviral particles.

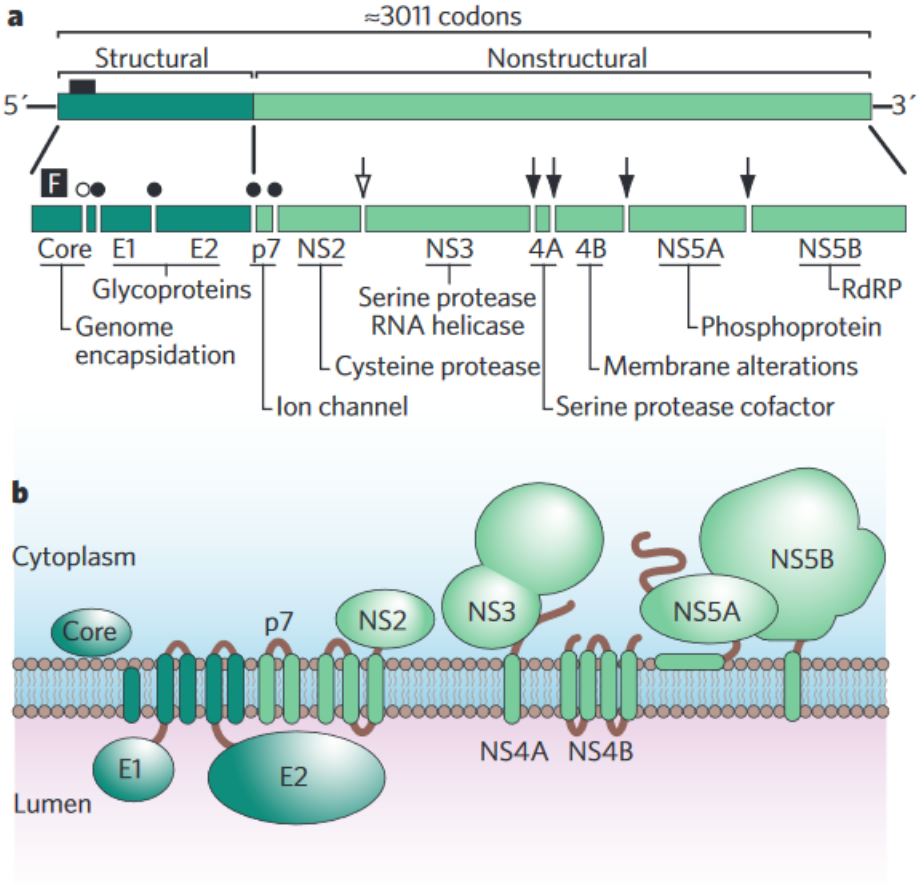


Figure 1. HCV genes and gene products.
A. The structure of the viral genome, including the long open reading frame encoding structural and nonstructural genes, and 5' and 3' NCRs. The polyprotein processing scheme is shown below. Closed circles refer to signal peptidase cleavage sites; the open circle refers to the signal peptide peptidase cleavage site. B.The topology of HCV proteins with respect to a cellular membrane. Lindenbach BD, Rice CM. Unravelling hepatitis C virus replication from genome to function. Nature. 2005 Aug 18;436(7053):933-8. Review. Reprinted with permission from Nature Publishing Group.

The virus attachment process is very complex and involves a number of cellular molecules such as the Very Low Density Lipoprotein Receptor, Scavenger receptor B1, CD81, Claudin 1 and Occudin. The reason for this complexity is poorly understood, but some are involved in lipid metabolism which the virus hijacks (146).

HCV has a great diversity. There are globally at least seven different major genotypes differing at least by 30% at the nucleotide level and within each genotype (except genotype 5a) and a large number of subtypes (a-z and beyond)

(147). The most conserved region is the 5´- untranslated region, which therefore is the most suitable target for RNA detection by PCR. The Envelope 2-region contains a hypervariable region that is used as a decoy by the virus to evade the immune system, since it undergoes continuous mutations and also at the same time has a variety of sequences (quasispecies). The viral core and the NS3 and NS4 protein are more conserved, making them suitable antigens in antibody assays.

It is not yet possible to culture HCV strains from infected patient sera in the laboratory, with very few exceptions. However, partial genomes, so called replicons, representing the entire viral enzyme machinery have been developed in human hepatoma cell lines. Such replicons and also recombinant systems representing all genotypes have become key components, enabling further research and the development of DAAs (148).

Diagnostic testing

Exposure to HCV is determined by serological methods, currently based on the presence of anti-HCV antibodies. The antibody assays have evolved from the first generation assays aimed at NS4 to utilize additional recombinant proteins in the HCV core and NS3-5 in the second and third generation tests (149). For many years, including during sample collection for the studies in this thesis, the Recombinant ImmunoBlot Assay (RIBA) test aimed at antibodies to specific antigens (C100, C33, C22 and NS5) was used to confirm the findings of the screening test (reaction to at least two of the antigens was required). Since 2013 the RIBA test has been replaced by detection of HCV RNA by PCR (150). HCV core antigen detection in serum can be used as a surrogate marker for viral replication, but HCV RNA PCR is gold standard for determination of a current infection. HCV RNA is detected either through amplification of target RNA using reverse transcription polymerase chain reaction (RT-PCR) or transcription mediated amplification (TMA). In the last decade RNA detection is done with quantitative real-time RT PCR tests.

Since HCV RNA is detectable 7-21 days after infection and the development of anti-HCV is delayed about 8 weeks (range 3-12 weeks) on average, early infection in this window phase is only detectable by HCV RNA PCR or possibly with the HCV core antigen test (which is about 10 times less sensitive than HCV PCR). Development of anti-HCV may be considerably delayed (months) in immunocompromised patients, such as transplant patients or individuals co-infected with HIV (151). Chronic HCV infection is defined as persistence of HCV RNA with >6 months interval.

In clinical praxis, genotype/subtype determination is performed by reverse hybridization or sequencing of a suitable subgenomic region such as the core, envelope 1 or the NS5B-region. Lately, analyses of the hypervariable regions or

even the full genome provide more detailed information if needed for outbreak analyses and scientific or forensic purposes (152).

Diagnostic challenges for PWID

Access to diagnostic testing may be limited or subjected to challenges on various levels for high risk groups, such as PWID, concerning recruitment to initial testing, follow up with feedback on test results and linkage to further examination and care. Studies from the UK have shown that the absolute majority (90%) of HCV infections acquired in the UK are among current or former PWID, while general practice is the single most important setting for HCV testing (30 % of all tests) and for identifying positive patients (30 % of all positive results) (153). Here, an intervention aiming at HCV case finding through practice staff training, use of an electronic patient record search to identify individuals at higher risk of HCV infection with subsequent offering of HCV testing was found to be cost-effective among those aged 30–54 in relation to quality-adjusted life years (QALYs). The intervention was more cost-effective for PWID with an ongoing risk of transmitting HCV. Through targeted case-finding, even previously diagnosed but "lost" patients may now be offered an opportunity for reconsideration of assessment and treatment (154).

Also, as diagnostic measures for liver fibrosis recently have been facilitated by the transition from liver biopsies to non-invasive transient elastography, the interest for assessment of liver damage from the patient's perspective should increase.

In the USA, targeted testing of the 1945–1965 birth cohort for HCV was found to be cost-effective above a certain threshold HCV prevalence (155). By screening individuals with a history of intravenous drug use, blood transfusion prior to 1992, immigration or elevated ALT levels 80% of unknown HCV RNA-positive cases are estimated to be found (156). In Sweden in 2007, the National Board of Health and Welfare recommended through public campaigns intensified identification and offering of HCV-screening to all whom 1965-1991 had been at risk through blood transfusion due to surgery, cancer or neonatal care. In 2010 women having received blood transfusions during pregnancy or delivery were included. The campaign resulted in >65 000 screening tests and diagnosis of 600 HCV infections (350 more than expected) transmitted through transfusions in Sweden before 1992 (most tests were from women transfused during pregnancy or delivery) (157).

Blood samples dried on filter paper (dried blood spots, DBS) taken through capillary blood sampling are an option when aiming at increased testing of high risk groups such as PWID. Besides being easier and more convenient than venepuncture for both patients and staff, they also facilitate transport logistics by not requiring prompt removal of the cellular components and by the fact that antibodies, many medications and their metabolites as well as nucleic acids

usually remain stable for long periods. False negative anti-HCV results have, however, been observed in some patients with presumably long since resolved HCV infections, which should be taken in consideration when applying DBS testing to high anti-HCV prevalence groups like PWID, with a substantial absolute number of people with resolved HCV infections (158).

Experiences from several outreach approaches for counselling and testing (C&T) among PWID performed and/or studied by researchers at the Burnet Institute in Australia have been summarized to "Our experience is that brief messages – reiterated over time – are more likely to be remembered by participants, as opposed to large amounts of information conveyed during long sessions" (159). Adapting to the individual's need, interest and available time for C&T is of uttermost importance, making general guidelines less applicable than for non-PWID settings. In Melbourne, the combination of established primary health care centres for PWID around drug 'hot-spots' with active and flexible outreach approaches including peer workers and permitting self-collected blood sampling contribute to improved diagnosing of HCV infections and linkage to further care. As for self-sampling, the number tested for STDs rose from 9% to 32% if self-collected sampling was allowed (160).

Evidence for behaviour change in PWID, reducing the potential for HCV transmission and other drug-related harm have been shown by inclusion of HCV high-quality counselling and testing through a NEP by staff and well-educated peer counsellors (161).

Pathogenesis and disease burden

There is strong evidence for the association of HCV with liver-related mortality based on cirrhosis and HCC and on other medical conditions such as type 2 diabetes mellitus, rheumatological diseases, psychiatric morbidity such as depression and quality of life impairments (162). In high-income countries, HCV-associated disease is the leading cause for liver transplantation and of HCC (163, 164).

Most acute infections are asymptomatic and may go by unnoticed, especially among PWID who might not seek health care for diffuse symptoms due to various reasons. Many chronic HCV infections are associated with at least subtle symptoms, sometimes only reported by recently cured patients who notice the disappearance of fatigue, myalgia or arthralgia.

A systematic review and meta-analysis estimated the prevalence of spontaneous viral clearance of HCV infection among PWID to 24.3 % (165). The clearance rate was higher (25.7%) among HIV-negative individuals compared to those HIV-positive (16.1 %). Male gender and HIV-positivity were significantly associated with lower chances of spontaneous viral clearance in multivariate analysis (but

since the temporal relation between HIV and HCV infections was unclear the effect of HIV on viral clearance of HCV could not be further established).

Polymorphism of interferon lambda 4 (IFNL4) genotype (SNP rs12979860; CC vs CT/TT) (formerly known as interferon lambda 3 and interleukin 28B) is associated with viral clearance (166). A study with pooled data from 9 prospective cohorts from different geographic regions (Australia, The Netherlands and the USA) consisting of individuals with viral clearance obtained either spontaneously or through viral suppression observed a HCV reinfection incidence of 12.3 cases/100 person-years. Peak HCV RNA levels were lower during reinfection than primary infection and the rate of reclearance 6 months after reinfection was high, at 52%. Reclearance was four times more likely to occur for females with the IFNL4 rs12979860 CC genotype (167).

A large meta-analysis (n >33 000) estimated stage-specific fibrosis progression rates and observed a non-linear disease progression, correlated to duration of infection. The estimated prevalence of cirrhosis at 20 years after the infection was 16%, factors associated with fibrosis progression including older age at infection, male gender, heavy alcohol use, HIV coinfection, study design factors, mode of acquisition and HCV genotype (168). Genotype 3 has been associated with induction of insulin resistance, steatosis and with more rapid fibrosis progression (169).

As many PWID are infected with HCV early (within a few years) after the debut of injection drug use, chronic HCV infection liver sequelae (including HCC) are estimated to occur in mid-to late-adulthood (average time to F3 26-38 years and to cirrhosis 34-46 years post-infection) (170, 171). The pooled incidence rates of compensated cirrhosis were estimated to 6.6, decompensated cirrhosis 1.1, and HCC 0.3 events per 1000 person-years, respectively (172).

Epidemiology

The global prevalence of anti-HCV is estimated to 115 million individuals (1.6% of the total population), of whom 70% (80/115 million) are chronically infected (173). Regional variations are substantial (Figure 2).

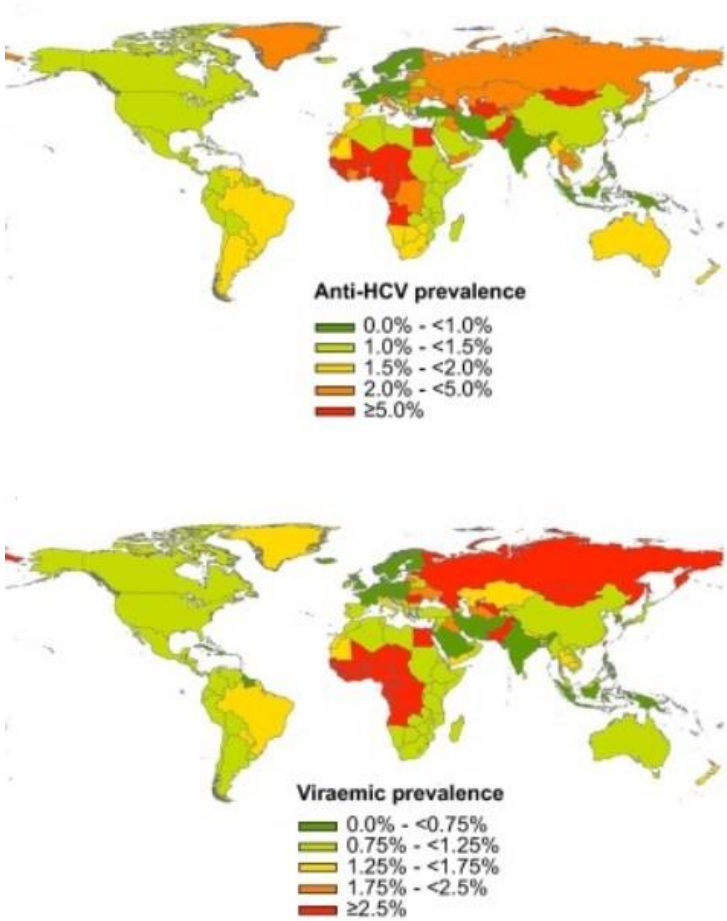


Figure 2. Reported and estimated HCV prevalence and infections among adults.
Above: Anti-HCV prevalence – adults (reported and estimated). Below: Viremic prevalence – adults (reported and estimated). Adapted from Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014 Nov;61(1 Suppl):S45-57. Permission to reprint through the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY NC ND).

Previous studies based on pooled, regional estimates found anti-HCV prevalence to be higher, at 185 million in 2005 (174). HCV infection is mainly spread parenterally and to a much lesser extent permucosally. An important route of HCV transmission before blood donor screening was implemented, was through transfusion of blood and blood products (175). Poor handling of percutaneous procedures have probably created many recognized and unrecognized transmission events and are still a reality in many low income settings (176-178). However, in

many world regions HCV transmission occurs mostly among PWID through shared needles, syringes and other drug paraphernalia. HCV infection is endemic among PWID with a high prevalence of 43-80% (165, 179). Approximately 10 million of the total 16 million PWID are anti-HCV positive (11). In Europe approximately 4.5 million PWID are estimated to be anti-HCV positive with 2.0 million viremic individuals (180).

Among European PWID HCV incidence rates range from 2.7–66 infections/100 person-years at risk (PYRs) (median 13100 pyr), depending on geographical regions and settings studied (181). Thus both the prevalence and incidence of HCV exceed that of HIV and HBV among PWID. Data for Sweden, although representing regional samples from high risk settings, show high anti-HCV prevalence (Figure 3).

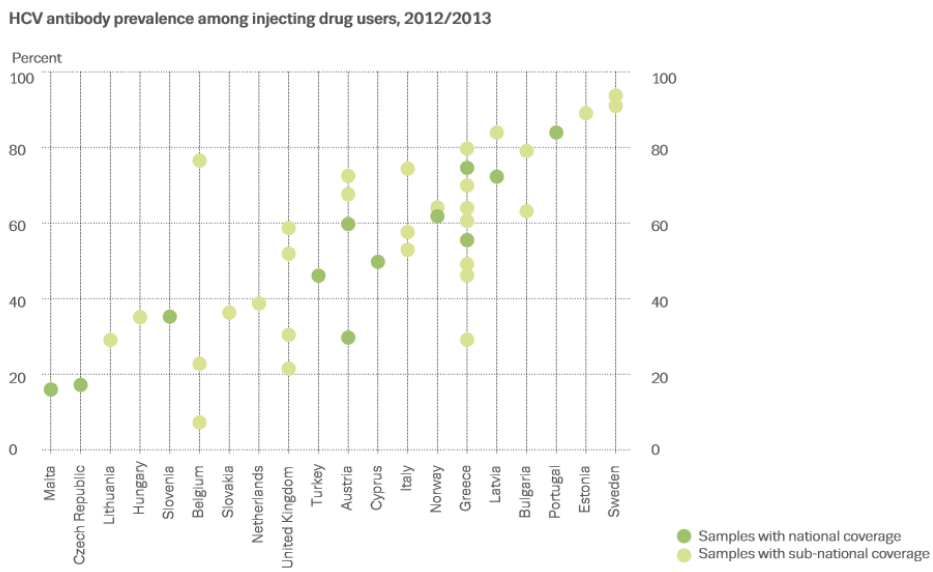


Figure 3. Anti-HCV prevalence among PWID in Europe 2012-2013.
European Drug Report. Trends and developments 2015. Reprinted with courtesy of European Monitoring Centre of Drugs and Drug Addiction (EMCDDA).

Although HCV transmission is mainly parenteral, sexual transmission may occur. Sexual HCV transmission is especially common among HIV-positive MSM and is linked to high risk sexual behaviour (182). A rising HCV incidence and high degrees of HCV-clustering among HIV-positive MSM has been described in several studies and overlapping clustering between MSM and PWID has also been observed (183-185). In a study investigating anti-HCV-discordant injection partners (anti-HCV or HCV RNA positive vs negative for both markers), over one

third (38%) reported sexual intercourse with their injecting partner (unprotected for >90%) (186). In that study, intimate injection partnerships (those who lived together and were also in a sexual relationship, both heterosexual and MSM as well as a low number of female-female partnerships) were independently associated with a 5-times greater risk of both receptive syringe sharing (RSS) and a 7-times greater risk of receptive cooker use (RCU) when compared to injecting only partnerships. During the study period, HCV incidence was high (23.8/100 pyr).

HCV is rarely transmitted vertically, but might occur in up to 5% of children delivered by chronically infected mothers (10% or higher if the mothers are co-infected with HIV) (187).

Globally, genotype 1 is the most prevalent (46 %), followed by genotype 3 (22%) and genotypes 2 and 4 (both 13%) and the remaining 6% consisting of the least common genotypes 5-7 (173). Regional variations have been observed; genotype 1 dominating in Australasia, Europe, Latin America and North America (53-71% of all cases) and genotype 3 representing 40% of all infections in Asia. Genotype 4 is most common (71%) in North Africa and the Middle East, but the distribution pattern changes to 34% genotype 4 and 46% genotype 1 in these regions if Egypt is excluded. Egypt has historically had a remarkably high iatrogenic transmission of HCV among the general population with a current prevalence of chronic infection at 7% (188).

Subtypes 1a and 3a are the most prevalent among European PWID (181). Phylodynamic studies of HCV in Europe have indicated that genotype 1 spread exponentially from the 1960s to 80s with subtype 1b preceding 1a, and that the spread of 1b being was more likely caused by iatrogenic measures and 1a through illicit drug use (189). A phylogenetic study on HCV transmission among HIV-positive PWID from seven European countries spanning 1984-2001 observed that 36 % of the HCV infected PWID had subtype 1a and 33% 3a (190). These authors also concluded that HCV had entered the PWID community through multiple introductions and had circulated in the PWID population for approximately 20 years longer than HIV.

Prevention

Similar to HIV, there is no vaccine against HCV. Due to viral factors, including the rapid selection of quasispecies and its intricate ability to hide partly embedded in VLDL-particles, HCV efficiently escapes both the humoral and cellular response of the host, explaining why vaccine trials have so far proved unsuccessful. Hitherto, prevention efforts are therefore focused on reducing the risk of parenteral transmission in different settings. As mentioned previously, screening of blood (using anti-HCV) and blood products has been successful in

almost eliminating transfusion-related transmission. Nosocomial transmission has been reduced by several measures, such as restricting the use of multidose medical vials (191, 192). However, many HCV infected patients have no known route of infection and may have been nosocomially infected through medical or dentistry procedures. However, given the asymptomatic manifestations of incident HCV infection, it is difficult to estimate the remaining risk of nosocomial HCV spread, especially in low and middle income settings. Similar principles as for nosocomial prevention may apply for reduction of HCV transmission among PWID. However, indications on harm reduction interventions being less efficient against HCV than HIV have been observed (193). This will be discussed further in the next chapter.

Recently, universal antiviral treatment for HCV-infected individuals with risk for onward transmission has also been proposed as a strategy for prevention (treatment as prevention, TasP). Mathematical models have suggested that scaling up HCV treatment among PWID will eventually result in ending transmission and finally complete eradication (194). The estimated time and resources needed to accomplish this is depending on several factors (baseline HCV prevalence, ongoing risk behaviour reflected in rates of reinfection). One unknown caveat is the degree of treatment adherence among PWID – irregular use of DAAs is a shortcut to resistance development, which would diminish the usefulness of these new drugs.

Treatment

There is strong evidence that sustained viral eradication of HCV can improve mortality rates (both all-cause and liver-related mortality) as well as quality of life among individuals with chronic hepatitis C (162, 195). Experiences especially from Asia (Japan, Taiwan) indicate on the beneficial effects of HCV treatment for liver disease, extrahepatic manifestations and overall well-being (196).

Interferon was the first approved treatment for chronic HCV infection. Apart from low rates of treatment response, subcutaneous administration combined with both somatic and psychiatric side effects made it unsuitable for many patients, particularly PWID (197). Even combined with ribavirin many clinical challenges remained (198). Pegylated interferon became available in 2001 and subsequently from 2002 to 2011, the standard of care treatment for chronic HCV infection was 24 or 48 weeks of therapy with pegylated interferon-alfa (PEG-IFN) and ribavirin (RBV) (199). In 2011, the HCV protease inhibitors telaprevir and boceprevir were launched to be used in combination with PEG-IFN and RBV for genotype 1 HCV infection, increasing the chances of SVR to 67-75% in treatment-naive patients (while being less successful in treatment-experienced null responders (200). This combination had however an increased risk for adverse effects requiring stringent monitoring, leaving great expectations for interferon-free regimens, which have been available in clinical practice in Europe since 2014. These direct- acting

antivirals (DAAs) have high cure rates (>95%), minimal adverse effects and short treatment duration (usually 12 weeks). Similar to other antiviral drugs, these drugs have lower or higher barriers to resistance and must be given in combinations and with high adherence (otherwise the usefulness of these drugs may be lost with replacement of wild-type with drug-resistant strains that can be transmitted onwards). DAAs have now practically revolutionized the whole concept of HCV treatment, including for patients with decompensated cirrhosis and other groups for whom interferon based treatment is contraindicated. In short, the DAAs are either nucleotide analogues (ending with –buvir), NS5A-inhibitors (-asvir) and NS3-4A protease inhibitors (-previr). Substances from a 4th class, non-nucleoside inhibitors of HCV RNA-dependent RNA polymerase (RdRp) Palm-1 inhibitors have recently been approved. DAAs are used in different combinations, sometimes with the addition of ribavirin depending on viral and host factors.

Previous treatment uptake for PWID with the peg-interferon and ribavirin regimen was low and injection drug use was even considered as a contraindication for anti-HCV therapy. Among Australian NEP participants an increase in treatment uptake was observed in 1999-2011 (still very low numbers, only 2.1% for current treatment and 8.6% for having ever received treatment) (201). Treatment adherence and sustained viral response (SVR) rates among PWID in both Swedish (OST patients), Dutch (multidisciplinary approach) and British (community-based clinics) settings have been well comparable to rates in the general population (202-205). The Dutch research group concluded that active drug use, including injecting, should not preclude access to HCV treatment. Australian researchers came to the same conclusion, upon studying adherence and responses to interferon-based therapy among PWID 2009-2012. Recent injecting drug use at the study baseline did not negatively influence SVR, while younger age and adherence were predictive of improved treatment response (206). Rates of re-infection after treatment among PWID have been higher among those who continued to inject after SVR, but lower than primary HCV infection in studies of PWID in similar settings (203). Reinfection rates for HCV after treatment were also lower among PWID than among HIV-positive MSM (207).

Increased uptake of treatment with DAAs among PWID is recommended by guidelines from CDC, EASL and other expert body organs (199, 208). Studies of HCV treatment with DAA of PWID receiving OST have shown promising results (209). According to modeling-based estimates from the Netherlands HCV treatment with DAA-containing regimens is considered to be a highly cost-effective public health intervention among PWID (210). The need for country-specific national strategies, resource allocation and implementation of global management policies is impending and also well underway in some regions (134, 211).

Harm reduction

General aspects

The concept of harm reduction encloses several interventions aiming at reducing harm and limiting damage linked to the use of potentially harmful substances, mainly illicit drugs, but interventions aimed at alcohol and tobacco may also be included.

The need for interventions became apparent already when the strongly addictive characteristics and other potential side effects of morphine were recognized. Restrictive measures were called for at an international meeting in Geneva in 1931. Prohibition of consumption of all drugs for non-medical reasons formed the basis for the restrictive drug policy supported by UN (at United Nations General Assembly Special Session on Drugs, UNGASS, in 1961, 1971 and 1988) and also supported by several nations including Sweden. At UNGASS 1998 the goal was set for governments to reduce drug production and consumption greatly within 10 years. At the evaluative meeting in 2009 for the UN organ Commission on Narcotic Drugs (CND) it was apparent that the goals had not been met (352). On the contrary; the production and trafficking of illegal opioids as well as other substances (central nervous system stimulants like amphetamines), transmission of blood borne viruses (mainly HIV, HBV and HCV) among PWID and organized criminal industry linked to drug trafficking had all increased. Peter Reuter at the School of Public Policy and Department of Criminology, University of Maryland, concluded (353):

> "No prevention, treatment or enforcement strategies have demonstrated an ability to substantially affect the extent of drug use and addiction; the best that government interventions can do is to reduce the damaging consequences of drug use and drug control."

At the UNGASS 2016 the assembly reiterated "the commitment to ending, by 2030, the epidemics of AIDS and tuberculosis, as well as to combating viral hepatitis and other communicable diseases among people who use drugs, including people who inject drugs" (resolution adopted by the United Nations General Assembly on 19th of April 2016).

In a global context, overall harm reduction coverage was found to be low in a review published in 2010 (92). Two needle–syringe units were estimated to be distributed per PWID per month, eight PWID out of one hundred received OST and four PWID out of 100 HIV-positive PWID received ART. For drug related mortality in Europe among 15-39 year olds in 2013, 3.4% of all deaths were opioid over doses, while opioids were detected by forensic analysis in 66% of fatal overdoses among PWID (348). The detection of opioids by forensic analysis in fatal overdoses had increased to 82% in 2014 (212). Also for 2014, the mortality rate due to overdoses in Europe was estimated at 18.3 deaths per million population aged 15–64 (with the highest rates in Estonia with 113 per million, Sweden 93 per million and Ireland 71 per million) (212).

Some countries have continuously expanded their harm reduction services and also implemented national action plans aimed specifically at HCV. Especially the Scottish Action Plan has provided fundamental insights into the management of HCV among PWID. Here, a 50% increase in diagnosed HCV cases, a sustained near 2.5-fold increase in the annual number of people initiating HCV treatment (with more pronounced increases among PWID and prisoners) have been observed since the implementation in 2006 (213). However, there are also examples of punitive drug policies with serious consequences. In the Russian federation, the government's refusal to meet its commitment to finance NEPs at the end of the Global Fund investment led to the closure of many NEPs in 2011, with a subsequent decrease by nearly 60% of the number of PWID with NEP access. In Russia, opioid substitution therapy (OST) is also prohibited (214). In the Philippines, despite a previously approved harm reduction program for PWID and an observed increased HIV prevalence among PWID, recently conflicting legislation and disregard of human rights have led to persecution of PWID as has been reported by media (215).

EMCDDA, the European Monitoring Centre for Drugs and Drug Addiction, promotes the idea that abstinence-oriented interventions can work in parallel with the other harm reduction interventions mentioned above, together aiming for a mutual goal (216).

The pharmacological foundation of opioid dependency treatment consists of providing opioid agonists in a structured way, based on either methadone, buprenorphine or buprenorphine/naltrexone (opioid substitution treatment, OST). Methadone maintenance therapy was subjected to heated debates for decades, after being launched in the 1960s by psychiatrist Marie Nyswander and metabolic disease specialist Vincent Dole in New York. They established the first officially sanctioned methadone maintenance clinics, which became models for such programs throughout the world. Their hypothesis was that individuals with an opioid dependency had undergone a metabolic change and needed opioids for

physiological reasons (217). In 1965, Swedish psychiatrist Lars Gunne worked as a research fellow in New York and became acquainted with the MMT concept, which he soon after introduced in Sweden, at the Ulleråker hospital in Uppsala and laid thus the foundation for the first MMT program in Sweden. He could later describe significantly higher rehabilitation rates and reduced morbidity and mortality among those who received methadone (218).

As described earlier, the jaundice (HBV) epidemics related to vaccination campaigns in soldiers during WW II emphasized the need of clean, unused syringes and needles for each individual. The importance of clean injection equipment and paraphernalia resurfaced when transmission of HIV among PWID was reported in the 1980s. A logical intervention was to make clean needles and syringes available for PWID through needle exchange programs. Subsequently other interventions, such as supervised injection sites, diacetylmorphine treatment and overdose prevention through opioid-antidote distribution have been launched at some sites as additional methods for harm reduction. Peer education and counselling are other important components. Harm reduction interventions are now available in several countries on different continents and are increasingly accepted in many places, but continue to raise controversy in some countries, including Sweden. Here, a traditionally restrictive view on drug policy, advocating for abstinence and a society completely free of illicit drugs have worked against the introduction of several harm reduction interventions (219, 220). Professor Vermund concludes in his paper "Global HIV epidemiology: A guide for strategies in prevention and care" that (89):

> "We have learned that reducing marginalization of drug users, implementation of non-judgemental and pragmatic sterile needle and syringe exchange programs, and offering of opiate substitution therapy to help persons eschew needle use altogether can work to reduce the HIV epidemic. Never has the urgency of stigma reduction and guarantees of human rights been more urgent; a public health approach to at-risk populations requires that to avail themselves of prevention services, they must feel welcomed."

Effects and risks of injection drugs

Overview

Globally, the most injected drugs belong either to the group of opioid-derived substances or to the amphetamine-group substances (central nervous system stimulants). Two other large groups, hallucinogens and tranquilizers, may cause various degrees of dependency and harm, but are usually not injected. However, the impact of the tranquilizers benzodiazepines combined with opioids should not be underestimated, especially in potentiating respiratory depression (221).

Polysubstance use may now be prevalent among many PWID populations, demanding efforts from care givers to understand and elucidate interactions and synergistic effects. In Sweden amphetamine has been most popular by tradition, with opioids gradually taking over the scene in many settings. During the time of studies I-III these two main groups of injection drugs were almost exclusively used by participants of the Malmö NEP. More recently, a wider range of drugs have been reported by new participants. However, heroin and amphetamine still remain by far the most prevalent at the Malmö NEP and their effects and potential to cause harm are discussed briefly below. No or very limited use of methamphetamine and crack cocaine has yet been observed among participants of the Malmö NEP.

Opioids

Opioids are divided into three subgroups; opiates (opium alkaloids, derived from opium), semisynthetic opioids and synthetic opioids. The EMCDDA estimated in its Drug Report for 2016 that 1.3 million Europeans 15-64 years of age were problem opioid users (212).

The opiate *morphine* was first isolated from opium (*Papaver somniferum*) in 1803 by Friedrich Serturner and has been used for medical purposes since 1814, as an analgesic supposed to be less addictive than the widely used opium (222). Morphine gathered, however, quickly many soldiers under its spell during wars in the 19th century. In the American Civil War the needle-gun was used for self-dispensing of morphine for the wounded. The British chemist Charles Wright derived diacetylmorphine from morphine in 1874, and in 1897 it was synthesized for medical purposes by German chemist Felix Hoffmann at Bayer AG, who chose to call it "heroin" for its "heroic" effects – which proved to be quite the opposite. In 1899, heroin was launched by medical company Bayer AG, as an oral remedy for a variety of symptoms and disorders (223). Soon the addictive effects of heroin were obvious and in the USA the Harrison Act was passed in 1914, introducing federal narcotic controls and limiting the amount of heroin in proprietary preparations. An outright ban was introduced in 1924 in the US as well as in many other countries. However, in 1929 the injectable preparation was added and the World War II at least 6000 soldiers were dependent on opioids.

The transition from oral or intranasal administration to intake of opioids by intravenous routes is driven by the craving for a more rapid opioid effect, as this is correlated with the sentiments of reward and reinforcement (224). The primary target for opioids is the μ-opioid receptor, which is present with highest density in brain areas modulating pain and reward (e.g., thalamus, amygdala, anterior cingulated cortex and striatum). Activation of μ-opioid receptors inhibits GABA-mediated tonic inhibition of dopaminergic neurons, initiating a cascade of effects not only related to reward, but also inducing a craving for more (225, 226).

A heroin injection gives an initial, short lived euphoric "rush" within a minute, followed by a stable period of some hours (354). Eight to twelve hours later symptoms of abstinence/withdrawal will occur, causing a strong yearning for the next dose. The initial withdrawal symptoms constitute of rhinorrhoea, sweating, yawning, irritability and tremor. After a further 2-3 days anxiety, insomnia, diarrhoea, muscle pains ("cold turkey"), tachycardia, hypertension, vomiting (potentially leading to dehydration and ketosis) may occur. For the following 3 to 10 days these symptoms slowly diminish. As most harmful effects have been described for overdosing, withdrawal symptoms may also have serious consequences (rhabdomyolysis, cardiomyopathy, kidney failure and gastrointestinal haemorrhage have been described in the withdrawal phase) (227, 228).

Three of heroin's metabolites (morphine, 3-monoacetylmorphine and 6-monoacetylmorphine) have opioid activity and since genetic variations control the formation of these metabolites, this could in combination with other drugs explain the individual's sensitivity to overdose (221). Respiration is controlled principally through medullary respiratory centres with peripheral input from chemoreceptors, mediated by neurotransmitters (glutamate and GABA as the major excitatory and inhibitory neurotransmitters, respectively). Opioids inhibit the chemoreceptors in the medulla via both μ- and δ-receptors. Since both benzodiazepines and alcohol facilitate the inhibitory effect of GABA (alcohol also decreasing the excitatory effect of glutamate at NMDA receptors) the potential interaction of opioids with benzodiazepines and alcohol on respiration is apparent (221). Respiratory depression may progress to loss of consciousness and hypothermia and eventually death. If an antidote, such as opioid antagonists (naloxone hydrochloride; naloxone) is given immediately, the life threatening situation may be reversed. In hospital settings repeated doses are often needed, due to a longer half-life of heroin than the antidotes (antidotes can for this reason be given both intravenously and intramuscularly/subcutaneously for slower release).

Central nervous system stimulants (amphetamine)

Amphetamine was synthesized in Berlin in 1887 as the first of several chemicals with similar structures and biological properties collectively known as amphetamine-group substances (including methamphetamine and methylenedioxymethamphetamine). As a very potent sympathomimetic drug, amphetamine increases the synaptic levels of dopamine, norepinephrine and serotonin. The stimulant effects are mediated primarily through dopamine and depend on the dopamine transporter (DAT). By inhibiting the degradative enzymes monoamine oxidase A and B (MAO-A and MAO-B) and disrupting vesicular storage of dopamine, accumulation in the cytoplasm occurs, enabling further transport into the synapse (229).

The initial effects are euphoria, hyperactivity, increased endurance and diminished appetite. Withdrawal symptoms may constitute of fatigue, hunger, depression and anxiety. Overdosing can cause paranoid thoughts, hallucinations, and hypertension. Serious adverse effects include psychosis and cerebral hemorrhage. Amphetamine has clinical indications in treating narcolepsy and its derivate methylphenidate in Attention deficit hyperactivity disorder (ADHD) treatment. Methylphenidate has a less addictive profile, due to a slower inhibition of dopamine.

Substitution treatment for amphetamine, in analogy with opioids, is more difficult to establish, although some trials with methylphenidate and dexamphetamine have shown promising results (230).

Psychotic episodes are rare at recommended doses as prescribed medication, but may occur in illicit use especially in the presence of pre-existing psychotic disease or genetic predisposition (231, 232).

Use of amphetamine-group substances (AGS) is more prevalent than use of opioids or cocaine. Data from 2007 estimated that 16–51 million people worldwide used AGS at least once that year. Recent data from EMCDDA estimate that 1.6 million (0.5%) of Europeans aged 15-64 years had used amphetamine during the past year (approximately 20% through injecting), while the prevalence of life time use was 12.0 million (3.5%) (212). The prevalence of AGS use is thought to be leveling out in at least some western countries, while increasing in East and South East Asia and the Middle East. Most use of AGS is non-injection (snorted, smoked, injected, swallowed or used rectally), the proportion of injecting varying substantially by region and risk population (e.g., men who have sex with men, heterosexual people, commercial sex workers) (233). Use of AGS has been particularly prevalent in some settings among HIV infected, homeless and incarcerated individuals (234-236).

The most common precursors for methamphetamine production are chemicals such as ephedrine or pseudoephedrine, and phenylpropanolamine or phenyl acetic acid for amphetamine production. Manufacturing is thus not dependent on suitable soil or climate as for opioid or cocaine precursors and it is estimated to occur in at least 60 countries. In Europe, benzyl methyl ketone (BMK; 1-phenyl-2-propapone) is mainly used to synthesize amphetamine, but elsewhere usually to manufacture methamphetamine. Especially in its crystalline form methamphetamine is known to cause dependency and have more health-related consequences, such as an increased risk of HIV seroconversion (237-239). For young injectors and NEP participants injecting methamphetamine, higher rates of injection risk behavior and higher HIV prevalence compared to injectors of other drugs have been reported, indicating the need of specific attention towards this subpopulation of PWID (240, 241).

Needle exchange programs (NEPs)

Objectives

The overall aim of NEPs is prevention of blood borne transmission between PWID through distribution of sterile injection equipment that enables PWID to avoid sharing and re-use of syringes, needles and other paraphernalia (filters, cookers, and solutions). Simultaneously, NEPs can provide a platform for other harm reduction interventions directed at PWID based on individual and community needs. Swedish legislation (Law 2006:323) states two main objectives; to prevent transmission of blood borne viruses and to work in a broad manner to divert the individual from (injection) drug use.

Background

Sharing of needles, syringes and other paraphernalia occurs in various contexts. It may include picking up syringes and other materials used and abandoned by others, social sharing within couples and social networks and by borrowing, renting or buying used syringes depending on the local access to such materials and the setting (in a “shooting gallery”, for instance, the risk of sharing contaminated material may be very high, whereas it should be non-existent at a safe injection site) (242).

Harmful sharing is illustrated by the findings from three American cities in the late 1980s; more than half of the study participants injected drugs daily and 70% shared needles with others, averaging 6.3 injection partners in the past six months. Furthermore, 86% had shared a cooker and nearly 50% injected in a "shooting gallery"(243). The choice of drug may also play a pivotal role in sharing (extreme injection frequency for instance for cocaine, up to 20 times daily). The injection setting is also important in correlation to the harm in each separate injection, as suggested for “microinjecting” (for instance, frequent injecting in a rush in public places) (244).

Contamination and transmission of pathogens is especially likely in cases of sharing drugs through “back loading”; transferring a portion of the solution into another syringe by removing the plunger of the second syringe and squirting some of the solution from the first syringe into the second syringe. In the opposite method, “frontloading”, the needle from the second syringe is removed and the plunger drawn back, in order to allow the first person to squirt the solution in. (23).

One syringe may also be used as a storage receptacle, where drug solution is prepared for several injections and stored to be consumed in several injections over a given period of time by one or more users (242). As the survival time for

HCV in water may be >3 weeks (up to 63 days in high void volume syringes), this method may have a significant input on transmission (245).

Understanding injection behaviour is essential in order to identify targets for further interventions. Differences in injection methods and settings may partly explain the higher prevalence of HIV and HCV that have been observed among women compared to men (246). Despite more men injecting drugs, a higher prevalence of HIV has been observed among women (247). In several studies among young PWID female gender is an independent predictor of exposure to HIV and/or HCV (248, 249). High incidences of both HIV and HCV among female PWID have been reported (250). Women are more likely to have a sexual partner who (also) injects drugs or to be initiated into injection drug use by male sex partners and more likely to be injected by others (251). This was also found at an interview study conducted at the Malmö NEP, presented at the INHSU-meeting 2016 (346). Women who inject with a sexual partner have an elevated risk of incident HCV infection compared to those without overlapping partnerships (sexual and injecting) (186). Women also have a higher prevalence of injection-related injury and disease compared to men, partly due to more harmful injection techniques as described earlier (252, 253).

Coverage

The coverage of NEPs is defined as the number of units (needle-syringe) dispensed per head of the target population (212). Between 2003 and 2007 a 33% increase in the number of units distributed through specialised programs in Europe were observed, with steady increases in most countries (except several countries in Northern and Central Europe, including Sweden). It was estimated that on average 50 syringes per year were distributed per person injection drugs, with wide regional variations (254) (Figure 4). Availability of sterile syringes is also dependent upon country-specific pharmacy regulations. In Sweden syringes are not available through pharmacies, whereas pharmacies play a significant role in needle distribution for instance in New Zealand (255).

By 2009, NEPs had been globally implemented in 82 countries and OST in 70 countries, both interventions were available in 66 countries (Mathers 2010). Regional variations on coverage were substantial, with by far the greatest rates of needle–syringe distribution seen in Australasia (202 units of needle-syringes per IDU per year) and low rates in Middle East and North Africa (0.5 units per IDU per year) and sub-Saharan Africa (0.1 units per IDU per year).

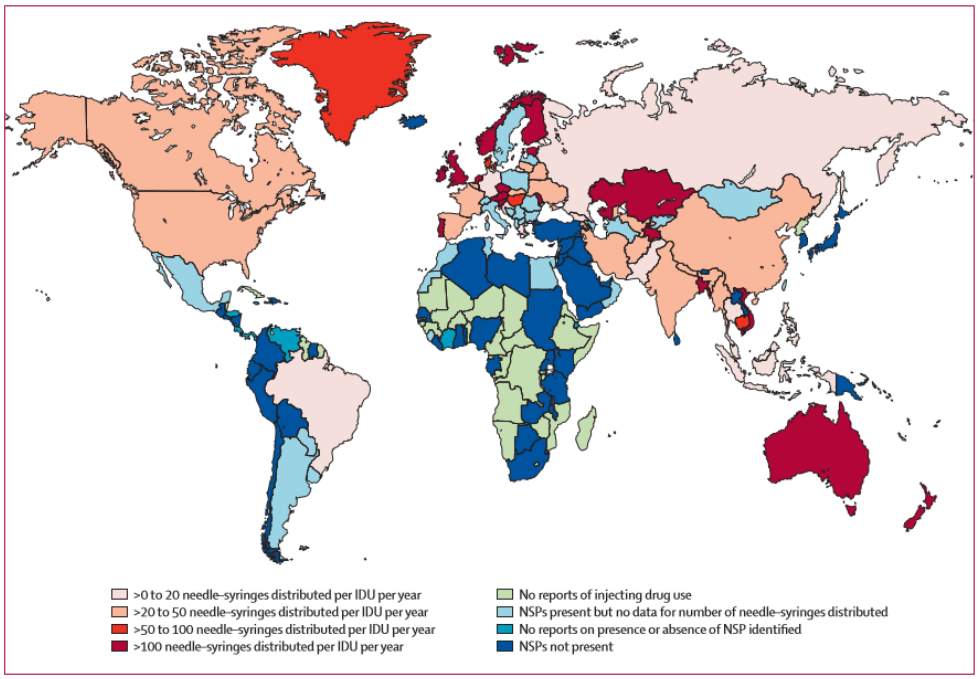


Figure 4. NEP coverage in 2007.
Estimations of number of needle–syringes distributed in a 12-month period per person injecting drugs. NSPs=needle and syringe programmes. Mathers BM, Degenhardt L, Ali H, Wiessing L, Hickman M, Mattick RP et al; 2009 Reference Group to the UN on HIV and Injecting Drug Use. HIV prevention, treatment, and care services for people who inject drugs: a systematic review of global, regional, and national coverage. Lancet. 2010 Mar 20;375(9719):1014-28. Reprinted with permission from Elsevier.

In Europe, approximately 30 countries supported harm reduction in policy or practice by all providing NEPs and OST in 2009. Furthermore, six countries also provided NEPs in prisons and 23 provided OST in prisons (216).

Further improved coverage was reported by the EMCDDA in 2015, when the reported number of syringes distributed between 2007 and 2013 increased from 43 million to 49 million in 24 countries (representing approximately half of the EU population). Still a large regional variation was observed, with approximately half of the countries reporting an increase in syringe distribution and half a decrease. For 12 countries with recent estimates of injection prevalence, the number of syringes distributed 2013 ranged from less than one in Cyprus to more than 300 per PWID in Estonia and Norway (348).

For Sweden, provision of syringes is low according to the EMCDDA (348). This must, however, be analysed in its correct context, with substantial regional variations. The Swedish perspective of harm reduction and NEP policy will be discussed further down.

It must also be noted that the modus operandi and services offered at each separate NEP might differ from others in the same country as well as between countries (as sites associated with hospital or at independent inner city settings, mobile clinics or other outreach forms, peer networks, pharmacies and unsupervised vending machines). Besides needles and syringes of different sizes, other paraphernalia such as filters, cups, swabs for skin disinfection and male condoms may be distributed. In most places (including other Nordic countries, albeit not Sweden, where identification is mandatory), NEP visits are often done anonymously.

Efficiency

Several evaluations of the impact of NEPs on HIV prevalence and incidence have been published and somewhat fewer on HCV incidence.

The methodological and other difficulties (such as the influence of the local social and ethnographic context of the NEP being evaluated) in evaluating the efficiency of NEPs in reducing the incidence of HIV and even more for HCV have been described (256). Compelling evidence of NEP efficiency, safety and cost-effectiveness have been presented as well as evidence supporting the effectiveness of harm-reduction programs including NEPs over punitive drug-control policies and the effect of NEPs reducing needle sharing (257-259). NEPs have also been shown to promote entry into OST (260, 261).

One of the most alarming and most cited studies on a negative NEP effect was "Needle exchange is not enough" by S. Strathdee et al in 1997 (262). Here, it was described how HIV incidence increased to alarmingly high rates (18.6/100 pyr) in 1994 in Vancouver despite a large, frequently visited NEP established six years earlier, in a low HIV prevalence setting at 1-2% among local PWID. Reasons for this increase were both suggested early on and then summarized fifteen years later (263). The lessons learned from this outbreak are essential to everyone working with NEP development. It was clearly demonstrated that NEP attendance is/was not driving the HIV outbreak. Contributing factors to the failure of the NEP to prevent the outbreak were the high risk profiles among frequent attendees of the Vancouver NEP with a continuous tradition of sharing injection equipment despite access to clean ones and a substantial transition to the more frequently injected substance cocaine. This was combined with an inadequate access to other harm reduction interventions such as drug and alcohol treatment, methadone maintenance treatment and counselling services. In detail, through the transition from injection use of heroin to cocaine daily injection rates could sky rocket from the usual injection rates from 2-4 times daily for heroin to up to 20 times per day for cocaine due to the drug's relatively short half-life (264).

It was estimated that to achieve adequate coverage, the Vancouver NEP would have to exchange 5–10 million syringes per year to meet the demand. Also

elsewhere in Canada there were concerns of insufficient local coverage and efficiency of NEPs (265). Subsequently, several improvements to Vancouver's NEP were introduced, such as removing syringe distribution limits and decentralizing and diversifying NEP services. This was made possible thanks to the declaration of the situation in the Downtown Eastside a public health emergency and substantially increasing funding to expand the services. The NEP opening hours were turned into a 24-hour operation, the amount of syringes distributed were increased and fixed sites, mobile vans and foot patrols for NEP were added. Reduction of HIV incidence was subsequently observed in Vancouver, although the efficiency of the harm reduction interventions on reducing HCV incidence was not evident (266).

Many reviews and even review-of-reviews have discussed the efficiency of NEPs. In the EMCDDA Monograph: Harm reduction: evidence, impacts and challenges from 2010, the authors conclude that there is tentative evidence to support the effectiveness of NEPs in reducing HIV incidence/prevalence among PWID (267). Many of the same authors expressed cautiously increased support for NEPs in a review from 2014; with tentative review-level evidence to support effectiveness in reducing HIV transmission, but still insufficient review-level evidence relating to HCV transmission (yet sufficient review-level evidence in relation to injecting related behaviour) (268).

Steep declines in HIV and HCV incidence rates were observed in the Amsterdam Cohort Study from 1985-2005 and it was subsequently shown that full participation in both NEPs and OST was necessary to achieve beneficial results on blood borne viral transmission among PWID (269, 270). These findings were corroborated in a study of approximately 3000 PWID in the UK in 2001-2009, where both receiving OST and high NEP coverage were associated with a reduction in new HCV infection. Full harm reduction (OST plus high NEP coverage) reduced the odds of new HCV infection by nearly 80%. Full harm reduction was associated with a reduction in self-reported needle sharing by 48% and mean injecting frequency by 20.8 injections per month (271).

Impressive reductions in HCV incidence among PWID were also observed in Scotland between 2008 and 2012 after implementation of their Action Plan in 2006. HCV incidence declined from 13.6/100 pyr in 2008-09 to 7.3/100 pyr in 2011-12. During this period increases in the coverage of OST and provision of sterile needles, syringes and other paraphernalia through NEPs and pharmacies had been implemented, with an observed decrease in the frequency of injecting and sharing of injecting equipment (272).

Another review focused on analysing findings from studies on structural-level NEPs (defined by a minimum 50% coverage of PWID and distribution of 10 or more needles/syringe per PWID per year) covering the years 1990-2009 in settings

in Europe, South East Asia, the United States, Canada and Australia. Decreasing HIV prevalence was reported from New York City, France, Spain, China, Vietnam and Australia. From New York, Montreal and Vietnam decreases in HIV incidence could also be reported. Decreasing HCV prevalence was observed in Scotland, Ireland and New York (in separate studies covering first 1990-2002 and then 2005-2007). In New York, a decrease in coinfections with HIV and HCV was also observed. In France and Spain, however an increase in HCV prevalence was observed during the earlier years of the study period (1993-2002). Altogether, the authors concluded that the findings indicated that large-scale NEPs can reduce HIV and HCV infections (273).

Other harm reduction interventions

Opioid substitution therapy (OST)

Opioid agonist maintenance treatment, with methadone or buprenorphine, are prescribed to divert and refrain from harmful use of illicit opioids, while antagonist therapy (in particular naltrexone), accelerates the detoxification process and is thereafter useful in preventing relapse as well as overdoses (274). To be successful, this intervention requires long-term commitment of both the patient and the caregiver. Successful OST requires continuous evaluation, optimizing of dosage and distribution intervals, varying from directly observed treatment to delivery via pharmacies with longer intervals for pharmacologically well stabilized patients with impeccable adherence.

Methadone is a full μ-receptor agonist (with a half-life of 15-57 hours). Buprenorphine is a partial μ-agonist, meaning it is sufficient to eliminate or significantly decrease the craving for heroin, while not inducing a "rush" or respiratory depression, regardless of more doses taken (the initial half-life being around 2-5 hours, yet due to metabolic reabsorption eliminated in 20-25 hours) (354). For methadone some interaction risks must be noted when treating infectious diseases (such as tuberculosis with rifampicin and with several ARTs). Methadone has also been associated with ventricular arrhythmias, (long QT-syndrome and "torsade de pointes"), sometimes making transition to buprenorphine a safer option (275). For buprenorphine interaction may occur with selected ARTs. DAAs for HCV treatment have no known risk for interaction with these opioid-agonists.

An estimation of global OST coverage in 2010 found that OST had been implemented in 70 countries, while being unavailable in 66 countries (no information was obtained for 15 countries) (Figure 5). Methadone maintenance treatment was the most frequent (61 countries), followed by buprenorphine maintenance treatment (47 countries).

For Europe, it was estimated that approximately half of opioid users (644 000 or 680 000 individuals including Norway and Turkey), received OST in the European Union in 2014. This was a decline of 50 000 since 2010, but may partly depend on methodological differences in data collection (212).

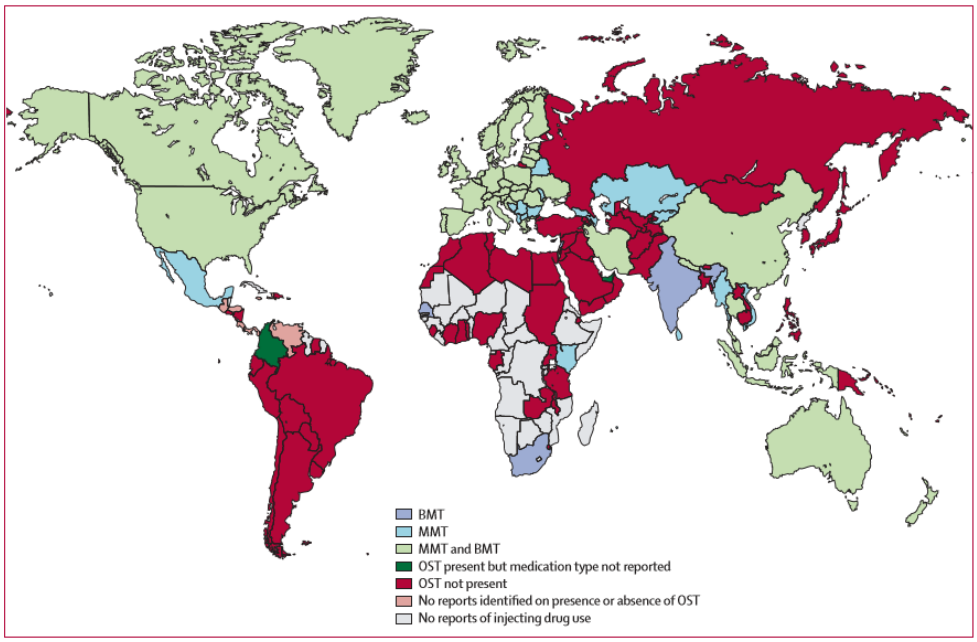


Figure 5: Availability of opioid substitution treatment.
BMT=buprenorphine maintenance treatment. MMT=methadone maintenance treatment. OST=opioid substitution therapy. Mathers BM, Degenhardt L, Ali H, Wiessing L, Hickman M, Mattick RP et al; 2009 Reference Group to the UN on HIV and Injecting Drug Use. HIV prevention, treatment, and care services for people who inject drugs: a systematic review of global, regional, and national coverage. Lancet. 2010 Mar 20;375(9719):1014-28. Reprinted with permission from Elsevier.

OST participation does not guarantee cessation of drug injection. A study based on data from the Australian Illicit Drug Reporting System since 2000, collected among others from NEP participants, observed high levels of heroin use and/or moderate levels of a broad range of other substances among PWID receiving OST. From a clinical perspective, among OST patients continuing to inject drugs and use a broad range of substances, drug related negative outcomes were not less compared to those not receiving OST (276).

These findings are partly contradicted by findings from a Norwegian survey among NEP participants. Here, fewer non-fatal overdoses among current OST patients compared to former/never OST users were also observed. But

additionally, several beneficial factors were identified among NEP participants simultaneously receiving OST; less frequent injecting, less daily or almost daily use of heroin and less engagement in theft and drug dealing in the past month. Overall, there was a high level of polysubstance use and no group differences on this measure. These authors concluded that NEP participants currently in OST have substantially reduced health risks and criminal activity than other NEP participants (277).

Supervised Injection Sites (SIS)

The objectives of drug consumption rooms or supervised injection sites are to reduce the mortality and morbidity associated with drug overdose, drug use in public places, inappropriately discarded injecting equipment, the risk of transmission of blood borne viruses and to act as an access point to drug treatment, health care and social welfare assistance (278). The first supervised drug consumption room was opened in 1986 in Bern, Switzerland. Subsequently, as open drug scenes were closed in the beginning of the 1990s more injection rooms were opened in Switzerland. By 2003, SIS had been opened in three more European countries (Germany, the Netherlands and Spain) as well as in Australia, over all 60 operational units in around 30 cities (278). Now there are also SIS in Scandinavia (Norway, Denmark, but not in Sweden). The largest SIS in North America has been operating in Vancouver since 2003 as an efficient harm reductive measure, to provide clean needles, syringes and other paraphernalia, to prevent and reverse and treat overdoses and to establish a health care contact with PWID. Evaluation of this facility found an array of community and public health benefits without evidence of adverse impacts (279). SIS have proved an obvious effect on overdose reversal, although the effect on HCV incidence is more difficult to determine (280, 281).

Diacetylmorphine (DAM) treatment

Several studies suggest that legal distribution of diacetylmorphine (heroin) in treatment settings could provide an effective alternative for socially excluded, opioid-dependent patients with severe physical and mental health problems due to drug dependency, when all available previous treatments have failed. Retention rates were often higher than in the control groups receiving oral methadone, leading to decreasing use of street heroin (282-285). Repeated Cochrane analyses have observed positive influences of well-supervised DAM-programs concerning factors such as physical health, HIV risk behaviour, street heroin use, and days involved in crime (286, 287). In a British setting, it became apparent early on that supervised injectable heroin treatment lead to significantly lower use of street heroin than treatment with supervised injectable methadone or optimised oral methadone (288).

Overdose prevention programmes

The immediate and ultimate harm for the opioid injecting individual is death by respiratory arrest when an overdose is injected. Heroin overdose is a leading cause of death among PWID (212). Naloxone is a short-acting, high-affinity μ-opioid receptor antagonist used to reverse unwanted opioid effects by medical personnel since the 1960s. The significantly shorter effect duration compared to opioids often necessitates repeated dosing. Naloxone can be injected intravenously, subcutaneously, intramuscularly or by nasal application. The intravenous route has the fastest effect, which in fact may be too abrupt, making the other routes both easier for the one applying the dose as well as for the receiving part (289). Reports from two trials of naloxone provided directly to PWID to give to their peers in the case of overdose events in the late 1990s showed promising results without any adverse effects and that 10% of the distributed naloxone had been used (and resulted in successful resuscitation) (290). Several naloxone distribution programmes are now operating in many countries.

Beneficial results in favour of the intranasal administration mode compared to the intravenous route were observed in a randomized controlled trial among patients with suspected opioid overdoses at an emergency care hospital (291). The intranasal application mode is especially appealing from many aspects; no risk for injection related complications, but a rapid onset, high plasma bioavailability, direct transport to the central nervous system across the olfactory mucosa and elimination of first pass metabolism.

As ex-prisoners have an increased risk of fatal opioid overdose directly following release (as high as 1706 per 100,000 person-years in the first week), naloxone provision upon release was introduced in Scotland in 2010 for all inmates with a history of heroin injection (292). A subsequent decline from 9.8 % of all opioid deaths for 2006–2010 to 4.7 % in 2013 as well as a decline in the proportion that occurred among former inmates in the 4 weeks after release was reported (293). An elevated risk of a fatal overdose has also been observed in individuals who had recently reported a non-fatal overdose, and should be specifically targeted with overdose preventive methods (including kits with naloxone) (294).

Swedish perspective of harm reduction

When the first reports on HIV infections among PWID in Stockholm and Copenhagen in the middle of the 1980s, clinicians in Lund – led by psychiatrist Kerstin Tunving and infectious disease specialist Bengt Ljungberg - decided to act, despite the political controversy surrounding NEPs. Collaboration between the social services and the department of infectious diseases had already been initiated in Lund (80.000 inhabitants), in southern Sweden (295). Thus it was only natural to announce the opening of a NEP at the infectious disease clinic on the message board at the social service bureau in the autumn of 1986 (295).

After an initial skepticism from the local PWID the program gained momentum and was also used by an increasing number of visitors from neighbouring Malmö (260.000 inhabitants). Here, with a busy harbour and close proximity to Copenhagen ensuring an influx of illicit goods, populations of PWID (mainly amphetamine users) had been established already in the 1960s (296). The Malmö NEP was opened at the Department of Infectious Diseases at the General Hospital of Malmö in the summer of 1987, led by Dr Torkil Moestrup and Dr Stig Cronberg. During 1986-1994 a total of ten NEPs were opened in Sweden, but after an evaluation the Swedish Board of Health and Welfare decided to only give permission to the NEPs in Lund and Malmö to operate. Thus, these two remained the only NEPs in the country for more than two decades, under strict scrutiny with inspections for further licensing every second year. Continuous evaluations of the programs have taken place, with a maintained low prevalence and incidence of HIV and without any negative results such as an increased recruitment of young individuals to injecting (297, 298).

After many political debates questioning the existence of Swedish NEPs (described in detail by other authors), the situation gradually changed with new legislation allowing NEPs to be started in counties that wanted to do so, with some restrictions (219, 220, 299, 300). The age limit would still be set to 20 years and the participants should fulfill certain criteria. The expansion process remained slow and not until 2010 did a third NEP get started (in the city of Helsingborg in Skåne County). One more NEP in Skåne County was opened in the town of Kristianstad in 2014. By 2015 there were six operating NEPs in Sweden; four in Skåne, one in Kalmar (2012) on the southeastern coast and one in Stockholm (2013). In January 2015 The Public Health Agency of Sweden announced their strong support for NEPs, resulting in two more NEPs opening in 2016 and more being planned for (16).

Despite being so few, the services provided at the Swedish NEPs are comparable to those of a health care center. All are located in association with Infectious disease clinics and continuously staffed or having access to a broadly competent

staff (nurses, physicians, social workers and midwives) and with close cooperation with drug dependency services or emergency medicine. All participants are registered by their national identity number (NIN) and agree to regular testing (every 3 to 6 months, depending on location) for relevant markers for HIV, HBV and HCV. Vaccination against HAV and HBV is offered, including follow-up testing for immunity. For the majority of the Swedish NEPs, all data are collected in similar databases. This enables data comparisons on a national as well as a regional level.

The Swedish Public Health Agency estimated the number of PWID in Sweden to 8000 individuals, with 1100 in Skåne county (range 996-1298) in 2008-2011, equaling 1.3/1000 PWID per inhabitant in Skåne (1.1/1000 for the whole country) (16). Since the number of participants in the Malmö NEP during these years was 750-900/year, at least 70% of the PWID in the region should have visited the Malmö NEP. A study on respondent-driven sampling originating from Malmö NEP participants did not reach beyond the group (301). It was estimated that approximately 204 000 syringes were distributed to 2266 clients in 2014 altogether through the (then six) NEPs in Sweden, which would indicate a relatively good coverage with 90 needle-syringe units per PWID with NEP access (although leaving two thirds of the estimated number of PWID outside) (304).

The restrictive attitude towards harm reduction did not only concern NEPs, but also other harm reduction interventions. In Sweden, a long period of controversy regarding OST led to strict regulations, issued by The Swedish National Board of Health and Welfare. OST can only be given at specialized psychiatric or drug dependence clinics. Regulation updates have, however, continuously moved towards a less restrictive model.

Prior to 2005, access to OST was limited, with requirements of documentation of at least 4 years of intravenous opiate addiction and three failed attempts (documented in medical records) at drug-free treatment. For those who managed to pass this bottleneck and qualified for OST (often after waiting for several years) strict rules accompanied the treatment. OST could be suspended for several reasons (absence from treatment for more than 1 week, repeated relapses into illicit drug use, excess alcohol use, manipulation of the weekly control urine samples, conviction of drug offenses). In case of suspension, the patient was barred from seeking readmission to OST. It was also mandatory to have a treatment plan with the social services before applying for OST. Such a restrictive treatment model had forced many PWID to manage their own OST, sometimes during several years, while waiting to pass the barriers into the licit OST-programs (302).

New regulations in 2010 allowed OST to patients over 20 years old with at least one year’s documented opiate (heroin, morphine, or opium) dependence (349).

This indicated exclusion of those dependent on other opioids (such as buprenorphine, fentanyl or methadone) and those with excess use of alcohol in quantities posing substantial medical risks and polysubstance use. The suspension period in the event of involuntary discharge had been gradually reduced (from two years in the 1990s to 6 months in 2005 to 3 months in 2010). In 2016, the regulations were adjusted to allow inclusion of all opioid dependent individuals; the requirements of both documentation of one year duration of opioid use and the suspension time after involuntary discharge were removed. The requirements of direct observed treatment to be used at least during the first 6 months was reduced to 3 months (303).

Effective referral directly from a NEP to OST has been shown through a pilot randomized controlled trial study; the Malmö Treatment Referral and Intervention Study (MATRIS) (261). NEP participants were randomized either to be included through traditional recruitment or with the aid of a semi-structured case management intervention, assessing the patients' strengths and needs and identifying practical aid needed to get to the first appointment for OST. For both groups the inclusion rates were very high (94% vs 95%, respectively). Transfer from the NEP waiting room to initiation of OST was done within 10 days and was very appealing to NEP participants. The project was operational 2011-2013, but has since been implemented as a regular service provided through the Malmö NEP, with OST personnel present for recruitment once a week.

In 2014 in Sweden, the drug-induced mortality rate among adults (aged 15–64) was 92.9 deaths per million, more than four times the most recent European average of 19.2 deaths per million (304). A significantly increasing trend in mortality has been observed among Swedish PWID during the last decade, but the increase was especially marked from 2013 (460 drug-induced deaths) to 2014 (609 drug-induced deaths). Toxicology reports were available for almost all cases, data indicating the presence of opioids in the majority of cases, although combined with other substances in a large proportion.

No supervised injection rooms or diacetylmorphine treatment are allowed in Sweden, nor has distribution of naloxone to PWID yet been introduced. In Copenhagen in Denmark, kits with naloxone for intranasal application have been distributed since 2011, together with education to PWID and staff at different sites (including the staff at Malmö NEP). Currently, interventions for reduction of the alarmingly high overdose mortality rates among Swedish PWID are underway, mainly through planned distribution of intranasal naloxone to PWID and their peers.

Materials and Methods

Setting

The Malmö NEP

Prerequisites for enrolment in the Malmö NEP are self-reported injection drug use, age >20 years, signs of recent injection and consent to HIV testing. During the study period for papers I-III, 1997-2005, participants were allowed to test anonymously for HIV, but the majority (approximately 70%) chose to use their Swedish 10 digit national identity number (NIN). NINs are used in all Swedish hospital and laboratory databases and in population registers. Since 2006, according to new legislation, all participants have to register with their NIN at the first visit.

All participants of the Malmö NEP undergo a baseline interview on sociodemographic data at NEP enrolment, such as onset age of drug use, drug type ever used (injection as well as additional non-injection), main injection drug, contact with authorities (social services, psychiatry and drug dependency care and institutionalized care), education, employment, housing, marital status and sexual risk behavior (prostitution and MSM). Data on self-perceived status for HIV, HBV and HCV are also collected at NEP enrolment, before the results of the first blood sample are available. Serum samples are obtained at baseline and tested for serologic markers of HIV, HBV and HCV, again approximately every three months to assess further seroconversions to any of these pathogens.

The opening hours are from 9-12 am and 1-4 pm on weekdays (except 9-12 on Fridays). The staff composition has varied slightly throughout the years, with the core consisting of assistant nurses, registered nurses, physicians specializing in infectious diseases (scheduled for drop-in consultations and available outside schedule for urgent cases), midwives (present one afternoon a week for consultations from women with concerns about STDs, contraception or pregnancy) and social workers.

Participants

Approximately 4800 individuals have enrolled in the Malmö NEP from the opening in 1987 to today (2016). The proportion of women has remained relatively stable at 25% throughout the years. The median age among new visitors is around 29 years and for all visitors approximately 44 years. The number of new visitors declined in the early 2000s, most markedly after 2006 (Figure 6).

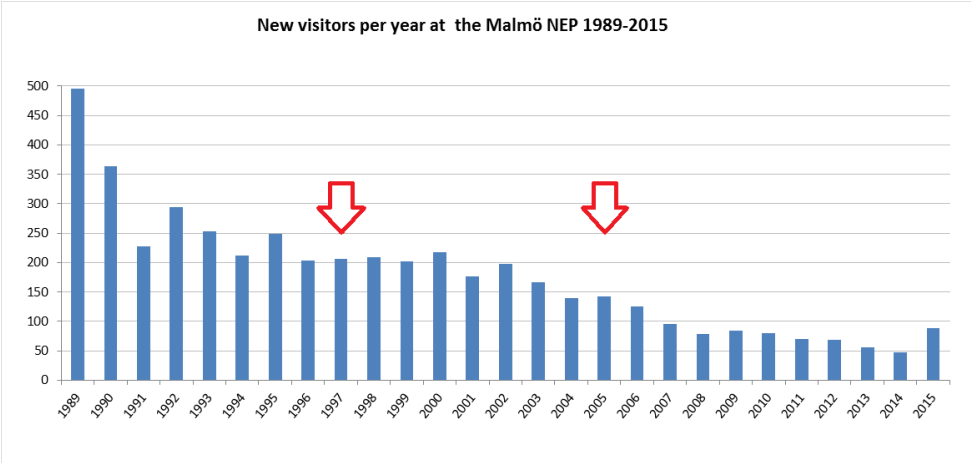


Figure 6 A. New visitors annually at the Malmö NEP 1989-2015.
Arrows indicating beginning and end of study period in papers I-III. (Published in the annual NEP report 2015, Isendahl P, Lanbeck P and MAB).

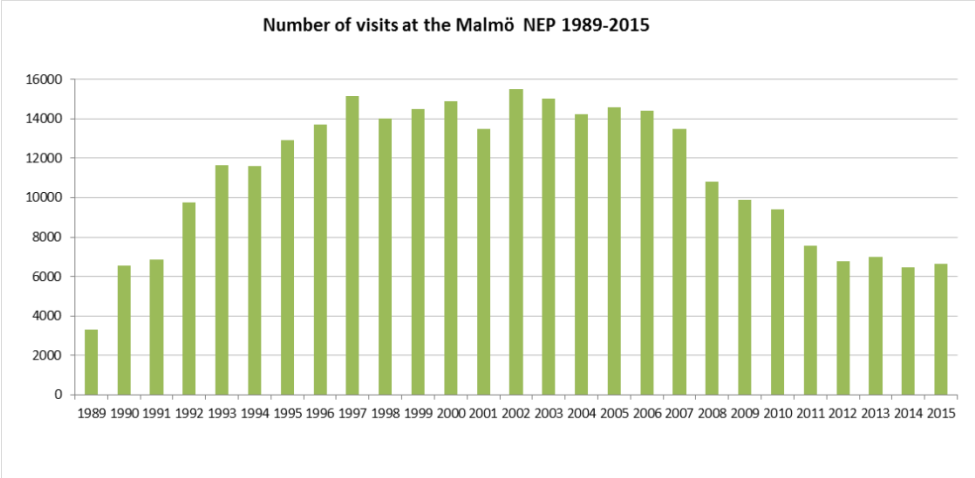


Figure 6 B. Number of annual visits at the Malmö NEP 1989-2015. (Published in the annual NEP report 2015, Isendahl P, Lanbeck P and MAB).

The decline in number of visitors and visits after our study period for study I-III may be attributed to several factors. One is the new legislation from 2006 requiring full identification (NIN) at first visit. The increased recruitment into OST as well as an overall increased access to OST in Skåne County during the past years are likely to be other contributing factors. Many participants of the Malmö NEP utilize the liberal harm reduction services in Copenhagen (anonymous collection of unlimited amounts of needles and syringes, safe injection sites etc.). An increasing trend in number of visitors has been observed from 2015 onward.

Study design

Paper I

Prevalence and incidence of HIV, HBV and HCV among participants of the Malmö NEP in 1997-2005

Participants

A total of 1661 participants registered in the Malmö NEP between 1 January 1997 and 31 December 2005 were eligible for inclusion in study I, and a National Identity Number (NIN) was available for 1183 of those individuals. The remaining 478 subjects were mainly sporadic visitors who refused both use of their NINs and blood sampling (n=454), or agreed only to blood sampling (n=24), and thus identification of serological markers was not possible. Among the 1183 persons who allowed use of their NINs, 831 provided two or more samples and underwent two or more registered needle/syringe exchanges, and they were included in the analysis of incidence and risk factors prevalent at enrolment; this group is referred to as the longitudinal cohort. The 352 persons providing only one blood sample were analysed regarding prevalence of viral markers, demographics and risk profile; this group is called the baseline-only cohort.

Virological testing

Serology

Virological results were retrieved from the mainframe computer at the hospital microbiology laboratory. During the study period, testing was progressively upgraded with new instruments and techniques (1997, chemiluminescence assay using avidin-coated tubes (Boehringer, Mannheim, Germany); 1998, bead-based ELISA (CobasCore; Roche, Mannheim, Germany); 2001, micro particle-based EIA (Abbott AxSym, Abbott Park, IL, USA).

The markers studied were as follows: for HIV, anti-HIV antibodies followed by immunoblotting if needed; for HBV, hepatitis B surface antigen (HBsAg), core antibodies (anti-HBc) and surface antibodies (anti-HBs); for HCV, anti-HCV antibodies followed by immunoblotting if needed. All assays were continuously evaluated in international proficiency panels. Sera were routinely stored at -20 degrees C, which allowed retrospect retesting of the earlier sera with the most sensitive anti-HCV assay (third generation Abbott AxSym).

Molecular tests

In attempt to define the time of HCV acquisition more exactly, all the latest available preseroconversion sera testing anti-HCV negative in the third-generation AxSym assay were retrospectively tested for HCV RNA in a TaqMan48 Roche assay (Roche Diagnostics). Because the sample volumes were limited and the TaqMan system requires 1 mL of starting material, participant sera were prediluted 1:10 in negative serum, which resulted in a detection limit of 150 IU/mL for HCV RNA.

Statistical methods

Odds ratios, Mann–Whitney U-tests and Fisher´s Exact tests were calculated to analyze differences between groups. Univariate and multiple logistic regression analyses were carried out to identify baseline risk factors associated with previous HCV exposure and with HCV seroconversion. Kaplan–Meier curves with log rank tests were plotted to visualize and test time to seroconversion between different 3-year periods. The statistical tests were performed in SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). P-values <0.05 were considered statistically significant. Odds Ratios (ORs) were considered as significant if the entire 95% confidence range either was above or below 1.0. The time point of seroconversion to HIV, HBV and HCV was defined as the midpoint between the last antibody-negative and the first antibody-positive sample. The time point of HCV infection was adjusted by the results of HCV RNA testing. The monitored follow-up period spanned from the first serum sample collected after registration in the NEP to the last sample taken before the end of 2005.

Paper II

Kinetics of viral levels of HCV around seroconversion and one year later

Participants

For study II, participants from study I with documented HCV seroconversion were eligible for inclusion. The HCV RNA data generated from the last anti-HCV negative and the first anti-HCV positive samples (irrespective of detectable

viremia), as well as a sample drawn about one year later, were interpreted quantitatively. Complete series of these three samples were available for 150 persons from a total of 186 individuals with documented anti-HCV seroconversion (paper I). For 32 of the remaining 36 participants one year follow-up samples were not available (due to death in 4 subjects) and in 4 cases the seroconversion samples were exhausted.

Virological methods

HCV RNA testing was done by Roche COBAS AmpliPrep/COBAS TaqMan HCV Test as described above but interpreted quantitatively. For the purpose of statistical analysis, cases with a positive but non-quantifiable PCR signal (below 150 IU/mL) were categorized as having HCV RNA levels of 149 IU/mL, and those completely non-reactive/negative one log10 lower (<15 IU/mL). Genotyping of the viral strains was performed by amplification and sequencing of a part of the HCV NS5B gene.

Statistical methods

Categorical variables were expressed as frequencies with percentages. Continuous variables were presented as median and 25% and 75% interquartile range of log10-transformed values. Comparisons of viral load within each subject between time-points were analyzed with Wilcoxon signed rank test. For categorical variables the exact binomial test was used.

Paper III

HCV transmission among NEP participants studied through phylogenetical analyses

Participants

A total of 685 individuals were diagnosed with HCV in the Malmö NEP follow-up study cohort; 499 of whom were already anti-HCV positive at NEP registration and 186 with incident, time-defined infection after NEP enrolment. For 412 individuals (60% of the initial study cohort of 685); 155 incident and 257 prevalent individuals, a partial NS5B sequence was obtained for analysis and included in study III.

Laboratory Methods

Viral RNA was extracted from 200 µl of serum using a MagNA Pure 96 system (Roche Applied Sciences, Basel, Switzerland), eluted into 50 µl of H2O and aliquoted and stored at -70^{o}C. A combined reverse transcription and first PCR followed by nested PCR targeting the NS5B middle region was performed as

described. The PCR product was purified on a spin column, labeled by ABI BigDye, underwent cycle sequencing and subsequently subjected to bidirectional Sanger sequencing (sequences sent to Genbank, accession numbers pending).

HCV genotyping and local transmission cluster analysis

The local sequences were aligned to HCV reference sequences in ClustalX2, edited to a final length of 321 bases (positions 8277-8597 in the HCV-H reference strain with Genbank accession number M67463) and subtyped by phylogenetic analysis (147, 305). A maximum likelihood (ML) phylogenetic tree was constructed using GARLI v2.0 utilising the GTR+I+Γ nucleotide substitution model as determined using Modeltest and branch support was obtained using aLRT-SH (approximate Likelihood Ratio Test Shimodaira-Hasegawa like) implemented in PhyML v3.1 (306, 307). An aLRT-SH value ≥0.9 was considered significant. To identify local transmission clusters, a background data set was first obtained by identifying the ten best scoring Genbank sequences for each local sequence by BLAST. The Genbank and the local sequences were aligned and ML phylogenetic trees were constructed for each subtype specific data set as described above. To reduce the likelihood of including Genbank sequences from the same patient or sequences in transmission clusters, the number of Genbank sequences was reduced by excluding all except one sequence in monophyletic clusters containing Genbank only sequences (defined as clusters with an aLRT-SH branch support of ≥ 0.9). Finally, an ML tree of non-redundant Genbank sequences and local sequences of the same subtype were constructed. Ten replicates were performed in GARLI v2.0 and the best scoring ML tree of each subtype was selected for transmission cluster analysis. Local transmission clusters were identified by analysis of ML trees from root to tips and were defined as clades in the phylogeny with an aLRT SH-like branch support ≥ 0.9, and contained at least 80% local sequences (308, 309). Local transmission clusters were further classified based on the number of sequences per cluster, into dyads (two sequences), networks (3-14 sequences) and large clusters (15 or more sequences) (308, 310).

Statistical analysis

Descriptive statistics are given as numbers with percentages and medians with quartile ranges (q1-q3). Fisher's exact test or Mann-Whitney U test was used to assess differences between groups. Multiple logistic regressions were also performed to assess relationships to cluster members and non-cluster members (311). Since the number of individuals associated with clusters was limited, the following backward stepwise procedure was performed. Variables with a p-value below 0.30 in univariate analysis were selected as candidates for the multivariable analysis (due to the limited number of individuals with subtype 2b associated with clusters, variables that had a p-value below 0.20 were selected here). The variable

that had the least association was taken out of the model. If the removed variable affected the remaining variables more than 20% it was considered as a confounder and it was returned to the model. This process was repeated until only significant covariates, $p < 0.05$, and confounders remained. Results are expressed as odds ratios (OR) with 95 percentile confidence intervals. The data set was dichotomized in correlation to the median age of the study cohort (31 years) and subanalyses were performed on the subset of younger and older participants.

Paper IV

Coverage and response to HBV vaccination among NEP participants

Participants

All participants enrolled in the NEP 1994-2013, without serological markers for HBV (negative for HBsAg, anti-HBc and anti-HBs) at baseline and with available National Identity numbers (NIN), were eligible for inclusion in study IV, which was a retrospective assessment of vaccination given as a part of the NEP services since 1994.

Vaccination

All HBV-susceptible NEP participants were offered a standard three-dose vaccination schedule with a commercially available vaccine (Engerix-B®, Glaxo-Smith-Kline, 20 µg/1 mL), given intramuscularly at months 0, 1 and 6). An option existed to accelerate the vaccination schedule if needed, as for close contacts to acute clinical HBV cases. This rarely used option consisted of three doses at 1, 7, and 21 days or 0, 1 and 2 months. Irrespective of schedule used, a serum sample was thereafter tested for anti-HBs, usually two months after the third vaccine dose. For individuals without serological vaccine responses following the standard vaccination course, up to three booster doses (each followed by anti-HBs analysis) were given.

Laboratory methods

The NINs were used to retrieve virological results from the Malmö Microbiology laboratory database for serological markers of exposure to HBV, indicating infection, prior HBV exposure or vaccination (HBsAg, anti-HBc and anti-HBs) as well as antibodies to HIV and HCV. Sera were routinely stored at -20^{o}C and traceable based on the associated NIN. Some additional, clarifying re-testing of samples was done for the few cases of vaccination breakthrough, but in the majority of cases our analysis was based on available test results. Data on clinical status and liver function laboratory tests were not systematically collected or available and hence not used in this retrospective study.

Statistical methods

Descriptive data based on sociodemographic information retrieved from the NEP database as well as virological results from the laboratory database were subjected to statistical analysis, with descriptive statistics presented as valid percentages or median values with interquartile ranges. The Fisher's exact test or the Mann-Whitney U test was used to assess differences between non-responders and responders. To examine the relationship between responders and HCV adjusted for age at first dose, multiple logistic regression was used. The statistical tests were performed in IBM SPSS Statistics 22 for Windows (IBM Corporation, Armonk, NY, U.S.). P-values below 0.05 were considered as statistically significant.

Ethical considerations

This study population can be considered particularly vulnerable for leakage of personal data outside the NEP setting. Many NEP participants express upon NEP enrolment a concern of their personal information made available for others. All participants are informed of the secrecy regulations in all hospital charts according to the Swedish law and especially including data collection in the database. All patients in Region Skåne are upon blood sampling informed of the storage of serum or plasma samples in the regional biobank and their right to decline from this. All studies were approved by the Regional Research Ethics Committee in Lund (no 195/2005 and 648/2015). After approval by the Regional Research Ethics Committee, the study purpose and procedure were announced on posters in the NEP and in advertisements in the local press, including a widely distributed free daily newspaper. An opt-out strategy was used, assuming consent for NEP participants who did not actively object to study inclusion. While all virological testing was done with access to a personal identity number, all subsequent handling of patient data was done under code. For study IV, NEP participants were informed at NEP enrolment about the voluntary vaccination and could decline at any time point. Written informed consent was not obtained since the HBV vaccination was part of the national prevention schedule targeting risk populations. The collection and analysis of data, however, was approved by the ethics committee.

Results

I. Prevalence and incidence of HIV, HBV and HCV

Baseline-only cohort vs the longitudinal cohort

The baseline prevalence of HIV, HBV and HCV markers among study participants enrolling in the Malmö NEP in 1997-2005 with a NIN, was analyzed for the whole group (n=1183); those who provided only one sample as well as those with at least two samples. Comparisons were then made to detect differences between these two groups; the baseline-only cohort (n=352) vs the longitudinal cohort (n=831).

The baseline prevalence of serological markers for all identified participants (n=1183) was very low (0.34%) for HIV, moderate (32%) for previous exposure to HBV (anti-HBc) and high (64%) for anti-HCV. Markers of previous exposure to HBV and HCV were significantly more prevalent in the baseline-only cohort compared to the longitudinal cohort for HBV (OR 1.82, CI 1.40–2.37; $p<0.001$) and for HCV (OR 1.85, CI 1.41–2.44; $p <0.001$). The baseline-only cohort also had a significantly longer history of injecting both heroin and amphetamine before enrolling in the NEP ($p<0.001$). This group also reported having spent time in police custody more often (OR 1.69, CI 1.21–2.38; $p=0.002$) or in prison (OR 1.50, CI 1.16–1.92; $p=0.002$), or had been subjected to care under the social services law (OR 1.73, CI 1.27–2.35; $p=0.001$).

Our findings illustrate a difference between those who continued to attend the NEP and those who did not. In general, those who visited only once were more exposed to HBV and HCV and had a longer duration of injection drug use. They had also had more contacts with the law enforcement system and social services.

Longitudinal follow-up cohort

Factors associated with HCV prevalence at baseline and with HCV seroconversion during follow-up is presented in Table 1.

Table 1 Study population at NEP enrolment with regard to anti-HCV antibody status at baseline (left) and HCV seroconversion status for participants negative for anti-HCV at baseline during follow-up (right). Data represent number of individuals and percentages within parentheses, or median values and 2.5–97.5 percentiles within parentheses.

	All (n = 831)	Anti-HCV negative (n = 332)	Anti-HCV positive (n = 499)	Univariate logistic regr. OR (95 % CI)	Multivariate logistic regr. OR (95 % CI)	Anti-HCV non-seroconv. (n = 146)	Anti-HCV seroconv. (n = 186)	Univariate logistic regr. OR (95 % CI)	Multivariate logistic regr. OR (95 % CI)
Males	618 (74)	261 (79)	357 (72)	**0.68 (0.49–0.95)**	**0.39 (0.26–0.58)**	114 (78)	147 (79)	1.06 (0.62–1.79)	–
Age at NEP enrolment	30 (20–52)	25 (20–48)	34(20–54)	**1.09 (1.07–1.11)**	**1.03 (1.01–1.06)**	26 (20–50)	24 (20–47)	0.99 (0.96–1.02)	–
Native Swede	720 (87)	287 (86)	433 (87)	1.03 (0.68–1.55)	–	128 (88)	159 (85)	0.83 (0.44–1.57)	–
Drug used									
Cannabis	725 (87)	297 (89)	428 (86)	0.66 (0.42–1.03)	–	125 (86)	172 (92)	**2.18 (1.02–4.65)**	–
Intravenous heroin	173 (21)	105 (32)	68 (14)	**0.34 (0.24–0.48)**	–	44 (30)	61 (33)	1.14 (0.71–1.82)	–
Intravenous amphetamine	293 (35)	107 (32)	186 (37)	1.18 (0.88–1.59)	–	58 (40)	49 (26)	**0.55 (0.34–0.87)**	–
Intravenous heroin and amphetamine	359 (43)	120 (36)	239 (48)	**1.65 (1.24–2.19)**	–	44 (30.)	76 (41)	**1.60 (1.01–2.53)**	**1.87 (1.15–3.02)**
Oral heroin	83 (10)	45 (14)	38 (7.6)	**0.52 (0.33–0.82)**	–	16 (11)	29 (16)	1.47 (0.76–2.83)	–
Oral amphetamine	300 (36)	101 (30)	199 (40)	**1.52 (1.13–2.04)**	–	53 (36)	48 (26)	**0.60 (0.38–0.96)**	–
Oral heroin and amphetamine	430 (52)	183 (55)	247 (49)	0.80 (0.60–1.05)	–	75 (51)	108 (58)	1.29 (0.84–2.00)	–
Duration of drug use before NEP (years)									
Intravenous heroin	1.0 (0−23)	1.0 (0–11)	1.0 (0–27)	**1.11 (1.07–1.15)**	**1.06 (1.01–1.11)**	0.0 (0–14)	1.0 (0–12)	0.99 (0.93–1.06)	-
Intravenous amphetamine	4.0 (0−33)	1.0 (0–26)	7.0 (0–35)	**1.11 (1.09–1.14)**	**1.06 (1.03–1.09)**	1.0 (0–28)	1.0 (0–20)	0.97 (0.93–1.01)	**0.96 (0.92–1.00)**
In police custody	641 (77)	257 (77)	384 (77)	0.97 (0.70–1.36)	–	109 (75)	148 (80)	1.32 (0.78–2.22)	–
In prison	387 (47)	112 (34)	275 (55)	**2.43 (1.82–3.25)**	**1.59 (1.10–2.29)**	39 (27)	73 (39)	**1.79 (1.12–2.86)**	**1.89 (1.14–3.12)**
Care under law (social services)	129 (16)	44 (13)	85 (17)	1.34 (0.91–1.99)	**1.59 (1.01–2.51)**	19 (13)	25 (13)	1.04 (0.55–1.98)	–
Residential treatment centre	441 (53)	160 (48)	281 (56)	**1.39 (1.05–1.84)**	–	69 (47)	91 (49)	1.07 (0.69–1.65)	–
HBV positive before NEP	236 (28)	35 (11)	201 (40)	**5.72 (3.86–8.48)**	**2.75 (1.76–4.32)**	15 (10)	20 (11)	1.05 (0.52–2.13)	–
HIV positive before NEP	1 (0.1)	0 (0.0)	1 (0.2)	–	–	0 (0.0)	0 (0.0)	–	–

Incidence of HIV, HBV and HCV markers in the longitudinal cohort

Overall, HIV incidence was minimal with only two observed new HIV infections among individuals enrolling in the NEP 1997-2005 (both cases occurring in 1999). Thus, the incidence of HIV was 0.082/100 pyr during a total time at risk of 2433 years.

HBV seroconversion was observed for 39 participants (21 by detection of HBsAg and anti-HBc, 18 with anti-HBc only). The follow-up time was 1160 pyr, yielding a seroconversion rate of 3.36/100 pyr. Nineteen (48%) of the incident HBV cases initiated vaccination with 11 having received only one dose and 2 two doses. Six subjects with HBV seroconversion had been fully vaccinated but had not achieved anti-HBs $\geq$10 IU/mL. No incident case of HBV infection occurred in vaccine responders. Five of the incident HBV cases developed chronic HBV infection. Thirteen (33%) of the 39 HBV seroconverters were susceptible to HCV, and 9 (69%) of those also seroconverted to anti-HCV. Drug use profiles did not differ significantly between those who were and those who were not infected with HBV during participation in the NEP.

A high HCV incidence was observed 1997-2005. During 486 person-years at risk, 186 anti-HCV seroconversions occurred among 332 anti-HCV-negative individuals, resulting in an incidence of 38.3/100 pyr. The overall incidence for HIV, HBV and HCV is shown in Table 2.

Table 2.Incidence of HIV, HBV, HCV incidence among NEP participants enrolled 1997-2005. Reprinted with permission.

Table 2 Incidence of HIV, HBV and HCV among injecting drug users in the cohort of new needle exchange programme participants 1997–2005 and comparison with the previous evaluation in 1990–1993

	Number of susceptibles	Number of seroconversions	Years at risk	Seroconversions/100 pyr	Compared to 1990–1993*
HIV	830	2	2433	0.08	0.00
HBV	588	39	1160	3.4	11.7
HCV	332	186	486	38.3 (31.5†)	26.3

pyr, person-years at risk; *Månsson *et al.*, *Scand J Infect Dis* 32:253–258, 2000 [18]; †Incidence adjusted to detect HCV viraemia in last anti-HCV-negative sample.

The majority of incident HCV infections occurred during the first 2 years after registration. To assess potential trends over calendar years, we divided the entire cohort into three groups composed of all susceptible subjects registered in the NEP during three successive 3-year study periods (1997-99, 2000-02, 2003-05, respectively). The time to event for those susceptible to HCV was calculated in Kaplan–Meier mode, but no significant differences were detected between these three groups (Figure 7).

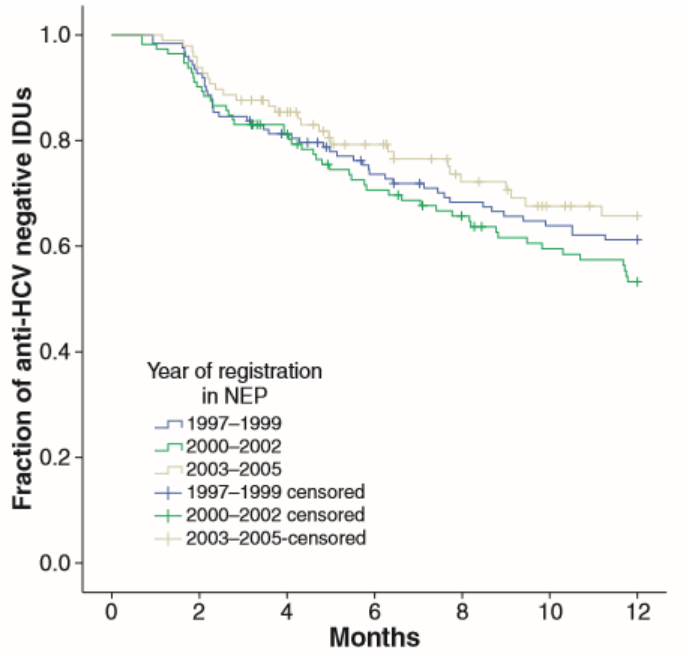


Figure 7. Time to HCV infection.
Anti-HCV-free interval during the first 12 months of participation in the Malmo¨ needle exchange programme among injecting drug users enrolled during three successive periods: 1997–1999, 2000–2002 and 2003–2005. Reprinted with permission.

HCV incidence adjusted for serological window phase.

Because anti-HCV antibodies appear months after infection, the high HCV incidence observed during the first months after enrolment might be explained by HCV infection acquired just prior to registration. Among 186 persons who showed anti-HCV seroconversion following enrolment, HCV RNA was detected in the last available anti-HCV-negative sample in 67 cases (Fig. 8). Thirty-seven of these were HCV viremic in their anti-HCV-negative enrolment sample, leading to a reclassification from incident to prevalent infection from the perspective of the protectiveness by the NEP in those subjects, with an adjusted incidence of 31.5 per 100 pyr.

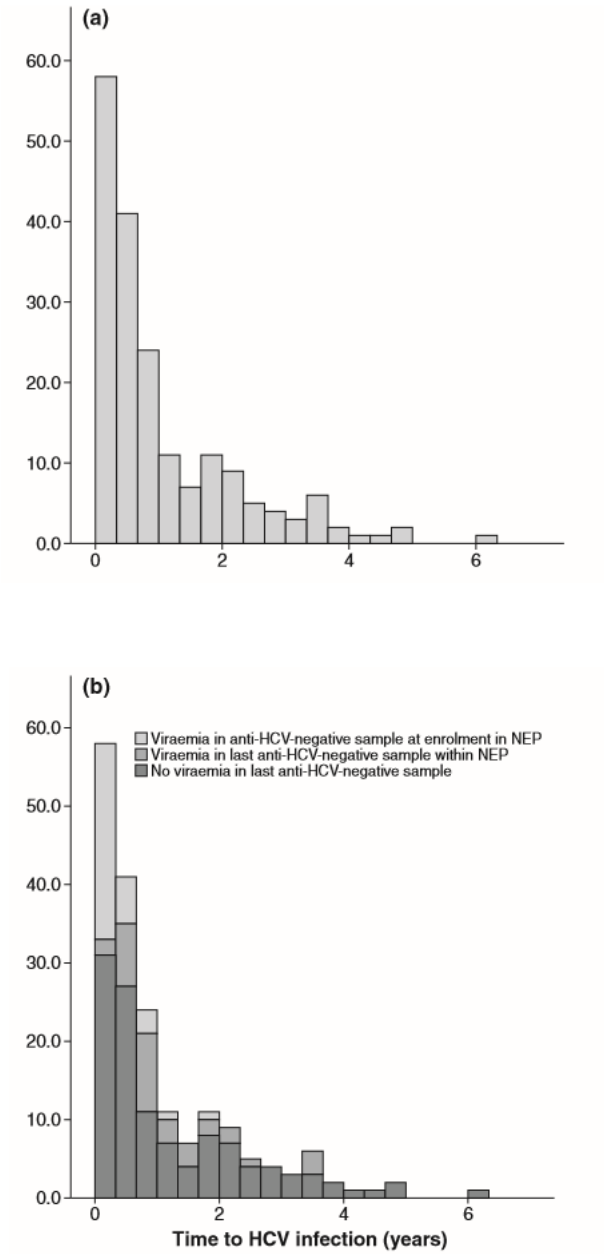


Figure 8. Time (years) elapsed before HCV-seroconversion after enrolment in the Malmö NEP.
Results shown without (a) and with (b) adjustment for viremia in the last anti-HCV negative sample. Reprinted with permission.

II. Viral kinetics at HCV seroconversion and one year later

Among the 186 individuals seroconverting to anti-HCV in the study described above in paper I, for 150 individuals samples were available from three defined time points (anti-HCV negative pre-seroconversion sample followed by the first anti-HCV positive sample and a third sample taken approximately one year later). Eight different patterns of viremia were observed. Spontaneous clearance at one year was noted in 48 cases (32%) and was associated with female gender (p=0.03, CI 0.17–1.00). In 13 cases HCV-RNA was not detected in any study sample. Among 61 subjects with pre-seroconversion viremia, viral load was significantly higher in the pre-seroconversion samples compared to subsequent samples. For the whole group, viral load declined to undetectable levels at seroconversion at the time point of the seroconversion sample in 28% of cases (but with recurrent viremia in 15%).

Baseline characteristics

The gender distribution among the 150 persons in our cohort of incident HCV infection was 119 (79%) males, while the median age at the first anti-HCV positive sample was 27 years (range 21– 53). Thirty four percent reported injecting heroin and 25% amphetamine, while the majority used both drugs (41%). The median duration of drug use prior to HCV infection was 2 years for heroin (range 0–14 yrs.) and 3 years for amphetamine (range 1– 30 yrs.) users, respectively. Prior exposure to HBV (defined as presence of anti-HBc) was observed in 16 persons, whereas no study participant had ongoing HBV or HIV infection.

Patterns of HCV viremia

Number of participants following each of the eight observed viremia patterns (A through H) are shown in Figure 9.

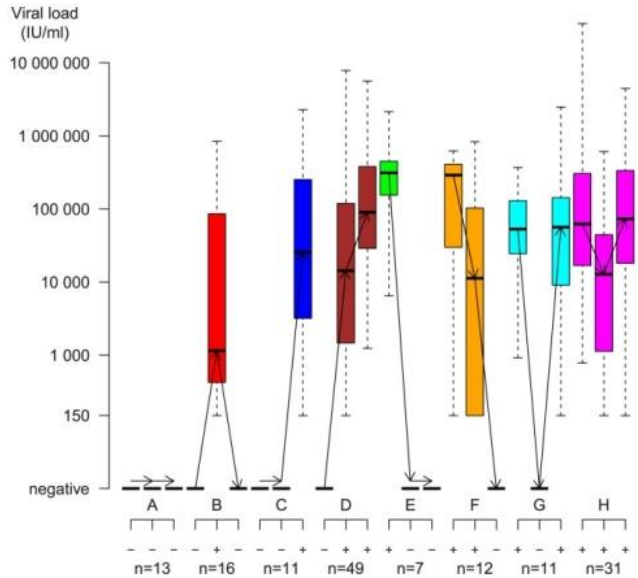


Figure 9: Patterns of viremia.
Groups are defined as described in Materials and Methods, depending on if viremia was detectable or not and indicated by + or −. Viral loads, presented as boxplot diagrams, in different colour for each group (A=black, B=red, C=dark blue, D=brown, E=green, F=orange, G=cyan/light blue, H=magenta) show median, 25% and 75% interquartile range and outliers. For each patient group, the first time point/column represents the last anti-HCV negative sample, the second first anti-HCV positive and the third the one year follow-up. For each group A-H the number of subjects is shown. Viral loads are presented in a log10 scale, and the samples that were RNA positive but below quantifiable as 149 IU/mL, to distinguish from samples where RNA was not detected, which were set one log10 lower at 15 IU/mL. Alanko Blomé, PLoS One 2014. Permission to reprint through the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY NC ND).

For the subgroup with pre-seroconversion viremia, viral load was significantly lower in the seroconversion sample than in the pre-seroconversion sample (p=0.001, CI 0.74–0.93). Since persons showing pattern G (n=11, pattern + to - to +) could represent cases of spontaneously resolved initial infection and subsequent re-infection at the time of obtaining the last sample, we also made a set of separate calculations with this group excluded from the analysis. Similar results were obtained for the whole cohort (now n=139, pre-seroconversion viral load lower than seroconversion viral load, p=0.003, CI 0.54–0.71) as well as for the subgroup viremic in the window phase (now n=50, seroconversion viral load lower than pre-seroconversion, p=0.001, CI 0.69–0.91).

Viral clearance at one year post-seroconversion

Spontaneous viral clearance (defined as absence of HCV RNA in the sample obtained one year after seroconversion) was observed in 48 persons; among these, 13 were persistently non-viremic in all our study samples (pattern A). Sixteen of those who cleared their HCV infection were only viremic in the seroconversion sample (pattern B). Seven had undetectable HCV RNA in both the seroconversion

and one-year follow-up samples (pattern E), whereas 12 of those who cleared the infection were viremic in both pre-seroconversion and seroconversion samples (pattern F). Among factors potentially associated with HCV clearance, only female gender was statistically significant (p=0.03, OR 0.412, CI 0.17–1.00) and we did not find any association with age, duration of drug use or HCV genotype. Nor did we observe a more pronounced decline of viral load between the pre-seroconversion and the seroconversion samples among women with pre-seroconversion viremia who subsequently cleared the virus compared to men with similar characteristics (data not shown).

Genotype distribution in study II

Determination of genotype was possible in samples from 106 of 150 individuals. In 19 cases genotyping was impossible due to undetectable viremia in all samples or a persistently low viral load (<150 mIU/mL). For 25 cases with detectable viremia we were unable to identify the genotype, neither by NS5B nor HCV core gene sequencing. Overall, the following genotypes were identified among 106 cases: 1a distribution with regard to patterns of HCV viremia was observed. Among the 103 individuals with viremia on multiple occasions during follow-up (patterns D, F, G, H), genotyping of two separate samples from the same individual was only possible to perform in 7 cases. In 6 of these 7 cases, the same genotype was found in the second sample, whereas only one showed different genotypes (2b to 1b).

III. HCV transmission studied by phylogenetic cluster analysis

Subtype distribution

Phylogenetic genotyping done as described in the methodology section revealed that subtype 1a and 3a were most predominant in the study cohort consisting of 412 individuals (42% and 38%, respectively) while subtypes 1b and 2b were found less frequently (6% and 14%, respectively). One case of genotype 4 (4d) was observed. Gender distribution was similar for all subtypes with males being more common (range: 75-78% for all subtypes).The number of individuals born in Sweden ranged from 78% (3a) to 93% (2b), the difference being statistically significant by univariate analysis for subtype 2b versus the other subtypes (OR 3,5, 95% CI 2.45-4.55, p=0.011). Exposure to HBV at baseline was associated with non-clustering by univariate analysis for the whole cohort (OR, 0.43, 95% CI 0.26–0.70, p= 0.001), but was only significant by univariate analysis for subtype 1a in subtype specific analysis (OR 0.44, 95% CI 0.20–0.96, p=0.039).

Cluster analysis

Cluster analysis was performed for all subtypes. Among the 412 individuals, 133 (32%) were part of 32 local transmission clusters while the remaining sequences outside clusters most likely represented infections with no or limited local spread. Fourteen clusters formed networks (3-14 members) and one formed a large cluster (18 members), while the majority of clusters were dyads (17 of the total 32). Twelve clusters belonged to subtype 1a, one to subtype 1b, five to 2b and 14 to subtype 3a. Thirty six percent of individuals with subtype 1a belonged to a cluster, 9% of 1b (only one cluster, a dyad), 39% of 2b and 30% of 3a. The median time span from earliest to last sample within clusters was 4 years (range: 1-9 years) where eight clusters (4 for 1a, 1 for 2b and 3 for 3a) contained members with first diagnosis during the last two years (2004-2005) of the nine year study period.

Incident and prevalent HCV infection versus clustering

Among the 32 identified transmission clusters, eight were dominated by individuals with incident infections (two 1a, one 1b, one 2b and four 3a clusters) while eight clusters had a predominance of members with prevalent infections (two 1a, three 2b and three 3a clusters). The remaining 16 clusters were mixed, i.e. they were not dominated by individuals with either incident or prevalent infections.

For the total cohort, participants with incident infection were found more frequently in clusters compared to non-clusters and this association remained statistically significant in multiple logistic regression analysis (OR 2.80, 95% CI 1.75–4.47, $p<0.001$) (Table 3). In subtype specific multivariate analysis, incident infection was associated with clustering for subtype 1a (OR 2.69, 95% CI 1.33–5.42, $p=0.006$) and 3a (OR 2.84, 95% CI 1.32–6.08, $p=0.007$). For subtype 2b, the association between incident infection and clustering was significant by univariate analysis (OR 4.03, 95% 1.19–13.6, $p=0.025$), but did not remain significant in multivariate analysis. Birth in Sweden and exclusive injection use of heroin were identified as confounding factors in the backward stepwise model of multiple logistic regression analysis of the whole cohort. Although not statistically significant by themselves, in the multiple logistic regression analysis they are presented in Table 3 together along with the significant factors. Younger study participants had more often incident infection than older participants, both among cluster members (OR 6.01, 95% CI 2.77–13.0, $p<0.001$) and non-cluster members (OR 5.04, 95% CI 2.87–8.84, $p<0.001$). Among younger study participants, more cluster members than non-clustering individuals had incident infection (OR 2.95, 95% CI 1.61–5.39, $p<0.001$).

Table 3. Factors associated with clustering.
Uni- and multivariable logistic regression. Cluster vs Non-cluster.

	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Male	1.19 (0.72–1.95)	0.505		
Born in Sweden*	0.71 (0.43–1.19)	0.196	0.84 (0.47–1.52)	0.567
Age at registration < 31 years (median)	2.48 (1.62–3.80)	<0.001		
Age at HCV positive	0.96 (0.94–0.99)	0.003		
Incident case	3.38 (2.19–5.19)	<0.001	**2.80 (1.75–4.47)**	**<0.001**
Previous exposure to HBV	0.43 (0.26–0.70)	0.001		
HIV	2.11 (0.13–33.9)	0.599		
Drug characteristics				
Iv use of amphetamine only	0.45 (0.26–0.77)	0.004		
Iv use of heroin only*	2.74 (1.59–4.73)	<0.001	1.28 (0.67–2.47)	0.451
Iv use of heroin and amphetamine	0.97 (0.63–1.48)	0.879		
Declared main iv drug (n=386)				
Amphetamine iv	Ref.		Ref.	
Heroin iv	2.97 (1.89–4.66)	<0.001	**2.22 (1.34–3.70)**	**0.002**

Maximum number; No n=279, Yes n=133. *) Indicate variables that where confounders in the backwards elimination model.

Age and clustering

Age at NEP enrolment for all study participants was in median 31 years (q1-q3: 24–40 years). The median age for individuals outside transmission clusters at NEP enrolment (33 years, q1-q3: 25–42) was significantly higher than for those within clusters (28 years, q1-q3: 23–36, p<0.001). The same trend was seen for the age at the first known anti-HCV/HCV RNA positive sample where the total group median age was 30 years (q1-q3 25–38) while the median age for cluster members was 28 years (q1-q3 24–35) and non-cluster members was 31 years (q1-q3: 25–39), (OR 0.96, 95% CI 0.94–0.99, p=0.002; individuals within clusters lower).

Young study participants were found more frequently in clusters compared to old participants, 63% vs 41%, OR 2.95 (1.61–5.39), p<0.001. Young participants were also associated by univariate analysis with clustering for subtype 1a (OR 2.30, 95% CI 1.20–4.40, p=0.012), subtype 2b (OR 3.33, 95%CI 1.03–10.8, p=0.045) and 3a (OR 2.79, 95% CI 1.38–5.66, p=0.004).

Drug profile and clustering

In univariate analysis, exclusive injection use of heroin was more common among cluster members compared to non-cluster members (OR 2.74, 95% CI 1.59–4.73, p<0.001), while the opposite was seen for exclusive injection use of amphetamine (OR 0.45, 95% CI 0.26–0.77, p<0.005). In multiple logistic regression analysis,

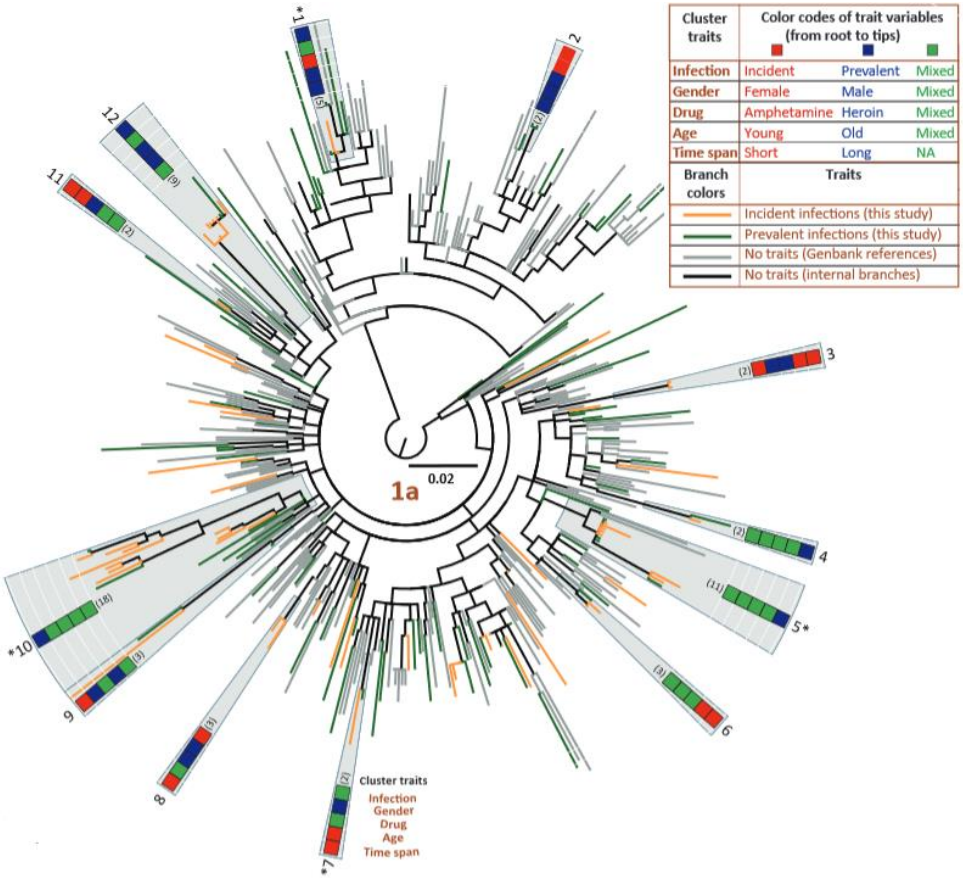
use of heroin as the self-declared main injection drug remained statistically significant among cluster members compared to non-cluster members (OR 2.22, 95% CI 1.34–3.70, p=0.002).

Self-perceived HCV status at NEP enrolment

For 89% (229 of 257) of the individuals, data on the self-perceived HCV status at baseline was available. About two thirds (69.4%) of anti-HCV positive individuals perceived their status correctly while one third (30.6%) recognized their HCV status incorrectly. Conversely, the majority (96.4%) of anti-HCV negative individuals perceived their status correctly while only 5.4% realized their status incorrectly. More younger anti-HCV positive individuals than older (52% vs 21%, $p<0.001$) incorrectly perceived themselves as anti-HCV negative. The differences in self-perceived HCV status was however not statistically significant between cluster and non-cluster members among the incident cases.

The phylogenetic trees for genotypes 1a and 3a are displayed with colour codes indicating distribution of drug type/gender/age/incident and prevalent members within clusters (Figure 10).

10 a.


Cluster traits
Color codes of trait variables (from root to tips)
Infection Incident Prevalent Mixed
Gender Female Male Mixed
Drug Amphetamine Heroin Mixed
Age Young Old Mixed
Time span Short Long NA
Branch colors Traits
Incident infections (this study)
Prevalent infections (this study)
No traits (Genbank references)
No traits (internal branches)
1a
0.02
Cluster traits
Infection
Gender
Drug
Age
Time span

10 b.

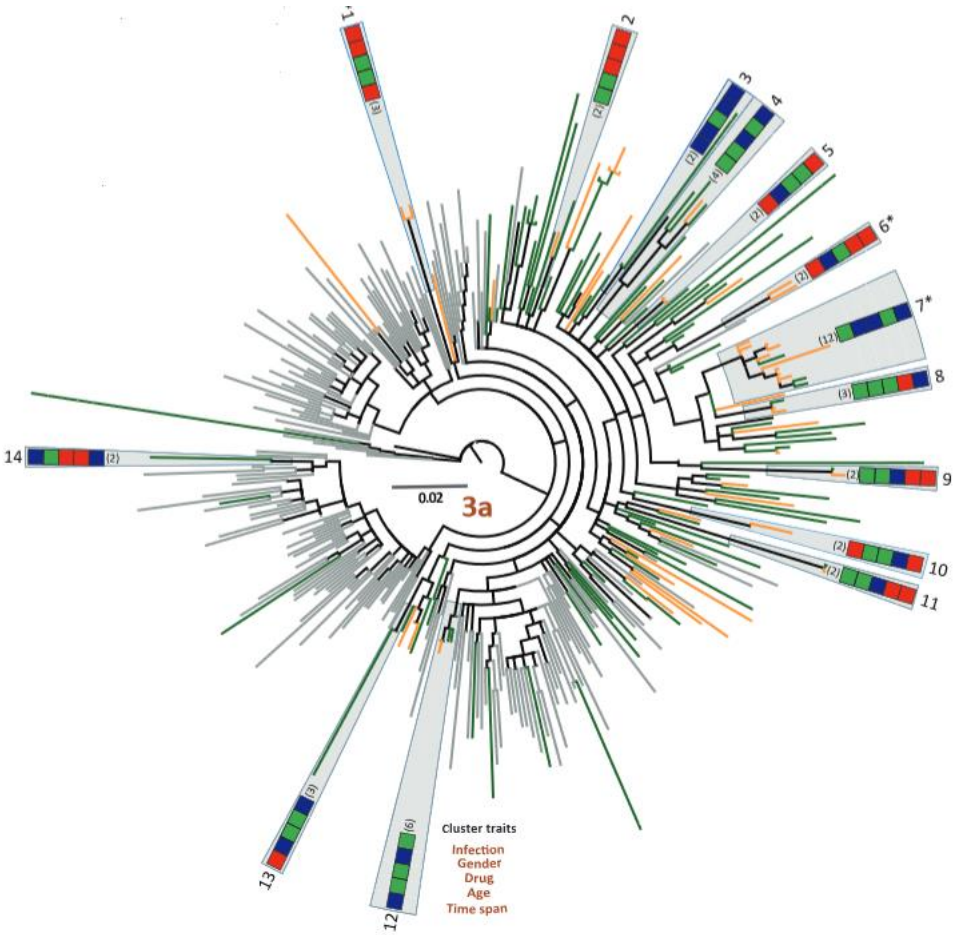


Figure 10.
Phylogenetic trees of subtypes 1a (fig. 10 a) and 3a (fig 10 b), generated as described in Materials and Methods. Clusters indicated by gray boxes.Clusters labeled as indicated in the colour code chart. Prevalence >80% of a certain trait (incident/prevalent infection, gender, main drug, age below or above/equal to the median age of 31 years, time span for HCV detection within clusters < or >4 years) is indicated by red/blue. Traits <80% within clusters are termed "mixed" and coloured green.

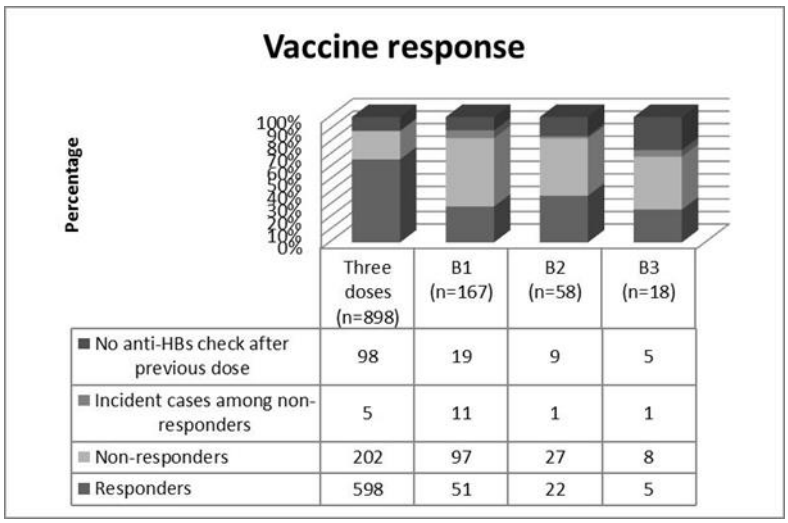
IV. HBV vaccination coverage and efficiency

Response to the basic vaccination schedule

HBV data was available for 2352 identifiable individuals enrolled in the NEP in 1994-2013. Among these, 1516 (64%) had no markers for previous exposure to HBV infection or vaccination and 1142 (75%) agreed to initiate vaccination. Of them, 898 (59%) completed the standard vaccination schedule. Anti-HBs levels were obtained in 800 cases, with 598 (75%) responding to the basic vaccination schedule.

Incremental responses to 1-3 booster doses

One hundred sixty seven of the 202 (83%) non-responders to the standard schedule received a first booster dose, subsequent testing of anti-HBs levels was done in 148 (89%) cases. At this step, 51 (34%) persons had achieved protective anti-HBs levels. Fifty eight of the 98 (60%) non-responders received a 2nd booster, followed by anti-HBs testing in 49 (84%) cases and with 22 (45%) showing anti-HBs seroconversion. A 3rd booster was given to 18 non-responders, with anti-HBs tested in 13 cases, 5 (38%) responding. The responses to the standard vaccination schedule and subsequent boosters are shown in Figure 11.



	Three doses (n=898)	B1 (n=167)	B2 (n=58)	B3 (n=18)
No anti-HBs check after previous dose	98	19	9	5
Incident cases among non-responders	5	11	1	1
Non-responders	202	97	27	8
Responders	598	51	22	5

Figure 11.
Responses to the standard HBV vaccination schedule and each subsequent booster dose. The ratio between responders (anti-HBs >10 mIU/mL), non-responders and the number of cases without anti-HBs level check-up after the standard three dose schedule and boosters 1 to 3. Also shown are the number of incident cases among non-responders detected at each step.

After up to three booster doses a total of 676 (85%) achieved protective anti-HBs levels.

Factors associated with vaccination response

Of the 800 subjects who completed the standard vaccination schedule and for whom subsequent anti-HBs levels were available, 555 (69.4%) were male. The mean age was 29.5 years (median 27.4, range 16-64). The time point of anti-HCV prevalence (prior, during or after HBV vaccination) could not be determined for 24 anti-HCV positive individuals (21 responders and 3 non-responders) and they were thus excluded from the following analysis. For the remaining 776 individuals, anti-HCV was prevalent before the first HBV vaccine dose in samples from 399 (51.4%) individuals, while 101 (13.0%) seroconverted to anti-HCV during the vaccination period and 141 (18.2%) seroconverted after the third vaccine dose. Of the vaccine responders, 48.5% were anti-HCV positive before initiation of HBV vaccination, compared to 66.9% of the non-responders. Thus, anti-HCV seropositivity before initiation of HBV vaccination was significantly less prevalent among vaccine responders (OR 0.365, 95% CI 0.224-0.594. $p<0.001$). Seroconversion to anti-HCV between vaccine doses 1 and 3 or after dose 3 did not differ significantly for vaccine responders compared to non-responders.

Higher age at start of vaccination was significantly associated with non-response; median age was 31.0 years (IQR 25.3-37.2 years) in non-responders versus median age 26.9 years among responders (IQR 23.1-33.7), (OR 2.76, 95% 1.23-4.29, $p<0.001$).

Concerning socio-demographic data, incarceration prior to NEP enrolment was significantly less common among vaccine responders (OR 0.464, 95% 0.075-0.853, $p<0.001$). Other socio-demographic markers such as country of birth (Sweden or not), education, housing (stable versus unstable), main injection drug (heroin versus amphetamine) and engaging in commercial sex, were not associated with vaccine response.

Incident HBV seroconversion

During vaccination

Thirty participants developed incident HBV infection before completion of the standard vaccine schedule; 21 after the first vaccine dose and 9 after the second. Seventeen were detected through seroconversion to anti-HBc and 13 by the detection of HBsAg and anti-HBc. Of the latter, 2 developed chronic infection, 9 cleared their HBsAg and 2 died within 6 months of HBsAg detection. Both of those who developed chronic infection were to our knowledge asymptomatic at the

time point of HBV infection, but one of them developed a fulminant HDV hepatitis one year later.

Among non-responders

Eighteen incident cases of HBV infection were observed among the 202 vaccine non-responders by the end of 2013; five were among individuals who had received three vaccine doses, eleven had received one booster, one two boosters and one three boosters. Ten of these eighteen were identified by detectable HBsAg (among whom 3 developed chronic infection, 6 had documented HBsAg clearance and 1 was lost to follow-up). Two presented with symptoms, one did not and data on seven was missing (no information on the test referral sheet). Time to the detection of HBsAg and/or anti-HBc from the last vaccine dose was in mean 5.5 years (in median 3.4 years, range 0.2-15.8 years).

Post-vaccination serological breakthrough with anti-HBc development in vaccine responders

Long-time follow-up testing for anti-HBc and/or HBsAg was not routinely done for vaccine responders, and not part of our study aims, but later data was available for 140 (21 %) out of the 676 vaccine responders (taken in median 21 months after the test with anti-HBs >10 mIU/mL, range 0-200 months).Three cases where adequate anti-HBs titers had been achieved (16, 23 and 66 mIU/mL, respectively) and testing for anti-HBc was negative at the same time point, had developed anti-HBc 8-11 years later and had thus had postvaccination HBV replication at some time point. Thus, in 3/140 (2%) examined samples seroconversion to anti-HBc was observed among vaccine responders.

Anti-HBs levels for vaccine response

Overall, standard schedule responders achieved higher final anti-HBs levels than subjects who fulfilled criteria for seroprotection after receipt of booster doses (median 350 mIU/mL vs. 40 mIU/mL for 2nd dose responders and 34 mIU/mL for 3rd dose responders, respectively, as shown in figure 12.

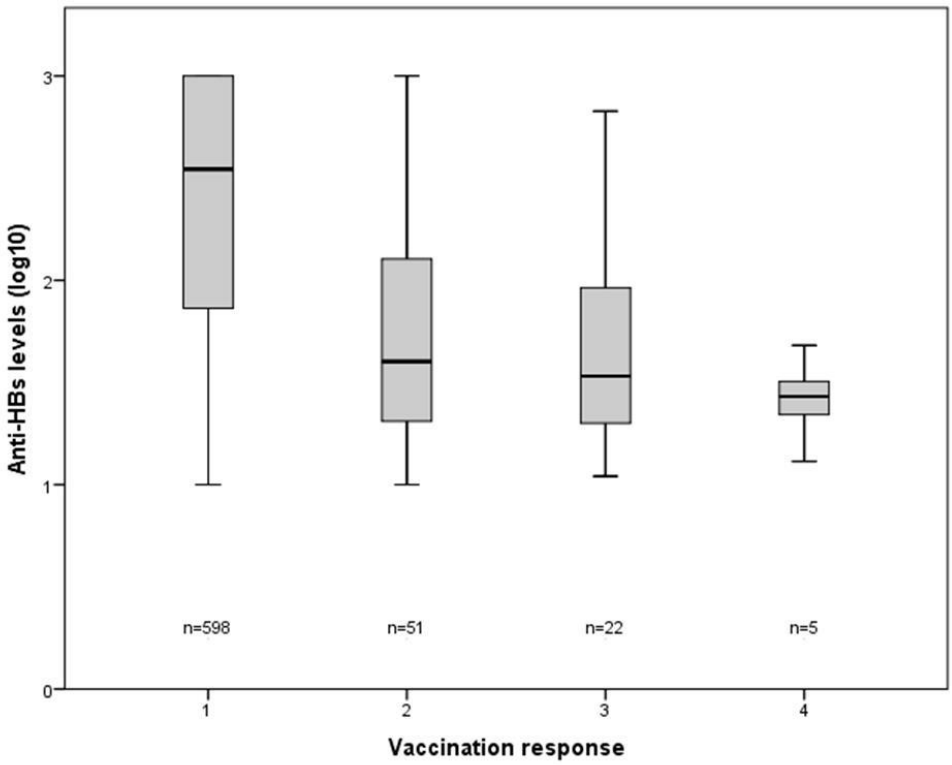


Figure 12. Anti-HBs levels corresponding to completed basic vaccination schedule and subsequent boosters among vaccine responders. Observed anti-HBs levels on a logarithmic scale at different time points: 1. After completion of the standard three dose vaccination schedule (n=598). 2. After the first booster (n=51). 3. After the second booster (n=22). 4. After the third and final booster (n=5). The levels of Anti-HBs decreased after each step, with median anti-HBs levels achieved after the standard schedule at 350 mIU/mL and after the third booster 34 mIU/mL.

General Discussion

If the prevalence of HIV is minimal in the PWID population under investigation, HIV seroconversion is not a reliable marker to detect blood borne transmission. Like in the 1990–93 study cohort of Malmö NEP, the incidence of HIV remained low in the 1997–2005 cohort (298). We have used HBV and HCV as surrogate markers of the potential risk of spreading HIV. It is obvious that sexual transmission and HBV immunization can confound the incidence of HBV and affect its usefulness as a surrogate marker. Apart from among HIV-positive MSM, HCV is not effectively transmitted sexually and thus mainly reflects the injection risk, making this virus a more suitable marker of blood borne transmission (182).

Our high HCV incidence during 1997-2005 remains alarming, but is in agreement with previous results from the Malmö NEP and from other studies during this time period (312, 313). Several reasons may contribute to this phenomenon, including the much higher background prevalence of HCV than of HIV infection among PWID, a higher viral load and greater physical virion stability of HCV compared to HIV (245, 314).

Thus, prevention of HCV transmission among PWID is indeed a challenge. This was already demonstrated in 2006 by a meta-analysis of 18 studies, with the authors concluding that neither NEPs nor OST alone affected the incidence of HCV among PWID (281). Subsequently, improvement of harm reduction measures by implementing high coverage and low threshold NEPs and OST have resulted in declining HCV incidence among PWID in the Netherlands and in the UK (270-272).

Declining HCV incidence has, however, not been observed in all settings. In New York City, expanded combined prevention programs were implemented during 2006-2013; e.g., OST, NEPs, expanded syringe access program through pharmacies (ESAP), the NYC Condom program and HIV Treatment as Prevention (TasP). HIV prevalence and incidence declined substantially among PWID during this period, while no significant decrease in HCV prevalence or incidence was observed (315). It was concluded that PWID in general, and new injectors in particular, had incomplete access to OST and incomplete sterile needle and syringe coverage (whether through NEP and/or ESAP), as well as a low HCV treatment uptake, resulting in some degree of sharing (193). Thus, what was

sufficient to have an effect on HIV transmission, still was insufficient for effective HCV prevention.

As mentioned earlier, massive scale-up of antiviral treatment among active injectors has been proposed as a strategy to prevent HCV transmission. Mathematical modeling taking into account the baseline prevalence of HCV among PWID has estimated the time and effort needed to achieve a decline of HCV prevalence and incidence. According to one mathematical model, for settings with higher baseline among PWID (such as Melbourne with 50% and Vancouver with 60%), it was estimated that the chronic HCV prevalence would decrease by 50% through a scale-up of treatment by 13–15 fold, with annual HCV treatment rates of 40 per 1000 PWID and 76 per 1000 PWID, respectively (194). For the participants in the Malmö NEP, this would, if approved, motivate actions at the level of those proposed for a high-prevalence setting like Vancouver.

The difference between those who continued to attend the NEP and those who did not was illustrated by the comparisons between the longitudinal cohort and the baseline-only cohort in study I. In general, those who visited only once showed signs of greater exposure to HBV and HCV in line with their longer duration of injection drug use. They had also had more contacts with the law enforcement system and social services. However, despite greater HCV prevalence, findings from our study III imply that this population is probably not drivers of the HCV epidemic and responsible for ongoing spread of HCV. Instead, clustering was significantly more frequent for those with incident infections, main injection use of heroin and by univariate analysis, also younger age. That could motivate DAA treatment, which is now introduced in many countries. From another more clinical perspective, case-finding of PWID and other individuals with chronic hepatitis C in the community is of great importance since many of these may have unrecognized advanced liver disease and are in need of antiviral therapy in order to prevent disease progression and death.

Incident HCV infection

We observed in study I that the duration of injection use of heroin prior to NEP enrolment was in median one year (4 years for amphetamine users) and that most cases of HCV seroconversion occurred during the first year after enrolment. Similar data on time duration from onset of injection use to HCV seroconversion has been reported by Hagan et al (170). They found the median time to HCV infection to be 3.4 years overall, however, for 41% of new injectors (injection duration $\leq$2 years), time to HCV infection was estimated to 0.6 years. In their cohort 62% used heroin and 67% had visited a NEP.

Retrospective assessment of HCV RNA in frozen key samples from the longitudinal cohort revealed viremia in 37/332 (12%) of the subjects who were

anti-HCV negative at the time of enrolment. In an additional 30 individuals, window-phase viremia was detected in the sample obtained prior to anti-HCV seroconversion. After the initial phase of high HCV incidence following enrolment, new HCV infections became rarer in those still susceptible – a phenomenon which could be due both to behavioural changes with safer injection practices and less needle-sharing. Possibly genetic or immunologic factors may lead to mediated resistance to HCV-infection.

Among those with incident HCV infection, different longitudinal patterns of HCV viremia were found when studying HCV RNA profiles based on three sequential serum samples starting from before the time of documented seroconversion until approximately one year after the appearance of the first available anti-HCV positive sample. Viral load was highest in those cases that manifested positive HCV RNA in samples obtained prior to the development of antibodies, declined in the first anti-HCV positive samples, and rose again in the follow-up samples to median levels lower those than prior to antibody seroconversion. Others have found associations with high HCV RNA levels in early chronic infection and factors such as IFNL4 rs12979860 CC genotype, male sex, HIV coinfection and HCV genotype 2(316). A more pronounced decline of HCV viremia within the first months following infection has been reported among female PWID (but not male), with subsequent viral clearance (317). In contrast, we did not find that the pattern of early HCV kinetics influenced the chance of spontaneous clearance (for the whole group or for either gender) – among 42/150 (28%) persons with undetectable HCV viremia in the seroconversion sample, 22/150 (15%) had detectable HCV RNA at the one-year follow-up. Another study prospectively following PWID with apparently cleared infection, observed that viral intercalation (ie, intermittent recurrent bouts of viremia with homologous virus interspersed with nonviremic periods) occured with significant frequency (318). The rate of spontaneous viral clearance (32%) in study II was slightly higher compared to clearance rates reported from other studies (approximately 24% among PWID and 15% in HIV-positive MSM) (165). In our study, comparison between the groups regarding age at seroconversion, gender and viral genotype showed no other significant differences than a greater chance for women to spontaneously clear the virus ($p=0.03$, OR 0.412, CI 0.17–1.00). The median age at seroconversion was relatively low in our cohort (27 years), which has been suggested as a favourable factor regarding spontaneous clearance (319). Other factors have been associated with spontaneous clearance, for example the CC variant in the genetic locus Lambda4 (formerly designated IL28-B on chromosome 19) (167). Also, individuals homozygous for certain combinations of killer inhibitory receptor (KIR) genes and ligands have a higher chance of viral clearance than others, while HCV-specific memory CD4 and CD8 T cells have been found in blood from individuals with spontaneously resolved HCV infection

a long time ago (320). Thus, even with effective treatment options, there are still other aspects of HCV that needs to be elucidated and possibly enable vaccine development.

Based on an assumption that persons with incident HCV infection have high risk behaviour, and our finding of high-level viremia in this group, we hypothesized that such individuals could also be more likely to spread HCV onward. In order to explore this hypothesis, we examined partial HCV sequences from subjects with incident and prevalent HCV infection at NEP enrolment with phylogenetic methods. In our study III, incident infection was associated with phylogenetic clustering. Incident infection (anti-HCV seroconversion after NEP enrolment) was an independent, significant factor by multiple logistic regression for belonging to a cluster. The same degree of significance was also seen for heroin being the main injection drug.Younger age (below the median age for the whole cohort, 31 years) at NEP enrolment and at the first available HCV positive sample was also associated with belonging to a cluster in univariate analysis.

Our findings are in agreement with Canadian studies, where approximately one third (31-36%) of the PWID included belonged to clusters (321, 322). A recent report from one of these studies, the VIDUS cohort study in Vancouver, also identified incident infection as well as younger age associated with HCV clustering (323).

Thus, our findings and those from other researchers support the hypothesis that incident HCV infection may drive the HCV epidemic - the similarity with ongoing HIV spread is evident (324, 325). Apart from high-level viremia and high-risk behavior, incorrect perception and unawareness of one's own HCV status could contribute to transmission from young recently infected individuals. A statistically significant number (52% vs 20%, $p<0.001$) of those anti-HCV positive below the median age of 31 years in our cohort incorrectly perceived themselves as uninfected with HCV. This indicates further the need of early access to testing and linkage to care for PWID. Forwarding the most recent test results to the patient has been suggested to be beneficial in correlation to risk behaviour by most, but not all authors (326-328).

Specific drug use habits may have an impact on HCV transmission – an increased risk for HCV seroconversion has even been observed for injecting prescription opioids (also compared to heroin injecting) (329). Phylogenetic HCV clustering has been associated with the use of methamphetamine among street-involved youth in Vancouver (330). Clustering was associated with mainly injecting heroin in our study, in the city of Malmö, where heroin was introduced into the amphetamine dominated PWID network in the early 1990s. In 1990-93, injection use of amphetamine was reported by the majority (71 %) and heroin injecting by only 14 % of the NEP participants (15 % reported injection use of both drugs).

However, several mixed clusters included both amphetamine and heroin users, reflecting the increasing combined use of both opioids and amphetamine.

Our findings of high viremia levels prior to seroconversion in acute HCV infections and incident infection as well as main heroin injection use being independently linked to transmission clusters can be used to target the group with the highest risk of transmitting HCV within the PWID community. Although our data are retrospective, today´s PWID with these characteristics should have first priority if the concept of treatment as prevention is accepted and introduced (as it is used in HIV control). Obviously, this should be added to the current strategy of providing treatment to persons with liver disease, since it also addresses asymptomatic subjects with ongoing risk behavior who are at the greatest risk of onward transmission.

Genotype distribution

Our findings on genotype distribution in study III indicate circulation of several HCV genotypes (1a, 1b, 2b and 3a) among NEP participants during the study period; 1a and 3a were the most prevalent ones in the whole group as well as in clusters, which is consistent with the findings of others in our geographic region (190).

One inherent difficulty in studying HCV infection in PWID (in contrast to transfusion recipients or persons infected through needle-stick injuries) is that the time point of exposure leading to infection is usually impossible to determine. Furthermore, it is probable that a majority of PWID are exposed to HCV on multiple occasions. Infection with multiple HCV strains may therefore be common, although mixed infections are rarely detected with routine techniques. Even for individuals with access to a NEP, continuing injection drug use poses repeated risks of exposure to HCV. Estimates of reinfection rates have varied, but after treatment reinfection incidence appears to be lower than incidence rates of primary infection and chances of reclearance higher (167, 203).

Vaccination against HBV

PWID are recognized to be at high risk of HBV infection and are therefore a target group for vaccination in countries where routine childhood vaccination was not implemented when the individuals who later became PWID were children. However, PWID are often hard to reach for health interventions, and furthermore adherence to vaccination schedules and follow-up testing may be inadequate. Efforts have been made to reach PWID for vaccination in the streets, prisons or when entering into drug treatment facilities (331-333). In the Malmö NEP, routine HBV vaccination with systematic testing for assessment of vaccine response was introduced in 1994.

In our study IV, 74.8% of those receiving all 3 doses of the basic vaccination schedule achieved seroprotection. Response to HBV vaccination among PWID in our study was thus lower than that reported in general populations, where protective immunity is achieved in 90-95 % of healthy individuals (334, 335). Repeated booster doses led to achievement of seroprotective anti-HBs levels in 78/202 (38.6%) of initial non-responders, raising the total response rate to 676/800 (84.5%).

Genetic factors, immunosuppression, and various chronic illnesses (e.g. chronic renal disease, chronic HCV and/or HIV infection, alcoholic liver disease) as well as male sex, smoking, obesity, higher age (>40 years), incorrect site of vaccine injection or length of the needle have been associated with a poorer response to HBV vaccination (336-338). In a study among PWID in Texas, non-response was found to be associated with age >40 years and injecting multiple times a day (339). A review investigating the immunogenicity of HBV vaccine among PWID found divergent results among the twelve separate studies included; in three of them the vaccine was found to be less immunogenic in PWID (with the lowest anti-HBs seroconversion rates among PWID at 58%) (340). Another meta-analysis identified the time point of testing for anti-HBs levels to be a significant factor associated with classification of response (testing recommended at 2 months, compared to testing at 1 or 6 months after the third dose) (341).

In agreement with previous studies, we found vaccine response to be correlated to age, with higher response rates among younger individuals. However, we were unable to determine the impact of several of the abovementioned previously reported factors on vaccine response. Since the prevalence of HIV infection is minimal (<0.5%) in our NEP cohort, we could not assess its impact on vaccine response, suggested by others (342). In contrast, HCV exposure was common, and the presence of anti-HCV was independently associated with non-response to HBV vaccine, corroborating findings from other researchers (343).

We found that sequential booster doses incrementally improved the response rate among standard schedule non-responders. Using adjuvated vaccines with proven higher immunogenicity to groups with high rates of non-response, such as haemodialysis patients, might improve vaccine response among PWID as well (344).

In our study, the three cases with serological vaccination breakthrough after approximately 10 years of measured anti-HBs levels >10 mIU/mL <100 mIU/mL might indicate the need of testing individuals responding in this range after 10 years and perhaps providing an additional booster to those with anti-HBs levels that have declined below the level of 10 IU/mL. A recent long-term (30 years) study on anti-HBs levels and response to boosters among Alaska natives

concluded, however, that the absolute majority (>90%) had evidence of protection and no further booster doses were needed (345).

In our material no new HBsAg positive cases during 2010-13 were found, likely reflecting an achieved herd immunity effect. This is supported by findings from an unpublished survey conducted in Skåne County showing declining rates of HBV infection in geographical areas with NEP access; incidence rates were 3.96/100 000 inhabitants in areas without NEP access compared to 1.75/100 000 in areas with NEPs (RR 2.29; 95% CI 1.84-2.85; p<0.001, personal communication, Mattias Waldeck, Medical Officer, Regional Office of Communicable Disease Control and Prevention, Skåne County, Sweden).

Limitations

Cohort studies of PWID are challenging to perform. A balance between stringent protocols and representative study populations must be achieved. We chose to base our studies on participants in a NEP, which involved some limitations. Although approximately 70% of PWID in our uptake area are estimated to have enrolled in the Malmö NEP we have limited knowledge on PWID who do not enroll, and the findings from our studies should therefore be interpreted with some caution. Furthermore, only NEP participants who were fully identified could be followed, which might have affected the results. Some socio-behavioural data, especially on syringe and needle sharing and changes of drug injection risk profile or sexual risk factors over time, were limited since our behavioural data focused on baseline interview findings. In this real-life setting, blood sampling and vaccine distribution followed the sometimes irregular schedules of the participants. Thus follow-up samples were not available for all individuals seroconverting to HCV, nor was anti-HBs data after vaccination available for all participants and thus the response to all vaccine doses could not be measured. Nor could we assess some factors potentially associated with either vaccine non-response (such as BMI) or clinical data on symptoms and laboratory results on liver enzymes and other markers. Shorter sampling intervals according to a more strict study protocol could have described the viral kinetics around seroconversion in greater detail.

Strengths

To our knowledge, these studies are the first from a NEP setting in Scandinavia. The participants in these studies represent a majority of PWID in the uptake area, and the material is therefore not likely to be biased towards subgroups of PWID. A strength for the studies included in this thesis was the access to a large study material with longitudinal sample series collected following a structured protocol from all participants (from prior to HCV seroconversion as well as follow-up samples). HIV prevalence was low in this setting, which enabled us to study patterns of HCV viremia not influenced by HIV coinfection. Routine regular

collection and storage of samples allowed us to identify cases of incident HCV infection before anti-HCV seroconversion. By sharpening the criteria of observation intervals it was possible to measure the kinetics in the pre-seroconversion to seroconversion period. Also, we could over two decades in a real world situation describe HBV vaccination take-up among PWID and measure development of seroprotection both after a standard vaccination schedule and after adding booster doses.

Conclusions and future perspectives

The first Global Health Sector Strategy (GHSS) on viral hepatitis with an ambitious goal of eliminating HBV and HCV as public threats by the year 2030 was adopted in 2016 by the World Health Assembly. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has alerted, however, on gaps in the available data (estimates of the prevalence and incidence of viral infections among PWID including genotype distribution and the access for PWID to diagnostic testing and further linkage to care). Sweden has partly due to the restrictive harm reduction policy not reported complete data to the EMCDDA.

Through the NEP in Malmö, a well-established institution among the local PWID with longitudinally collected data over almost three decades covering virological as well as sociodemographic variables, the studies included in this thesis have been possible. We have described how the prevalence and incidence of HIV has remained minimal within this population, mainly thanks to the early introduction of the epidemiologic surveillance and access to sterile injection equipment. The prevalence and incidence of HBV have decreased following the broad vaccination approach implemented more than twenty years ago. Despite the efficiency apparently being lower among PWID than among the general population, a successful elimination of HBV has been achieved.

After study I we concluded that the persistently low incidence of HIV in the cohort studied may be explained by a protective effect of the NEP combined with low background prevalence of HIV. Although there were no signs that the NEP affected the incidence of HCV during 1997-2005, we could establish that the NEP contributed to the surveillance and control of HIV transmission in the target population.

The findings in study I with the alarmingly high HCV incidence prompted us to try to analyze in whom, why and when these HCV infections occurred. We realized that improved diagnostics beyond anti-HCV serology would offer improved means of case finding and further analysis. Thus testing for viremia with HCV RNA PCR was applied, to establish the rate of incident infections developing into chronic infection (equaling an ongoing risk for further transmission), which was done in study II.

For study II, we observed that several patterns of viral kinetics occurred among PWID with incident HCV infection. The highest viral loads were observed in the window phase, before the development of anti-HCV antibodies. Thus, basing the diagnostic measures on HCV RNA testing instead of on anti-HCV antibodies would enable detection of HCV infection already in the window phase.

Next, aiming at identifying risk factors for an ongoing HCV transmission among NEP participants, we applied phylogenetic analysis methods in study III. Here we could conclude that clustering was linked with incident HCV infection, main injection use of heroin and to a lower degree with younger age, suggesting that HCV transmission mainly occurs among PWID with acute HCV infection. Our study analysis was retrospective, but knowledge of HCV transmission clusters generated in real time today together with contact tracing and active screening by HCV RNA PCR could probably identify target treatment candidates or groups, those who are recently infected and have the highest risk for onward transmission. Interventions, such as expanded testing and linkage to further care (both for HCV infection and for opioid substitution), could be specifically aimed at PWID with these characteristics.

Our conclusion for study IV is that HBV vaccination through a NEP is feasible and should be recommended as a component of NEP interventions, especially in settings where childhood immunization is not implemented. Response rates might be further improved by distribution of adjuvated vaccines to non-responders, which should be studied in the future. With the vaccination failure rate in about a fourth of all who were followed up, it seems highly justified that efforts are made to follow up the standard 3 dose vaccination schedule with a test for achieved seroprotection.

Development after studies I-III (1997-2005)

The effectiveness of a NEP depends on several factors, which, in addition to the number of needles and syringes exchanged, include acceptance and accessibility by the target population, as well as provision of adequate health education and medical services. In the Malmö NEP, limited opening hours and an age requirement of $\geq$20 years for participation may have been counterproductive in limiting the spread of HCV. The broad range of services offered in a non-denunciative, user-friendly environment probably has had the opposite effect, by attracting PWID and enabling testing and linkage to further assessment and care. Findings from these studies have contributed to the further development of the Malmö NEP and its services.

During the past ten years the range of paraphernalia distributed at the Malmö NEP have gradually broadened. The cups were added approximately six years ago and

filters around four years ago, together with skin swabs. During the last two years we have implemented a more generous distribution of needles and syringes, taking into account the needs of the individual NEP participant, in order to become a high coverage NEP. Annual reports of HIV, HBV and HCV incidences from the Malmö NEP since 2010 have shown a continuously low incidence of HIV (compiled by MAB and other NEP staff). No incident cases of HBV have been observed after the study period for paper IV (2013).The number of notifications of new HCV infections have also decreased during the past few years.

HCV RNA PCR analysis has by now (January 1st 2016) been implemented in the routine testing at all four NEPs in Skåne in collaboration with the Regional Office of Communicable Disease Control and Prevention, Skåne County, Sweden. We have also improved the diagnostic assessment of liver damage by introduction of transient elastography with an on-site Fibroscan.

On the local level, access to OST has increased greatly in the Malmö area and the strict waiting lines have now vanished. Means for recruiting NEP participants with opioid dependency directly from the NEP to OST services have been implemented (no such option is yet available for amphetamine users). Further in-depth analysis is required to assess whether the improved NEP coverage and collaboration with OST will be sustainably reflected in the HCV incidence.

On the national level, an important factor is the change of the Swedish legislation in 2006 allowing NEPs in Sweden. Subsequently more NEPs have opened, facilitating the overall access for PWID to clean syringes and needles and additional services provided by the NEPs. The network of Swedish NEPs now have better opportunities to together improve the services and observe and compare regional trends in viral transmission, drug use and other important variables such as mortality. New legislation is planned to be implemented next year (2017), allowing for lowering of the age limit for participation from 20 years to 18 years. This will improve access to young injectors and may influence transmission.

On the global level, some countries such as Australia and Georgia have taken steps to eliminate HCV from their territories according to the WHO global action plan and thereby treating PWID is a key component. However the overall efficacy of TasP has yet to be proven at a larger scale, not only shown in modeling. It seems, however, as if the awareness of the long term consequencs of HCV infection is on the rise among PWID. Still and if reinfection becomes a topic elimination may not be achievable without a protective vaccine. Vaccination studies have met many challenges, but are ongoing (350).

The next logical step should be to offer treatment with DAAs on-site at the Malmö NEP, combined with individual support and long-term follow-up. We believe that

the NEP setting could be used for studies on this approach, linked with surveys on HCV incidence among non-infected participants and surveillance of drug resistance and reinfections, as well as cost-effectiveness analyses. We are confidently looking through the windows of our NEP as through a window of opportunity.

Populärvetenskaplig sammanfattning

Individer som injicerar droger är utsatta för flera olika sorters hälsorisker; överdoser, infektioner med både bakterier och virus, våld och olycksfall, vilka alla bidrar till en ökad sjuklighet och dödlighet inom denna grupp. Psykiatrisk samsjuklighet, både primär och sekundär, är vanlig och kan få allvarliga konsekvenser. Sociala relationer och den personliga ekonomin kan vittra sönder. Injektionsmissbruk är socialt stigmatiserande och många individer riskerar att slinka igenom de skyddsnät samhället spänt ut. Om drogpolitiken baseras endast på kriminalisering, utan att folkhälsoaspekten eller de mänskliga rättigheterna tas i beaktande, ökar hälsoriskerna för denna grupp ytterligare. Detta gäller även risken att smittas med blodburna virus, såsom HIV, hepatit B (HBV) och hepatit C (HCV). Även i Sverige, där skyddsnätet har täta maskor och där klimatet gör livet som hemlös extra svårt, lever många individer med injektionsmissbruk under marginaliserade förhållanden.

Man uppskattar det totala antalet individer som injicerar droger till 15.9 miljoner, med ca 3 miljoner smittade med HIV, 6.4 miljoner med HBV (varav ca 1.2 miljoner med kronisk, pågående infektion) och ca 10 miljoner smittade med HCV (ca 7 miljoner med kronisk infektion). Beroende av opioider och central stimulerande medel (såsom amfetamin) är vanligast förekommande.

När HIV-epidemin uppdagades i mitten av 80-talet belystes den utsatta situationen för personer som injicerar droger. Även om de första fallen upptäcktes hos män som har sex med män (MSM) och patienter som erhållit blodprodukter (t ex blödarsjuka), insåg man snabbt att genom injektionsmissbruk kunde HIV snabbt spridas både bland dem som injicerade droger, men även utanför den gruppen genom sexuell smitta.

”Harm Reduction” eller skadelindring/skadereduktion innebär flera åtgärder som tillsammans strävar efter att minimera riskerna och begränsa skadorna av ett skadligt bruk av droger (men även bruk av alkohol och tobak kan ingå). Detta koncept har stöttats av Världshälsoorganisationen (WHO) sedan flera decennier. För personer som injicerar droger innefattar det tillgång till rena kanyler (nålar), sprutor och andra injektionstillbehör (drogparafernalia) genom sprutbytesprogram. I Sverige har tillgången till sådana varit begränsad. Under tiden som materialet till arbete I-III i denna avhandling samlades in (1997-2005) tilläts endast två officiella sprutbytesprogram i Sverige (i Lund och Malmö). Rena sprutor och kanyler kan

heller inte erhållas från svenska apotek utan recept. Efter en lagändring 2006 tillåts fler sprutbyten att öppnas, men fortfarande idag finns endast åtta stycken i landet (fyra i Skåne, en i Kalmar och en i Stockholm samt nyöppnade i Karlskrona och Jönköping). Det här innebär att det inte finns några sprutbyten norr om Stockholm. Folkhälsomyndigheten utannonserade starkt stöd för sprutbytesverksamhet under 2015 och flera sprutbyten planeras nu att öppnas. Folkhälsomyndigheten beräknar att ca 8000 individer som injicerar droger fanns i Sverige 2008-2011.

Den höga förekomsten av blodburna infektioner såsom HIV, HBV och HCV bland personer som injicerar droger är oroväckande. HIV kan numera räknas som en kronisk, hanterlig sjukdom med minimal risk för vidare smittspridning hos välbehandlade individer. Mot HBV finns ett säkert och effektivt vaccin, som i många länder införts i barnvaccinationsprogrammet. Det håller just nu på att införas i barnvaccinationsprogrammet även i Sverige. Riskerna med HBV och HCV utgörs av att de på sikt kan leda till allvarlig leverskada; ärrbildning, skrumplever och levercancer. Även om HIV och HBV kan fås under kontroll bland personer som injicerar droger bl a med hjälp av ett sprutbytesprogram, har det visat sig vara svårare att uppnå effekt mot HCV. Detta beror på flera olika faktorer; en högre förekomst, symtomfrihet med okunskap om både egen och andras smitta och virusets egenskaper. Tills nyligen har också behandlingen av HCV varit besvärlig, med långa behandlingstider, svåra biverkningar och dålig utläkningsgrad. Nu har helt nya läkemedel kommit ut på marknaden, med kortare behandlingstid (ca 12 veckor), få biverkningar och mycket hög utläkningsgrad (>95 %). Man talar t o m om att införa ”behandling som smittprevention”.

Både för den drabbade individen och för smittspridning inom hela gruppen är det viktigt att dels kartlägga antalet smittade med HIV, HBV och HCV och graden av nysmitta hos en grupp som har tillgång till rena sprutor och kanyler och dels studera hur smittan i så fall sprids inom gruppen. Vad gäller HBV är det viktigt att mäta hur många av sprutbytesdeltagarna som kan nås och skyddas genom vaccination, vilket ett av delarbetena i den här avhandlingen handlar om.

I den här avhandlingen har vi också studerat prevalens (förekomst) och incidens (nysmitta) med HIV, HBV och HCV. Vi har analyserat hur många av dem som smittas med HCV förblir kroniskt infekterade (och riskerar alltså att bli sjuka själva på sikt eller sprida smittan vidare) och vilka det är i gruppen som mest riskerar att smittas/sprida infektionen vidare.

Det vi kunde se i arbete I var att bpde prevalensen och incidensen för HIV var mycket låg, medan HBV incidensen minskat påtagligt efter införandet av vaccination 1994 (från 11.7 per 100 personår under risk till 3.4/100 pur). Även om gruppen i stort svarar lite sämre än den allmänna befolkningen på den sedvanliga grundvaccinationsserien om tre doser, kan man förbättra vaccinationssvaret med

upp till tre extra stöddoser (från 75 % till 85 %). Ett sämre vaccinationssvar sågs hos äldre deltagare och dem som var redan smittade med HCV (arbete IV).

Avseende HCV kunde vi observera att både prevalensen (förekomsten) och incidensen (nysmittan) var hög under de åren materialet samlades in (1997-2005). Av 332 mottagliga individer smittades 186 med HCV, vilket gav en incidens på 38.3 fall per 100 personår under risk (pur). När vi justerade detta för viremi vid inskrivningen i Sprutbytet (dvs att man redan bar på viruset, men inte ännu hunnit utveckla antikroppar, vilket dröjer ca 8 veckor), sjönk incidensen till 31.5/100 pur – fortsatt en hög siffra. Vi kunde se att nysmitta med HCV var kopplat till injektionsbruk av både amfetamin och heroin vid inskrivningen och till dem som varit fängslade före inskrivning.

Vi kunde också mäta att ca en tredjedel av alla nysmittade läkte ut sin infektion inom ett år (men man får inget skydd efter en genomgången infektion utan kan smittas på nytt vid fortsatt riskbeteende). Chansen för utläkning var högre för kvinnor. Vi fann att virusnivåerna var som högst precis i början av infektionen (redan innan man utvecklat antikroppar) och att de sedan följde flera olika mönster under det första året efter infektion (arbete II).

Därefter studerade vi spridningen av olika subtyper av HCV bland sprutbytesdeltagarna, både dem som var nysmittade och dem som redan var smittade före inskrivningen i Sprutbytet. Detta gjordes genom en phylogenetisk analys och där kunde vi se att ca en tredjedel av alla dem vi studerade (412 individer) hörde till en s.k. ”kluster” av närbesläktat virus. Vi identifierade 32 stycken sådan klustrar, främst tillhörande subtyp 1a och 3a. De som hörde till en kluster var oftare nysmittad och använde heroin som huvuddrog. Genom att uppmärksamma dessa riskfaktorer kan vi förhoppningsvis fokusera specifikt på individer med dessa kännetecken för att förbättra sprutbytets preventionseffekt på även HCV. Detta förutsätter utvidgad diagnostik (viruspåvisning utöver påvisning av HCV-antikroppar). För att kunna åtgärda HCV på individnivå bör infekterade personer ges möjlighet till utredning av graden av leverskada och vidare behandling inom både infektions- och beroendevård.

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