Chapter 6

General discussion
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Epidemiology and harm reduction

HCV infection in injecting DU

Injecting drug users (DU) are at high risk for acquiring hepatitis C virus (HCV) infection via exposure to infected blood through the sharing of needles and syringes.\(^1\)\(^2\) HCV prevalence in injecting DU populations can be up to 90%.\(^3\)\(^4\) By using a statistical method that was based on coalescent theory, it was estimated that HCV has been circulating in DU populations in Europe since the 1960s, and that the introduction of HCV was possibly even earlier than that.\(^5\)\(^6\) Although HCV prevalence remained high and relatively stable in ever-injecting DU from the ACS (69-93\% between 1985 and 2005), we found that the HCV incidence declined substantially in the past two decades, from 27.5/100 person years (PY) in the late 1980s to 2/100 PY in more recent years.\(^7\) The decline of HCV incidence was comparable with the decline in HIV incidence observed in the Amsterdam Cohort Studies (ACS) in the same period, although HCV incidence was always higher than the HIV incidence.\(^7\)\(^8\) This can be partially explained by the natural course of an epidemic: after the introduction of a pathogen in a certain population, the number of infected individuals and the incidence increases. While this happens, the number of individuals at risk decreases, and therefore the chance for an infected individual to transmit the pathogen to uninfected individual also decreases. It is therefore the natural course of an epidemic that when the density of people at risk reaches a certain threshold below which the number of susceptible individuals cannot sustain an ongoing epidemic, incidence peaks and then starts to decline.\(^9\) The observed decrease in HIV incidence in the ACS among DU was most likely due to depletion of individuals at risk, along with a reduction in risk behaviour. For HCV on the other hand, which had already been circulating for a longer time than HIV in DU in Amsterdam, it is less likely that the natural course of the epidemic was the only cause for the decline of the incidence.

There are several other important factors that most probably influenced the declining HCV incidence: the observed decrease in injecting risk behaviour at the population level might have had a greater impact on HCV than on HIV. The number of new injectors declined, which decreased the size of the susceptible population.\(^8\) Furthermore, since HCV is mainly transmitted via blood-blood contact, and not via sexual contact, the number of susceptible individuals for HCV decreased more than for HIV. In addition, mortality of the highest risk individuals in the population, who were often co-infected with HIV, may have contributed to the declining HCV transmission, since they were no longer a source for new infections.

Within the ACS, there is the opportunity to investigate factors that determine the time from start of injection drug use to HCV seroconversion. We found that this window period is longer for injecting DU that started injection drug use in the 1990s than in the 1980s.\(^7\) Knowledge on determinants of the window period between start of injection drug use to HCV infection is important, in view of the fact that prevention measures should be applied and are only effective in this period.

Harm reduction

While HCV incidence decreased in Amsterdam, HCV incidence remained high in DU populations in many developed countries.\(^1\)\(^4\) Probably the prevalence of injecting risk
behaviour declined more in Amsterdam compared to elsewhere. Murray et al. demonstrated by mathematical modelling that the level of risk behaviour determines whether HCV incidence decreases.\textsuperscript{10} They calculated that if injecting risk behaviour is sufficiently decreased (through intense needle exchange programs and/or harm reduction strategies), then HCV incidence and prevalence will accordingly decline.

Methadone and needle exchange programs in Amsterdam and the rest of The Netherlands are incorporated in the so-called harm reduction approach and were readily available in Amsterdam since the end of the 1970s. The ultimate goal of harm reduction is to stop drug use, but until this is possible, the policy is to minimize the damage DU inflict on themselves and the society at large. This comprehensive harm reduction approach has probably had a major impact on the HIV and HCV epidemic in Amsterdam. We found that only methadone provision or only participation in needle exchange programs did not decrease the risk of HCV or HIV infection, while combining the two measures resulted in a two- to threefold reduction in risk of acquiring HCV and/or HIV.\textsuperscript{11} This implies that harm reduction programs should be comprehensive, widespread, and easily accessible. This has major implications for countries with new and sometimes explosive outbreaks of both HCV and HIV in DU, like China and countries of the former Soviet Union,\textsuperscript{12-14} since providing only methadone or only needle-exchange facilities will not be enough to curb these epidemics, but such measures have to be combined with social and medical care.

Non-injecting DU

Self-reported never-injecting DU in the ACS had a much higher HCV prevalence (6.3\%) than the general Dutch population.\textsuperscript{15,16} Several studies have shown an association between HCV infection and non-injection drug use paraphernalia, indicating that HCV might also be transmitted via this route in non-injecting DU.\textsuperscript{17} However, we could not confirm this in the ACS.\textsuperscript{15} Phylogenetic analysis showed that the HCV strains circulating in HCV-infected never-injecting DU are interspersed with strains derived from HCV-infected injecting DU, indicating that they were probably infected from the same pool. The incidence in never-injecting DU was very low, with only one HCV seroconversion in >2,000 person years of follow up.\textsuperscript{15} This increased risk of prevalent HCV infection and the discrepancy between HCV prevalence and incidence in self-reported never-injecting DU could be related to underreporting of injecting drug use, next to household or sexual transmission from injectors to non-injectors. Whatever the route of transmission is in never-injecting DU, these findings stress the need for HCV testing of DU who report regular hard drug use, especially given the potential to treat HCV infection effectively. It should be noted that the never-injecting DU included in this study, were DU with regular use of hard drugs (i.e., heroin, cocaine, methadone or amphetamines at least three times per week) and with a high prevalence of polydrug use.\textsuperscript{15}

Although there have not been many new HCV infections in DU in recent years, there is still a large pool of chronically HCV-infected DU. Most chronically HCV-infected DU have not yet been treated for their chronic HCV, as many physicians question the effectiveness of HCV treatment in DU due to the perceived lack of adherence and the risk of re- and superinfection, which may negatively influence treatment outcome. However, it is important to treat DU not only in their own interest, but also since they can be a source of HCV, even though it is unknown how much transmission there actually is from DU to the general population. Within the ACS it has been shown that HCV treatment for chronically infected DU is feasible using a multidisciplinary approach in which directly observed therapy is combined with methadone provision and where there
is a close collaboration between research staff, hepatologists, addiction specialists and psychiatrists.18

Natural History

Estimates of spontaneous HCV clearance

Acute hepatitis C virus infection is asymptomatic in many patients, and therefore it is hard to study factors associated with spontaneous viral clearance.19 Furthermore, there are many factors that can influence the estimates of spontaneous viral clearance. Several of these factors are discussed below.

Definition spontaneous viral clearance

Chronic HCV infection is usually defined as persistence of detectable HCV RNA at least six months after acute HCV infection and it is generally accepted that spontaneous clearance of HCV takes place within six months after clinical presentation or HCV antibody seroconversion.20,21 However, definitions of spontaneous clearance of HCV can vary between studies, which makes it difficult to compare them. Since HCV-RNA levels can fluctuate around the level of detection of the used assay,22 multiple samples should be tested for the presence HCV RNA before chronic infection can be ruled out. Whether undetectable HCV-RNA levels in serum really means that HCV is fully eradicated from the body, remains unclear. There are some studies that suggest that several immune cells can remain HCV infected even after spontaneous clearance or successful treatment with pegylated interferon (PEG-IFN) and ribavirin (RBV).23 Whether this very low-grade persistence of HCV-RNA has clinical consequences has to be analyzed in prospective longitudinal studies of individuals that spontaneously cleared HCV or successfully treated individuals.

Late clearance

Although HCV clearance is supposed to occur within six months after infection, late spontaneous clearance has been described: up to 24 months after HCV infection, and even after that in Alaskan-natives.24-26 To evaluate the prevalence and determinants of late clearance in the ACS, more longitudinal data on HCV-RNA load are needed. The comparison between individuals with and without late clearance, and the evaluation of determinants of late HCV clearance might add to the knowledge how to turn the insufficient innate immune response and HCV-specific immunity around into an adequate immune response which is capable of eradicating the virus.

Continuous risk behaviour increases risk of super- and re-infection, and HIV co-infection

In retrospectively identified HCV seroconverters from the ACS among DU the overall rate of spontaneous viral clearance was 33.0%. However, we know that the rate of HCV re- and superinfection is high in DU with continuous injection drug use in this and other cohorts.27,28 Therefore, the observed rate of spontaneous viral clearance might actually be an underestimation of the ‘true’ clearance rate. To have a better estimate of spontaneous viral clearance, one would need a prospective cohort of HCV seroconverters with preferably shorter sampling intervals and sequencing of (part of) the HCV genome for phylogenetic analysis, which provides the possibility of discrimination between different HCV strains.
Another factor complicating estimations of viral clearance in active injecting DU is that continuous risk behaviour not only increases the risk on HCV re- and superinfection, but also increases the risk for subsequent HIV infection which is associated with lower clearance rate and with HCV superinfection. Continuous risk behaviour might result in an underestimation of the viral clearance rate. In the ACS, originally designed for HIV research, frequent longitudinal HCV-RNA measurements have not yet been established. Currently, HCV-RNA measurements are available for all HCV seroconverters and injecting DU included within two years after start of injection drug use around seroconversion or entry and at the end of follow up. However, more longitudinal measurements are needed to answer important questions regarding the natural history of HCV. These questions include the natural kinetics of HCV RNA during acute and subsequent chronic HCV infection, variation per genotype and whether there is an association between viral load and the progression to liver disease or liver-related death. Furthermore, a more accurate estimate of the incidence of HCV re- and superinfection in comparison to incidence of initial infection would be possible.

Factors influencing HCV clearance

So far, no reliable predictors for spontaneous viral clearance have been defined, but female sex, non-African race, younger age, high and broad HCV-specific T cells, certain HLA haplotypes, and symptomatic presentation have been associated with spontaneous viral clearance. These associations were mainly derived from cross-sectional studies, which may have biased the results. In the ACS among DU, we had the unique opportunity to study factors associated with viral clearance in retrospectively identified HCV seroconverters. Below the main factors associated with viral clearance are discussed.

Female sex

For many infectious diseases it has been described that the severity of disease is less in women compared to men. In addition, women are more likely to suffer from many auto-immune diseases, conditions in which the immune system reacts against self, implying a more reactive immune system in women than in men. In the ACS, female sex was the most important predictor of spontaneous viral clearance in HCV seroconverters. This is in line with a high rate of spontaneous viral clearance that was found in pregnant women that had received contaminated anti-D immunoglobulins. For HIV, it has been described that women have lower HIV-RNA loads compared to men. Interestingly, this effect disappears after the menopause. Although a difference in HCV viral load between men and women has not yet been described, our results suggest that women tended to have a lower HCV viral load than men, but this did not reach statistical significance. It has been shown that HCV clearance is comparable in girls and boys, which suggests that sex hormones might be important in HCV viral suppression. It would be very interesting to address this question in further research.

HIV and hepatitis B virus co-infection

As expected, HIV co-infection at the moment of HCV infection was associated with a lower odds of spontaneous clearance of HCV in HCV seroconverters from the ACS among DU, however this effect did not reach statistical significance. HIV infects CD4+ T cells, and during the natural course of HIV infection these cells are depleted. This depletion is attributed to heightened immune activation, and it is thought that microbial
translocation (i.e., increased systemic translocation from microbes and/or microbial products without evident bacteraemia from the lumen of the gut) contributes to this systemic immune activation.\(^{40}\) We and others have found indications that HIV co-infection leads to impairment of the development of a fully competent HCV-specific T-cell response, even before clinically overt immunodeficiency (as evidenced by the occurrence of opportunistic infections) has developed.\(^{41-44}\) Furthermore, we have shown in a small group of DU that especially HCV-specific CD4\(^{+}\) T-cell responses directed against non-structural HCV proteins are negatively influenced by HIV infection.\(^{45}\) However, larger numbers of prospectively sampled patients with HIV and HCV seroconversion are necessary to clarify the influence of HIV on HCV-specific T-cell responses.

In several cross-sectional studies that looked at the effect of hepatitis B virus (HBV) co-infection on HCV clearance in both HIV-infected and -uninfected individuals,\(^{26,46-51}\) presence of HBV surface antigen (HBsAg) was associated with higher odds of undetectable HCV RNA in only two studies.\(^{46,52}\) However, the cross-sectional design of these studies limits the ability to draw causal relations. One small longitudinal study and two case reports of chronically HCV-infected individuals with subsequent acute HBV infection show that some individuals cleared both HBV and HCV infection after acute HBV.\(^{51,52}\) We found that chronic HBV infection (as evidenced by positive HBV-Core antibodies and HBV-surface antigen expression) was associated with higher odds of spontaneous clearance of HCV, although this did not reach statistical significance.\(^{35}\) The interaction between these two hepatotropic viruses is interesting, and deserves further study. Since both viruses infect hepatocytes, possibly there is a direct competition for target cells in the liver. It could also be hypothesized that the local inflammatory environment that is induced by the second infection gives a local immunological boost and thereby enhances the capability of the immune system to eradicate not only HBV-, but also HCV-infected cells. It has not been reported whether HBV superinfection in HBV-infected individuals is also associated with viral clearance of both viruses. Furthermore, interaction between viral proteins from the two viruses might be an explanation, in which one viral protein inhibits a viral protein crucial for replication of the other virus.

### Clinical symptoms and HCV clearance

In other persistent viral infections (e.g., Epstein-Barr Virus and HIV), severe clinical symptoms during acute infection are related to clinical problems later in life, including Hodgkin’s lymphoma, and a faster progression to AIDS and death for HIV, respectively.\(^{56,57}\) Clinical symptoms in acute EBV infection (infectious mononucleosis, a self-limiting lymphoproliferative disorder), are thought to be caused by massive expansion of virus-specific CD8\(^{+}\) T cells. Interestingly, in acute HCV a large expansion of HCV-specific T cells seem advantageous and is associated with spontaneous viral clearance.\(^{19,58-66}\) It has been shown that clinical jaundice and alanine aminotransferase (ALT) rise in exposed individuals was coincident with expansion of HCV-specific T cells,\(^{66,67}\) which suggests that the symptoms in acute HCV are mediated by the HCV-specific immune response, although numbers of patients were very small. We did not find evidence for the previously described association between clinical symptoms and HCV clearance.\(^{35}\) This is in line with Okayama et al. (14 HCV seroconverters, none reported jaundice), Cox et al. (62 HCV seroconverters, none reported jaundice),\(^{68,69}\) and in contrast to hospital-based studies by Corey et al. (that reported high occurrence of symptoms in 28 HCV infections in 24 patients), as did Tilman Gerlach et al. (60 acute
HCV, no asymptomatic patient cleared HCV. These findings, together with our observation that individuals with spontaneous viral clearance do not have higher CD8 counts than individuals that develop chronic HCV, suggests that the importance of clinical presentation as a predictor of viral clearance might be overrated. However, as the numbers in the studies mentioned are small, future studies should include more patients or pool data from several observational cohorts to gain statistical power.

### HCV-specific T-cell responses

Both HCV-specific CD4+ and CD8+ T cells play an important role in spontaneous viral clearance in acute HCV infection. Depletion experiments in chimpanzees have shown that HCV clearance could not be achieved after CD4+ T cell depletion and subsequent re-infection. Since acute HCV infection is usually asymptomatic, patient numbers have usually been limited in studies on HCV-specific T cell immunity during acute HCV, ranging from 3 to 38 patients. We and others have shown that HCV-specific T-cell responses mainly targeting nonstructural HCV proteins are beneficial, and are associated with spontaneous viral clearance. However, spontaneous clearance has also been described in the absence of a strong CD4+ or CD8+ T-cell response. In conclusion, the exact correlates of immunological control over HCV are still unknown, and future research should focus on the quantity and quality of HCV-specific T cells, preferably in a larger cohort of patients. Furthermore, standardization of used assays would allow for better comparison between different laboratories and different patient cohorts.

We and others have shown that both injecting DU and MSM can have HCV-specific T-cell responses before HCV antibody seroconversion. However, it is still unclear whether these responses are due to exposure to the virus, or due to actual infection, replication and successive clearance of the virus. Furthermore, it needs to be elucidated whether these responses play a role in protection against HCV infection and if so, what qualities of these T cells are important for the protection.

### Remodelling of HCV-specific T-cell responses during chronic HCV infection and supposed re-exposure

Usually, professional antigen presenting cells (APC) like dendritic cells (DC) take up antigen in the periphery, and then migrate to a lymph node. There, naïve CD8+ T cells are primed by the APC, after which they differentiate in cytotoxic T lymphocytes (CTL) with effector functions and memory CD8+ T cells. These CTL are released to the peripheral compartment and home to the site of inflammation to find the antigen they are primed for and kill the infected cells when they recognize one. When the HCV-specific T-cell response develops and quickly wanes or does not develop at all in acute HCV infection, viral persistence usually evolves. This waning of the immune response can have several causes. First of all, HCV seems to influence DC by suppressing CCR7 expression, which is a lymphoid tissue homing marker and thereby ‘trapping’ the DC in the liver. When T cells are not properly primed by DC, the T-cell response will be less, favouring HCV persistence. Viral escape mutations may render the HCV-specific T cells that are present non-functional, since they do not recognize the epitope anymore. Although the HCV genome is highly variable, Neumann-Haefelin et al. have shown that only half of the T-cell responses found in the liver were associated with viral escape mutations, and thereby also showed that the other half of the HCV-specific T cells recognized epitopes that were still present in the circulating virus.
During chronic HCV infection and supposed re-exposure by continuous risk behaviour, antigen-specific T-cell responses are constantly evoked and boosted. We show in chapter 5.1 that skewing of hepatitis C virus (HCV)-specific T cells to Core responses in chronic HCV infection in injecting drug users is associated with the presence of HCV RNA. Since these Core-specific T-cell response are associated with viral persistence, future studies should focus on whether targeting of Core by T cells is less efficient than targeting NS proteins or whether the observed skewing of these responses can be attributed to high antigenic pressure, with more presentation of Core to T cells and thereby skewing of the HCV-specific T-cell response.

During chronic HCV infection, liver fibrosis and -cirrhosis can develop. Development of liver-related morbidity and mortality is multifactorially determined, some of these factors are known, for instance alcohol consumption and HIV and HBV co-infection, while others remain to be elucidated. It is assumed that the development of liver fibrosis and -cirrhosis is mediated by chronic inflammation of the liver. Although there are many competing causes of death in the ACS, there are individuals that die of liver-related causes. Although liver-related death occurs only in a limited number of HCV seroconverters, a comparison between longitudinal HCV-specific T-cell responses in those who progress to HCV-related causes of death and those who do not, might identify host (T cell) characteristics that are associated with progression, especially since it is possible to correct for confounding factors like alcohol consumption and HIV co-infection within the ACS.

Forced viral clearance during treatment of HCV with PEG-IFN and ribavirin

Our findings on female sex and HIV co-infection suggest that HCV treatment can be postponed in HIV-negative women to await spontaneous viral resolution, unless the patient wants to be treated immediately. However, although the optimal regimen for treatment of acute HCV in HIV-infected individuals is unknown, treatment should not be postponed in individuals and groups who are HIV-infected, since treatment outcome is less favourable in HIV co-infected individuals. Furthermore, it has been shown in HCV mono-infected patients that the chance of achieving an SVR declines significantly within a year after acute infection, and there is no reason to assume that this will not occur in HCV/HIV co-infected individuals.

HCV genotype is the most important baseline predictor of HCV-treatment outcome, however, the underlying mechanism for this is yet unknown. Factors negatively influencing HCV treatment outcome are HIV co-infection, higher baseline viral load, and African-American race. It is thought that during treatment of HCV with PEG-IFN and RBV, HCV-specific T-cell responses are enhanced. However, so far only one study has been able to show this, while others did not find a boost of HCV-specific T cells during treatment. In chapter 5.2, we examined HCV-specific T-cell responses in HIV co-infected patients with HCV genotype 1 and 3, and we observed that HCV-specific T-cell responses at week 12 after start of treatment were more often undetectable in genotype 3 than in genotype 1 infected patients. Although this study was performed in a limited number of patients, this decline of HCV-specific T-cell responses suggests that the observed HCV-specific T-cell responses are dependent on continuous antigenic stimulation, which is in line with Capa et al. This implies that the role of HCV-specific T cells in viral clearance during treatment is limited and that forced HCV clearance is mainly achieved by the antiviral effectiveness of PEG-IFN and RBV.
Future perspectives of antiviral drugs

Next to the available PEG-IFN and RBV treatment, several new compounds are very promising and are expected to be registered within the next three years. Two protease inhibitors, telaprevir (VX-950) and boceprevir (SCH503034) have recently entered phase III clinical trials. Treatment regimens that include one of these new-generation anti-HCV drugs, referred to as STAT-C (specifically targeted antiviral therapy for HCV) have achieved SVR up to 65-75% and 50% in treatment-naïve genotype 1 infected and treatment-experienced patients who were nonresponsive to PEG-IFN/RBV, respectively. Future treatment of chronic HCV will probably be more effective and shorter and consist of a combination of PEG-IFN and RBV together with one or more new drugs. During treatment with these new compounds the viral decline of HCV is very fast. In analogy to HIV, it has been shown that HIV-specific T-cell responses weaken very fast after initiation of HAART. Since there is no antigen present, there is no need for HIV-specific T cells anymore. It can be expected that this will happen to HCV-specific T cells during STAT-C as well. However, so far no studies have been done that support this hypothesis.

At present, there is no vaccine available for HCV. However, studies on natural clearance of HCV have shown that a robust, multispecific and lasting T-cell response is very important. Furthermore, it has been shown in a cohort of German women that were infected by a batch of infected anti-D immunoglobulins, that early development of broadly targeted neutralizing antibodies was associated with viral clearance. Therefore, for an HCV vaccine to be successful, it will most likely have to elicit both a T-cell response and a humoral response.
References

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