Hepatitis C virus: risk factors and disease progression
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Chapter 4

General discussion
General discussion
The studies in this thesis were performed to improve our understanding of the risk, the disease progression and the long-term complications of HCV infection in PWID and HIV-coinfected MSM.

Changing epidemiology
Declining trends in PWID
People who inject(ed) drugs (PWID) are at increased risk of contracting hepatitis C virus (HCV) through exposure to infected blood. Using sequence analysis it was estimated that the epidemic of HCV infection among PWID in Europe started between 1924 and 1953 [1]. With the rise of injecting drug use in Amsterdam in the late 1960s, HCV-infection prevalence peaked to over 90% among PWID in the late 1980s [2]. From 1990 onwards we observed a steep decline in the HCV incidence among PWID followed in the Amsterdam Cohort Studies (ACS), see figure 1.

Although the decrease was comparable for human immunodeficiency virus (HIV) and HCV infection, the HCV incidence was about four times higher than the HIV incidence [3]. The decreasing trend in HIV incidence is in line with other longitudinal studies and data from surveillance systems on drug using populations in other high-income countries [4,5]. In chapter 2.1 we updated the data from the ACS and confirmed the continued decline in HIV incidence and injecting risk behavior among drug users. In contrast to an earlier observation in the ACS, where sexual risk behavior remained substantial, we found a decline in sexual risk behavior in recent years [6]. The declining trend in self-reported use of needle exchange, as observed in this study, is confirmed by a reduction in the absolute number of exchanged needles per calendar year in Amsterdam, which peaked in 1992 with 1,100,000 needles, whereas since 2012 only around 150,000 needles have been exchanged per year [7].

The Netherlands is known worldwide for its low threshold harm-reduction programs with interventions targeted at minimizing harm to PWID at the individual level but also minimizing harm from drug use to society at large. The decline in incidence of HIV and HCV infections seem inextricably linked with harm-reduction programs. A study to evaluate the effect of harm reduction by needle exchange programs and opiate substitution therapy on HIV incidence among PWID from the ACS found that the combination of these services was associated with a lower risk of
acquiring HIV and HCV infection [8]. This has been confirmed by recent studies [9-11]. However, it must be noted that several other factors can explain these declines. First, the decline could reflect the natural course of an epidemic. If a population is more or less stable in number and a pathogen is introduced the incidence will follow a Gaussian distribution. When the number of individuals increases, the number of individuals at risk will decrease, ultimately resulting in a decrease in incidence. Second, due to premature mortality of those with the highest risk behavior the chance of spreading pathogens to uninfected individuals also decreases over time. A modeling study by de Vos et al, based upon data of PWID from the ACS, demonstrated that a substantial part of the decline in HIV and HCV incidence and HIV prevalence could indeed be explained by a decrease in the number of high-risk individuals in the population due to HIV-related mortality [12]. In addition, availability of treatment for HIV and HCV infection might contribute to a declining trend [13]. However, it was estimated that use of combination antiretroviral therapy (cART) reduced the HIV incidence in Amsterdam with only 2%, as especially PWID in the early cART era received cART at low CD4 T cell counts, thereby increasing the time they could affect others [14,15].

Alternative explanations for the decreases in HIV and HCV incidence could include the ageing population of the ACS and Amsterdam PWID in general, with an average age of about 50 years [7]. With increasing age the risk behavior declines [16]. Unlike other countries the Netherlands has a low incidence of new injectors. Whether young people are discouraged from injection drug use through (in)direct education, fear of the “junky-status” [17], or that other (non-injecting) alternative drugs are more popular, more easily available, and fulfill the expectations sufficiently remains to be investigated.

Clearly the situation in the Netherlands is unique and often serves as a best practice for countries with ongoing epidemics of HIV/HCV infections. However tides can change through macro-economic changes affecting social structures, public health implementations, and vulnerable populations. In 2011 a staggering 1600% increase of HIV infections among PWID was observed in Athens, Greece, which is likely to be associated with the global financial crisis that had (has) a particularly large negative impact on the gross domestic product of Greece [18]. In addition, Romania reported an HIV and HCV infection outbreak among PWID in 2011 and 2012 [19]. Even though it is difficult to demonstrate a relation between an economic crisis and an increased infection rate, it does emphasize that new outbreaks of infectious diseases among PWID could be waiting in the wings. Adequate coverage of harm-reduction programs is essential, not only for maintaining stable low-incidence rates of HIV and HCV infection at today’s levels, but also for monitoring new outbreaks and trends in drug use. Moreover, harm-reduction programs give access to screening and to treatment of HIV, HCV, and other infections [20].

New epidemic among MSM
Since the year 2000, a rapid increasing incidence of HCV infection in HIV-infected men who have sex with men (MSM) has been observed in Asia, Australia, Europe, and the United States of America [21-25]. An international phylogenetic study revealed a large international MSM-specific HCV transmission network [26]. This network differed from networks associated with injecting drug use, but coincided with the introduction of cART. In the mid 1990s, following the introduction of cART, an increase in sexual risk behavior and sexually transmitted infections was observed among MSM [27,28]. Mucosal damage through sexual practices (e.g., fisting, toys) and STIs are considered to be risk factors for HCV acquisition [29]. In addition, although cases of sexually transmitted HCV infections have been reported in HIV-negative MSM, HIV is likely to be an important factor in
transmission [30]. However, it remains difficult to prove sexual transmission as an infection mode of HCV, as multiple risk factors for HCV infection often coexist in individuals at risk. Current evidence suggests that, during the 1980s, there were multiple independent introductions of HCV from PWID populations into the MSM population [29]. However, if injecting drug use had been a major transmission mode among MSM during the 1980s, one would have expected a higher HCV incidence than observed in this group during that period. It remains intriguing that in Amsterdam the HCV incidence declined to less than 1 case per 100 person-years among PWID from 1996 onwards but increased drastically among MSM from 2000. In contrast to other countries such as the United States of America and Switzerland, the HCV incidence among MSM in Amsterdam seems to be leveling off [31]. Whether this is due to public health interventions and increased awareness, a decreased number of uninfected individuals in the susceptible pool (saturation effect), or a temporal trend remains unclear. Monitoring of the HCV incidence and prevalence among MSM and regular testing of HIV-infected MSM remains important. HIV-negative MSM who inject drugs should also be tested for HCV.

Risk of HCV reinfection

Although PWID account for the majority of new (80%) and existing (60%) cases in high-income countries [32], recent estimates show an annual HCV treatment uptake of 1–6% among PWID [33,34]. There are still barriers to receiving treatment, including concerns about treatment adherence, HIV coinfection, and the potential for reinfection following successful HCV treatment. Studies on the risk of reinfection among PWID after successful treatment are scarce. In order to overcome this barrier we investigated the risk of reinfection following successful treatment among PWID in Amsterdam in chapter 2.2 where we found a low risk of 0.76 cases per 100 person-years. The incidence increased to 3.42 cases per 100 person-years when we only included PWID who were actively injecting in our analysis. These results were lower than those found in a recent meta-analysis on the incidence of reinfection after successful treatment [35]. The pooled estimate of reinfection among all study participants was 2.36 (95% confidence interval (CI) 0.91-6.12) per 100 person-years. When the analysis was stratified to those who reported injecting drug use post-treatment, the pooled estimate of HCV reinfection was 6.44 (95% CI 2.49-16.69) per 100 person-years. This difference between the pooled and Amsterdam estimates among all study participants could be explained by the decreased risk behavior of ACS participants. Moreover, those treated might be a selected group at lower risk of reinfection. The local HCV epidemic situation, education, and counseling on the risk of HCV reinfection might also explain differences between studies and regions.

In our study, chapter 2.2, we found that 10% of PWID were actively injecting drugs during HCV treatment, and after treatment this increased to 20%. Unfortunately, our study size was too small to further investigate re-initiation of injecting drug use following treatment. Future studies are needed to investigate this and whether this relapse is associated with treatment failure or severe side effects from PEG-IFN. It is anticipated that due to future scale-up of (interferon-free) HCV treatment, high-risk individuals are more likely to be treated, thus stressing the importance of counseling reinfection risk and monitoring reinfections.

In contrast to previous studies we used phylogenetic analyses to distinguish HCV relapse from reinfection in case of infection with same HCV subtype. The re-emergence of viral RNA during or following treatment does not necessarily indicate treatment failure (relapse) but could be due to reinfection, which clearly has a different clinical implication. Using part of the NS5B gene to discriminate between viral strains should be sufficient in an established epidemic as is the case for
HCV infection among PWID. However, in a relatively small and emerging HCV epidemic such as that which occurred among HIV-positive MSM following the introduction of cART, identification of new infections with the same genotype as the original one can be challenging, in particular for patients with persistent viremia or a relapse following treatment. The NS5B region has an insufficient phylogenetic signal for discriminating re-infection with the same subtype from relapse in these settings. Therefore it is necessary to sequence a fragment of the viral envelope that contains a more variable part of the viral genome such as the hypervariable region 1 (HVR1).

Only two studies to date have investigated the rate of HCV reinfection among HIV-positive MSM following successful HCV treatment. The reinfection rate was 9.6 cases and 15.2 cases per 100 person-years in studies from the United Kingdom and the Netherlands respectively [36,37]. In Chapter 2.4 we extended the latter study and estimated the incidence of new infections with the original or different genotype in both persistent and cleared acute HCV infections among MSM using frequent sampling. We sequenced a genetically highly diverse fragment of the second envelope gene (E2) that includes HVR1. First of all, we found a reinfection rate following spontaneous and treatment induced clearance of 14.5 cases per 100 person-years, in line with the previous finding. Interestingly, additional analyses showed a significant reduced hazard of new infections (i.e., reinfections) with the original genotype as compared to new infection with a different genotype. This could be suggestive of a partial protection against the genotype present at the primary infection. The comparison of new infections with the original genotype versus new infections with a different genotype to the original using a competing risk framework has not been previously studied. Our finding of reduced hazard of reinfections with the original genotype should be repeated in larger studies with frequent sampling, phylogenetic testing, and also among other risk groups. An alternative explanation for reduced hazard of reinfections with the original genotype could be that MSM do not change their risk behavior but enter a different MSM network where other HCV subtypes are circulating. However, separate epidemiological HCV transmission networks were not revealed among MSM in Amsterdam [38].

**Figure 2.** Hypothetical model of the innate immune response contributing to increased rates of spontaneous clearance among females and individuals with the favorable interferon lambda 3 genotype. HCV-infected hepatocytes (green) present viral RNA to pDCs, mDCs, and Kupffer cells. Upon stimulation interferons (IFNs) and Interleukin (IL) are produced. The thickness of the arrow represents the relative contribution. These IFNs inhibit viral replication, activate the JAK/STAT pathway, and subsequently lead to the upregulation of interferon stimulating genes (ISGs). Female sex hormones could stimulate the innate immune response through binding of 17-β-estradiol to the estrogen receptor (ER).
Taken together, the data suggest that these days the risk of reinfection following successful treatment is lower among PWID than MSM in Amsterdam, in line with the patterns of HCV infection spread among the naïve population. Therefore, active drug use should not preclude access to HCV treatment. In contrast to PWID in Amsterdam, MSM are treated in the acute or early phase of infection. This means that they might have had less time to adapt their risk behavior and might more easily fall back into the risk behavior practices of their network. Investigation of the impact of HCV treatment on risk behavior and the duration of risk reduction could inform prevention programs targeted at risk reduction during and post treatment of HCV infection.

**Disease progression**

Understanding the natural course of infection of HCV can lead to important insights into the immunopathogenesis of the infection and may provide clues valuable for antiviral treatment or vaccine development. Treatment success with PEG-IFN and RBV are higher when individuals are treated during acute HCV infection than when they are treated during chronic infection. However, as spontaneous clearance of HCV infection can occur during acute infection, treatment may not be appropriate. The decision not to treat prevents unnecessary toxicities and saves costs. As the new therapeutic options are very expensive, it is even more important to be able to discriminate between acute infection and chronic infection and determine factors associated with spontaneous clearance.

**Spontaneous clearance of HCV**

Spontaneous clearance of HCV, defined as undetectable concentrations of HCV, generally occurs within the first six months following acute infection. However, cases of spontaneous clearance have been observed two years following infection [39]. A systematic review including cross-sectional studies and clinical cases reported a clearance rate of 26% [40].

In a longitudinal and community-acquired setting among PWID, we investigated the clearance rate of HCV infection in seroconverters (chapter 3.2). The spontaneous clearance rate of 33.0% is comparable to a recent study among nine prospective cohorts of PWID with observed seroconversion, including PWID from the ACS [41]. It is of interest to investigate which factors are associated with rapid viral clearance, as this may lead to insight into the causal mechanism(s) of viral clearance. It is evident that the likelihood of answering this question is highly dependent on finding acute cases and the frequency of testing intervals. If testing intervals are too wide, spontaneous clearance might be missed and persistence of HCV RNA might be classified as chronic infection instead of a reinfection. In addition, if the test intervals are too wide, uncertainty increases regarding the precise date of infection and seroconversion. This could have implications for interpretation of results from immunological and virological investigations during the acute phase of HCV infection.

**Factors associated with spontaneous resolution of HCV**

Our insight into the immune pathogenesis is indeed hindered by a lack of acute and well-defined cases with sufficient and frequently collected blood samples. Simultaneous evaluation of the innate and adaptive immune response in one study with frequent time points would provide insight into the dynamics of each of these branches of and which factors are essential for viral clearance. Viral clearance of HCV infection is a multifactorial process of host and pathogen-associated factors, including female sex, younger age, favorable interferon lambda 3 (IFNL3) genotype (formerly known as interleukin 28 B), HCV genotype 1, robust CD4+ and CD8+ T cell responses, chronic hepatitis B infection, and no infection with HIV [42]. Most of the observed associations with viral
clearance were derived from cross-sectional studies with unknown duration of disease and thus may be biased by inclusion of reinfections following spontaneous clearance. In our cross-sectional study with retrospectively identified PWID with acute HCV infection (chapter 3.1), we found that females with the favorable IFNL3 genotype (here defined as rs12979860 CC) had increased odds to resolve their infections compared to females with the unfavorable (defined as rs12979860 CT/TT) genotype and men with either the favorable or unfavorable genotype. In addition, we found that fever, which is also associated with acute infection, was associated with viral clearance. Although we found increased odds ratios, our study did not have enough power to demonstrate in multivariate analysis that being HIV-negative or being chronically infected with HBV are independently associated with viral clearance. Larger studies are needed to investigate these possible associations.

The interaction between IFNL3 genotype and sex has not been described previously. In figure 2 we propose a hypothetical model to explain this finding. Key players of the HCV innate immune response in the liver are plasmacytoid dendritic cells (pDCs), myeloid DCs (mDCs), and Kupffer cells [43]. When viral RNA is transferred via exosomes from infected hepatocytes to pDCs, a robust production of IFN-alpha and to a lesser extent IFN-lambda could be generated via toll like receptor 7 (TLR7) and retinoic acid-inducible gene 1 (RIG I) ([44]. Kupffer cells are the dominant macrophage population in the liver. Uptake of HCV RNA triggers an inflammatory response via TLR7 in these cells [45]. As stated before, females do not only differ in sex hormones but also in IFN-α responses via stimulation of TLR7, the gene of which is located on the X-chromosome. Stimulation of TLR7 in peripheral blood mononuclear cells from females results in significantly higher IFN-α responses as compared to males, independent of 17β-estradiol [46]. Whether this observation also holds true in specific immune cells of the liver is unknown. We hypothesize that TLR7 stimulation in pDCs and Kupffer cells of females results in increased IFN production as compared to men, thereby increasing the likelihood of viral clearance.

In chronic HCV infection, high intrahepatic levels of ISG expression are associated with the unfavorable IFNL3 genotype and are a predictor of a poor HCV treatment outcome. The net increase of upregulation of ISGs could be higher for those with the favorable IFNL3 genotype as compared to those with the unfavorable IFNL3 genotype [47]. Moreover, a high increase in ISG expression during acute HCV infection could also benefit spontaneous clearance of HCV. Due to the asymptomatic nature of HCV infection it will be very difficult to demonstrate the effect of IFNL3 genotype on ISG upregulation during spontaneous clearance. A recent in vitro study demonstrated that a subtype of mDCs produced substantial amounts of IFNL3 upon HCV infection [48]. But more importantly, this response was IFNL3 genotype-dependent, where the release of IFNL3 was superior among those with a favorable IFNL3 genotype. An in vitro study using a HCV replicon system demonstrated that cotreatment with both IFN alpha and lambda enhanced the antiviral activity, suggestive of a synergistic interaction [49].

| Table 1. Factors associated with high HCV RNA levels and HCV disease outcomes. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | Acute infection                | Chronic infection               |                                 | Liver disease                   |
|                                | High RNA                     | Persistence                      | High RNA                       |                   | Male             |
| Sex                             | =                            | Male                             | Male                            | Male              |
| Age                             | =                            | Old                              | Old                             | Old                |
| BMI                             | ?                            | ?                                | High                            | High               |
| HBV                             | ?                            | Negative                         | Positive                        | Positive                  |
| HIV                             | ?                            | Positive                         | Positive                        | Positive                  |
| IFNL3                           | CC                           | CC                               | ?                               | ?                   |

BMI: Body mass index; HBV: hepatitis B virus; HIV: human immunodeficiency virus; IFNL3: interferon lambda 3 genotype, rs12979860 CC allele (favorable), and CT/TT allele (unfavorable). Question mark indicates unknown; equal sign indicates no difference. Associated factors presented in this table are based on results from this thesis and [55,65,66].
In general, for many infectious diseases, males are affected more frequently and more severely than females [50]. This is also the case for HCV, as clearly demonstrated by spontaneous clearance rates of up to 54% among pregnant women infected with contaminated anti-rhesus D immunoglobulins [51]. Sex hormones including testosterone and 17β-estradiol are believed to explain the difference in immune responses between males and females. These sex hormones can bind to specific receptors and enhance the function of CD-4 T cells, macrophages, natural killer cells, and plasmacytoid dendritic cells (pDCs) [50]. Further support for this hypothesis is the observation that clearance of HCV infection is comparable between girls and boys who have not reached puberty [52]. It is unclear whether pre-menopausal women have lower viral load levels than post-menopausal women, as has been observed for HIV [53]. If so, this would support the hypothesis that the difference in immune responses could also be driven by sex hormones.

Favorable IFNL3 genotype is associated with spontaneous clearance but also with high levels of HCV RNA during acute infection. In chapter 3.2 we confirmed this association in the largest well-defined cohort of acute HCV infection cases, with data pooled from nine individual cohorts among PWID. The exact mechanism through which a favorable INFL3 genotype might lead to high HCV RNA levels remains to be elucidated. Moreover, it is of great interest to investigate whether high levels of HCV RNA could trigger stronger innate immune responses as suggested by Liu et al [52]: they found a significantly more rapid evolution of HVR1 among those who resolve HCV infection compared to those who become chronically infected with HCV [52]. However, the rapid evolution of HVR1 was not correlated with markers of innate or adaptive immune response.

The possible interaction between female sex and IFNL3 genotype suggests that decision to start HCV treatment, at least with PEG-IFN and RBV, in acute infection could be postponed for females with the favorable IFNL3 genotype, as they have the greatest likelihood to resolve the virus themselves. Bearing in mind the costs of the new IFN-free regimens, the cost of an IFN lambda test (about 60 Euro) is insignificant. HCV treatment regimens might benefit more from TRL7 stimulation instead of PEG-IFN-alpha. Promising results have already been made as a phase 1b study of the TLR7 agonist isatoribine prodrug resulted in significant declines in viral load among chronically infected individuals and was better tolerated than PEG-IFN-alpha [54]. However, as HCV treatment probably moves towards PEG-IFN (either alpha or lambda) -free regimens, the clinical value of these genetic polymorphisms could be redundant in the long run. In addition, it is likely that the decision to start treatment as soon as possible is superfluous, as cure rates for chronic cases will reach more than 90%.

Figure 3: Hypothetical model of telomere decrease. Acute infection with HIV or HCV leads to acutely decreased telomere length with further decline in the long run (grey striped line). Addition of cART could lead to return telomere decline to the normal rate (grey solid line) but never to telomere length of healthy individuals (black line).
Factors associated with increased HCV RNA levels during chronic infection

Quantification of viral load has proven to predict disease progression in chronic viral infections such as HIV and HBV. To date, there is no evidence that HCV RNA levels are associated with HCV disease progression. However, knowledge of the natural course of HCV RNA levels is limited and factors associated with high HCV RNA levels are mainly derived from cross-sectional studies. In the ACS we had the unique opportunity to study HCV RNA levels and factors associated with HCV RNA levels up to 23 years following HCV seroconversion (chapter 3.3). In line with other studies [55,56], we confirmed that male sex and favorable IFNL3 genotype were associated with higher HCV RNA levels. Notably, we found that increased body mass index (BMI) was associated with higher HCV RNA levels. This association has previously only been described in two small studies [57,58].

It remains puzzling that HCV RNA levels have never been correlated with disease progression. On the one hand we know that factors such as male sex, HIV coinfection, and metabolic conditions (BMI and/or diabetes and/or steatosis) are associated with increased liver disease [59,60] and with increased HCV RNA levels. On the other hand, IFNL3 genotype has not been associated with disease progression [61] but is associated with high HCV RNA levels. In addition, HCV genotype 3 is associated with increased fibrosis progression [59], but also with lower HCV RNA levels [55]. Taken together, this points towards a multifactorial interaction between associated factors of liver disease that might have contradictory effects on HCV RNA levels. Therefore HCV RNA levels might not be associated with disease progression. However, due to the cross-sectional nature (or limited follow-up) of most studies, a cumulative effect of high HCV RNA levels could be missed. Using the same principle as pack-years for smoking ((number of cigarettes per day * years smoking) / (20 cigarettes per day per year)), HCV RNA level years could be calculated. It would be very interesting to see if “HCV-RNA-level-years” do matter. In chapter 3.3 we also demonstrated that HCV RNA levels fluctuate during the course of infection, therefore it could also be that peak HCV RNA during the chronic phase might serve as a marker for disease progression. In order to investigate these hypotheses, a cohort of HCV seroconverters with regular clinical follow-up is necessary. Such questions could be best answered in a study that investigates fibrosis/cirrhosis progression of the liver during the course of HCV infection using transient elastography or noninvasive serum markers and collects data on cause-specific mortality. Unfortunately, the ACS has too little data on clinical endpoints such as liver fibrosis or cirrhosis to test these hypotheses. In chapter 3.5 we compared the degree of liver fibrosis at two and ten years later following acute infection between chronically HCV-infected PWID and PWID who spontaneously cleared HCV. We used an algorithm from the fibrotest, which is a validated test assessing the degree of liver fibrosis in HCV infection [62]. In our study we found no difference in fibrotest outcomes between the two study groups. These results suggest that HCV has limited impact on liver fibrosis during the first 10 years. Alternatively, it could imply that the fibrotest is not accurate or applicable in PWID. Over the years there have been quite a few validated noninvasive tools (e.g., fibrosis-4 score [63]), that use serum markers to predict the degree of liver fibrosis [64]. Although the results are promising it remains uncertain whether these scoring systems are applicable to different populations (e.g., different genetic backgrounds, different viral strains, comorbidities, or coinfections).

As mentioned before, HCV RNA levels are high during the acute phase and are associated with spontaneous clearance during the acute phase [67], which could be explained by favorable IFNL3 genotype (chapter 3.1). Although our study size was limited, we found no difference in HCV RNA levels during the acute phase between men and women. However, in the early stages of the chronic phase early on during the chronic phase there is already a significant difference in HCV RNA levels.
between men and women [68]. In table 1 an overview is given of factors associated with high HCV RNA, persistence, and increased progression to liver disease. Most factors associated with development to viral persistence during acute HCV infection are associated with high HCV RNA levels and increased disease progression. One would expect IFLN 3 CC genotype to be associated with increased disease progression, however the evidence is conflicting [69,70]. On the other hand the unfavorable IFLN3 CT/TT genotype is associated with higher ISG levels and possibly higher levels of inflammation contributing to enhanced disease progression. Of interest is the anomaly between spontaneous clearance of HCV infection and the enhanced disease progression of chronic HCV infection in HBV-coinfected individuals. Likely there is a complex interplay between these two viruses and this topic requires more investigation. Based upon the available evidence it seems that high HCV RNA levels during acute and chronic infection seem to be two different entities. This could be explained by the active innate and adaptive immune responses during the acute phase and an exhausted immune response during the chronic phase with continued but lower levels of inflammation [71]. Associated factors with high HCV RNA might therefore have differential effects during disease progression.

**Immune senescence**

With the advent of cART for HIV, the decline in drug-related causes of death, and the unpopularity of starting to inject in Amsterdam, the mean age of PWID is increasing and PWID are now at premature risk of developing multimorbidity and mortality from causes commonly observed in the elderly [72,73]. There is increasing evidence that HCV infection contributes to morbidity from cardiovascular disease, renal disease, and (systemic) autoimmune diseases [74,75].

As these inflammatory diseases involve activity of the immune system, we hypothesized that changes and increased levels of inflammation, and ultimately immune senescence, could form the basis of this premature burden of morbidity among ageing PWID. In Chapter 3.3 we demonstrated that in the early stages of follow-up HIV/HCV coinfection was already associated with significantly reduced telomere lengths in both CD4+ and CD8+ T cells. The use of cART in these individuals did not reduce telomere length decline to levels comparable to healthy donors of the same age. However, we could not rule out that cART, via telomerase inhibition [76], negatively affects telomere length. A recent cross-sectional study demonstrated no association between low telomere length and cART exposure [77]. Even though cART might have a direct negative effect on telomere length, the reduction of inflammation through cART outweighs the side effects of cART with respect to telomere length.

Unfortunately, we were not able to investigate the additional effect of HCV in HIV/HCV coinfection. Therefore we should have investigated PWID infected with HIV only. However, these individuals are hard to find considering HCV is much more infectious than HIV through blood-to-blood transmission. This research question could be answered among MSM infected with HIV and HIV/HCV as HIV infection usually precedes HCV infection in this group. Interestingly, HCV-monoinfected PWID had lower CD4+ T cell telomere lengths than healthy donors, suggesting that HCV on its own may have an effect on immune senescence. In figure 3, three scenarios with regard to telomere length decline are depicted. Acute infection with HIV or HCV results in an acute decrease of telomere length with a further long-term decline (grey striped line). Addition of cART or HCV treatment could return telomere decline to the normal rate (grey solid line) but never to the level of telomere length of healthy individuals (black line). As telomere length decline is associated with many lifestyle factors common among PWID (e.g., smoking, alcohol, low BMI) [78] it is important to further investigate the effect of HCV on immune senescence comparing.
chronically HCV-infected individuals with HCV resolvers instead of healthy controls. Another strategy could be to investigate whether chronically infected individuals who are successfully treated have increased telomere length as opposed to individuals not treated for HCV. In addition, the effect of timing of treatment and acute or chronic HCV infection on telomere length could be studied. If HCV truly has an impact on immune activation and immune senescence this would warrant early treatment initiation.

**Mortality**

It generally takes two to three decades to progress from HCV seroconversion to advanced liver disease (including liver cirrhosis, end-stage liver disease, or HCC). In many high-income countries, including the Netherlands, the HCV incidence among PWID peaked in the 1970s and 1980s. Therefore, without therapeutic intervention the future burden of disease is likely to rise over the next decade(s) [2]. However, to predict this expected rise of HCV related disease burden and the future treatment needs, accurate estimates morbidity and mortality due to infection with HCV are necessary, especially among PWID who account for most HCV infections worldwide but also are at increased risk of premature mortality due to their lifestyle.

Therefore, competing mortality should be taken into account when evaluating the impact of chronic HCV infection on mortality, particularly for PWID [79]. Doing so, we demonstrated no impact of chronic HCV infection on overall mortality among PWID in the first decade following seroconversion, as compared with HCV resolvers (see chapter 3.5). The latter group is the ideal control group as lifestyle is comparable between the two groups. However, during the second decade after infection, HCV-chronically-infected participants are at increased risk of all-cause mortality as compared with HCV resolvers. High rates of mortality were evident among those with chronic HCV infection but were predominantly attributed to non-natural causes and HIV/AIDS. Participants with a chronic HCV and HIV coinfection were at greatest risk of premature mortality. This finding could be explained by the observation that HIV-coinfection accelerates chronic HCV disease progression [80]. However, being coinfected with both viruses could also be a proxy for very high-risk behavior with premature drug-related mortality such as overdose and suicide as a consequence [81]. Due to the still limited follow-up with respect to HCV-related mortality and the presence of competing mortality, we observed only one liver-related death. The low incidence of HCV-related mortality is concordant with another longitudinal study among PWID [81]. This finding does not necessarily indicate that HCV infection has a low impact on mortality. Many studies investigating mortality are based upon links with national death registries. It is known that cause-specific deaths are often misclassified and it seems that the accuracy of physicians has not improved over the years [82]. However, the causes of death in our study were actively obtained from hospitals, general practitioners, and coroners, and reviewed and classified by two physicians.

A strong causative effect of chronic HCV infection on mortality from endpoints such as chronic liver disease, cirrhosis, and HCC is widely accepted. In order to estimate the true effect of HCV on cause-specific mortality, large cohort studies with follow-up of more than 25 years following HCV seroconversion and regular screening for fibrosis, cirrhosis, and HCC are needed. Since the average age of drug users from the ACS is now over 50 years we might expect an increase in ageing drug users dying “from” HCV instead of dying “with” HCV infection [2].

**HCV treatment and prevention**

Over the past decades our knowledge about HCV has advanced greatly. Fundamental basic research into the lifecycle of HCV has paved the way for numerous new drugs. These include NS3/4A
protease inhibitors (e.g., telaprevir, boceprevir, simeprevir), NS5A replication complex inhibitors (e.g., daclatasavir), nucleotide polymerase inhibitors (e.g., sofosbuvir).

The availability of sofosbuvir and other treatment regimens is changing the therapeutic landscape with activity against genotype 2 & 3 without PEG-IFN and acceptable tolerability [83]. Unfortunately a treatment regimen with sofosbuvir has an estimated cost of €55,655 to €111,741 in the Netherlands, dependent on genotype and consequent treatment duration [84]. Fortunately these new treatment regimens have been approved in the Netherlands and hopefully this will reduce the future disease burden among HCV-infected individuals. However, for 2015 these treatment regimens are only indicated for patients with severe fibrosis, severe extra-hepatic manifestations, patients waiting for liver transplantation, or patients post-liver transplantation [84].

With the success of these new treatment regimens the agenda has moved from curing HCV-infected individuals to a global HCV elimination and even eradication. Elimination of a disease is defined as a reduction of the incidence to zero in a defined geographical area as a result of deliberate efforts, but continued intervention measures are required during outbreaks [85]. Eradication implies a permanent reduction to zero incidence without any outbreaks requiring intervention (e.g., smallpox) [85]. HCV treatment can reduce the HCV disease burden, but can also serve as a preventive measure by reducing the transmission risk, prevalence, and incidence. From this point of view it might be more beneficial to treat HCV-infected individuals with the highest risk behavior, especially in regions that still have a low prevalence [86], instead of those with advanced disease progression and presumably a low risk of spreading the virus. A modeling study demonstrated that if the annually HCV treatment uptake with DAA is over 75/1000 PWID, it could reduce the chronic HCV infection prevalence by more than half in the next 10-15 years [87]. It is critical that HCV treatment is combined with opiate substitution therapy and high-coverage needle and syringe programs [13,86]. In the HIV-infected MSM population uptake of HCV treatment is quite high among those in clinical care, but there is a high risk of reinfection as mentioned before. Apart from targeting high-risk individuals for HCV treatment, these results stress the need for risk reduction strategies through, for example, counseling and promotion of condom use.

Unfortunately, many countries will not be able to afford the new HCV treatment regimens or treat the HCV-infected population sufficiently to achieve treatment as prevention. Apart from lowering the price, investments need to be targeted at screening programs, education, and vaccine development. Development of an effective vaccine could be one of the branches of global HCV eradication, however a successful vaccine is not on the market yet and implementation of an effective vaccine can be difficult for marginalized groups such as PWID [88].

**Concluding remarks and future perspectives**

The landscape of HCV has changed drastically over the recent years with major improvements in HCV therapy. Extensive research into lifecycle of HCV and developments by the pharmaceutical industry have paved the way for all-oral, pan-genotype, and interferon-free combinations of drugs with cure rates over 90%. If HCV treatment could be targeted at individuals with high-risk behavior such as MSM and PWID, HCV treatment could serve as a prevention strategy. However, before expensive treatment is initiated, the likelihood of spontaneous and treatment-induced clearance and also the risk of reinfection should be considered. It would be helpful to have very good predictors of these events. Further investigation into spontaneous clearance of HCV infection and the interaction between female sex and favorable IFNL3 genotype might aid the development of a successful T cell vaccine.
As more and more people will be cured, it is of great interest to investigate whether this impacts comorbidity and cause-specific mortality in the long run. For those not treated for HCV infection it is likely that we can expect premature comorbidities and mortality over the next few decades. Worldwide, PWID are the main drivers of the HCV epidemic, accounting for the majority of new infections. In this marginalized population treatment uptake is inadequate and scale-up is urgently needed. However, with treatment scale-up of risk groups there should be increased vigilance regarding reinfections in all risk groups due to the continued risk of exposure or ongoing risk behavior. Ultimately the combination of education, prevention measures, targeted screening, vaccination, access to care, and treatment with highly effective but affordable HCV treatment regimens are needed to eliminate HCV. Over 25 years of extensive research in the HCV field has resulted in better insights of the epidemiology, HCV lifecycle, disease progression, and drug development. Continued research and investments are of paramount importance and should include disease monitoring, cost effectiveness studies of combined interventions, and basic research. The multidisciplinary approach ranging from basic science to public health is a major drive for innovations, which will ultimately lead to the global eradication of HCV.
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