Chapter 1

General introduction
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Hepatitis C virus
Hepatitis C virus (HCV) is a single-stranded RNA virus and was first identified in 1989 as a cause for transfusion-associated non-A, non-B hepatitis [1]. Transmission of HCV occurs predominantly via blood-to-blood contact. After acute infection about 75% of those infected progress to a persistent infection and become at risk for liver cirrhosis, end-stage liver disease, or hepatocellular carcinoma (HCC) [2,3]. In contrast to hepatitis B virus (HBV), there is no effective vaccine against HCV. Over the past two decades HCV treatment has rapidly improved and new direct acting antiviral agents (DAA) will further improve its effectiveness and tolerability.

Epidemiology
HCV infection is a major global health issue with an estimated anti-HCV prevalence of 2.6%-3.1%, affecting 170-210 million individuals worldwide [4]. The distribution of HCV infection varies greatly per country. Egypt has the highest HCV infection prevalence among the general population (15%-20%) as result of a mass campaign to eradicate schistosomiasis in which injection material was reused without adequate sterilization [5]. In East Asia the estimated prevalence of HCV infection is 3.7%, which corresponds to an absolute number of > 50 million HCV-infected individuals [6]. In most high-income regions the prevalence of HCV infection is considered to be moderate and varies between 1.5% and 3.5% [4]. It must be noted that in many cases, the national estimates are based on limited data and are derived from selected populations that are not representative for the general population. In the Netherlands, the prevalence of HCV infection is estimated at 0.22% (range: 0.07%-0.37%), corresponding to 28,000 individuals (range: 9,600-48,000) who are anti-HCV-positive [7].

HCV is transmitted most efficiently through percutaneous exposure to contaminated blood [8]. Individuals who received blood products through transfusion (i.e., hemophiliacs) before 1991/1992 were at increased risk of HCV infection. Since the first introduction of the serological assay for the detection of anti-HCV in 1991, the incidence of transfusion-acquired HCV infection has drastically declined in regions of the world where donor blood is screened [9,10]. Effective screening of blood products has led to a shift in the primary transmission mode of HCV. In high-income countries injecting drug use replaced transfusion-acquired transmission as the primary source of new infections [11,12]. Worldwide there are 6 major genotypes and more than 50 subtypes [13]. The global distribution of HCV genotypes and subtypes reflects differences in transmission modes and temporal trends of

Figure 1. Relative prevalence of each HCV genotype by global burden of disease region. Size of pie charts is proportional to the number of seroprevalent cases as estimated by Mohd Hanafiah et al [4]. Reproduced with permission of the author [25].
transmission, see figure 1. The majority of infections in Europe, Northern America, and Australia have been acquired through contaminated blood products (HCV subtypes 1b, 2a, and 2b) or through sharing of injecting equipment among people who inject drugs (PWID) (subtype 1a and 3a). The prevalence of anti-HCV among PWID is estimated to be 67% worldwide, ranging from 10%-97% across regions, representing an estimated 10 million PWID [14]. In high-income countries, the incidence among PWID ranges from 2-66 cases per 100 person years [15-18]. In a few countries, including the Netherlands, a decline in incidence of HCV infection among PWID has been observed from 1990 onwards [15,16,19,20], which could be due to the high coverage of comprehensive harm-reduction programs, including needle exchange and opioid substitution [21-23]. In contrast, in Eastern Europe the PWID-related HCV epidemic started in the early 1990s and harm-reduction interventions have remained limited [24].

During the past decade several outbreaks of HCV infection among human immunodeficiency virus (HIV)-positive men who have sex with men (MSM) have been reported [26-28]. Based on molecular clock analysis, it is estimated that the HCV epidemic among HIV-positive MSM started in the (mid-) 1990s [29]. This finding was further supported by data from observational cohort studies [30]. Sexual transmission of HCV rarely occurs among heterosexuals [31] and transmission of HCV among HIV-negative MSM also seems to be low [32-35]. The risk for HCV transmission among HIV-positive MSM is multifactorial, and is likely to be associated with high-risk sexual behavior (fisting, group sex, toys) and biological mechanisms (coinfection with HIV and STIs) possibly leading to a reduced mucosal barrier [36,37]. PWID used to be the main risk group in the Netherlands for acquiring an acute HCV infection, however, in the last ten years there has been a shift towards HIV-positive MSM [7].

Among pregnant HCV-monoinfected women, the vertical transmission rate of HCV infection is 4%-7% [38-40] and this rate increases to 20% when the mother is coinfected with HIV [41]. In highly endemic countries, new HCV infections are often healthcare-associated but can also be community-acquired [42] through, for example, shaving at a community barber [43]. Knowledge regarding precautions for blood-borne pathogens by community healthcare providers [44] as well as barbers [45] might be limited. Consequently, migrants born in HCV-endemic countries with a high prevalence of HCV infection are at increased risk of carrying HCV [46]. In the Netherlands migrants presently account for the majority of prevalent cases, but transmission within the Netherlands is limited [7,46].

Molecular epidemiology
HCV is characterized by its genetic heterogeneity due to a lack of a proofreading ability of the viral non-structural gene 5B (NS5B) encoded viral RNA-dependent RNA polymerase. As a result, HCV exists as a spectrum of closely related genomes that have been classified as 6 major genotypes and more than 50 subtypes, as previously mentioned [13]. The degree of genetic diversity among different HCV variants provides information regarding the time that has passed since two viruses separated from a common ancestor, and hence the likelihood that two strains were acquired in the context of the same transmission network [47]. The total difference between HCV variants in a certain viral region can be used to calculate the genetic distance. When the genetic distance is small, viral strains will be closely related. The genetic distances can be plotted in a phylogenetic tree where closely related viral strains will form a cluster, see figure 2.

The combination of epidemiological data and phylogenetic analysis can be helpful in determining a common source of infection, transmission route, and distinguishing reinfection from relapse. In certain cases or specific populations, the genetic diversity of HCV may be limited to a few, often highly similar circulating clades. 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. This part of E2, a gene encoding one of the envelope proteins, is the region with the greatest genetic variability, allowing discrimination of homologous strains with an intra-host diversifying virus, from heterologous strains from the same viral subtype.
Disease progression of HCV infection

Acute infection

Upon infection with HCV, HCV RNA becomes detectable within 7 to 21 days and quickly elevates to a plateau phase during the first 2 months [48]. Usually alanine aminotransferase (ALT) levels follow the same pattern as HCV RNA, with levels more than ten times the normal upper limit [6]. Primary infection with HCV is usually asymptomatic, although 15%-30% of individuals develop flu-like symptoms 2 to 12 weeks following the initial infection [49,50]. In a minority of cases jaundice is the presenting symptom.

For about 25% of individuals, HCV infection is a self-limited disease [2] and for the majority of cases HCV RNA becomes undetectable within 6 months [51,52]. Several factors are associated with increased rates of spontaneous clearance and include: female sex; younger age at infection; negative HIV-status, favorable interferon lambda 3 (IFNL3) genotype (formerly named interleukin 28 B (IL-28B)), and HCV genotype 1 [51,53-55]. However, the majority of studies on spontaneous viral clearance have been conducted among anti-HCV-positive individuals, for whom the exact moment of anti-HCV seroconversion is unknown. These prevalence studies are subject to selection bias, which can potentially lead to biased rates of viral clearance and risk estimates. Early predictors of spontaneous viral clearance are needed to decide whether early treatment should be indicated, as treatment success rates with pegylated interferon (PEG-IFN) alpha and ribavirine (RBV) are higher when individuals are treated during acute HCV infection, while the high expense of new treatment options is also an important factor. In addition, a better understanding of factors associated with spontaneous HCV clearance or persistence is necessary for HCV vaccine development.

Chronic infection, morbidity, and mortality

Once chronic infection is established, people become at risk of liver fibrosis, liver cirrhosis, and/or HCC. In the majority of patients, chronic HCV infection remains clinically silent for decades [56]. Liver fibrosis and cirrhosis progression is accelerated with increasing age, male sex, obesity, alcohol consumption, and HIV coinfection [6,57]. Over time the probability of progression to cirrhosis increases exponentially. It is estimated that without treatment, on average liver cirrhosis occurs in 16% of patients after 20 years of chronic HCV infection, and in 41% of patients after 30 years [57]. Among patients with cirrhosis, HCC develops at an annual rate of 2%-4% [58]. HCV infection also has a role in extrahepatic morbidity, such as cardiovascular disease, likely through a similar mechanism of immune activation as that described for HIV infection [59,60]. Chronic infections, such as those demonstrated for HIV, are also thought to play a role in age-associated changes.

Figure 2. Example of maximum likelihood phylogenetic trees of 340 basepair NS5B sequences from people who inject drugs, before and after unsuccessful treatment of HCV infection.
in the immune system (immune senescence) resulting in impaired immunity through (long-term) continuous immune activation [61,62]. Whether chronic HCV infection also has an impact on mortality by inducing immune senescence is not known. Of interest, HIV/HCV-coinfected individuals do not only seem to be at increased risk of liver disease progression [63] but progression to AIDS is also accelerated [64], which suggests that both viruses could enhance each other's disease progression [65]. It is evident that individuals with chronic HCV infection can die from their disease. However, data on the long-term outcomes of HCV infection are still difficult to interpret. Studies on the impact of chronic HCV infection on mortality among PWID are also complicated by an increased risk of premature mortality due to HIV or lifestyle-related causes of death (e.g., overdose) [55]. Moreover, most of the studies published might be biased by their study design (e.g., study population comprises patients in a hospital setting including severe cases, a retrospective study design, and the unknown duration of HCV infection). In addition many studies do not compare chronically infected individuals with individuals who resolved HCV infection in order to adjust for lifestyle. Given the slow progressing nature of chronic HCV infection and the high incidence of HCV infection in the 1970s and 1980s among PWID, we can expect a significant rise in liver disease and mortality over the next two decades [66]. Accurate estimates on disease progression in PWID are crucial for estimating the future burden of disease in this group.

The immune response to HCV

After transmission of HCV, the innate immune system is the first non-specific defense system that HCV encounters. Hepatocytes are the main target cells of HCV and the innate response to HCV is the immediate induction of cytokines and interferon (IFN) alpha, beta, and lambda. These IFNs activate interferon-stimulating genes (ISGs) that amplify the IFN-alpha and IFN-lambda responses. The release of IFNs and proteins from ISGs leads to an antiviral state in uninfected liver cells and inhibits further replication [67]. Following the first four weeks of infection HCV RNA levels remain relatively stable, suggesting that a balance is achieved between viral replication and innate immune pressure [68]. Plasmacytoid DCs (pDCs) are thought to play an essential role in sensing HCV during the initial phase, increasing IFN production (via cell-to-cell contact), and activating the adaptive immune response.

The adaptive (specific) immune system responds to HCV with a humoral and cellular response. Most individuals who get exposed develop neutralizing antibodies (nAb) during the course of infection. One study demonstrated that spontaneous resolvers induced earlier, broader, and more vigorous nAb responses than those who developed chronic infection [69]. However, in hypogammaglobulinemic patients spontaneous clearance of HCV infection also occurs, which may suggest that the nAb response plays a minor role [70].

The importance of a vigorous T cell response is considered to be essential in the clearance of HCV. CD4+ T cells recognize viral peptides presented by major histocompatibility complex (MHC) class II molecules on antigen presenting cells. CD8+ T cells recognize viral peptides presented via MHC class I molecules on the surface of hepatocytes and mediate viral clearance via cytoxic activity [71]. Within the first eight weeks following infection, broad and vigorous HCV-specific CD4+ and CD8+ T cell responses are observed, irrespective of outcome [72-74]. After 10-12 weeks of viremia a dichotomization seems to occur in the HCV-specific CD4+ T cell response. Broad and vigorous HCV-specific CD4+ T cell responses remain stable for individuals who spontaneously resolve their infection, but these cells rapidly become less functional and disappear for those who progress to chronic infection [72,75]. Depletion studies in chimpanzees revealed that CD8+ T cells are essential for viral clearance [76,77]. During the acute phase it seems that there is no qualitative difference between individuals who resolve HCV infection and those who progress to a persistent infection. Therefore HCV has to escape from HCV-specific CD8+ T cells during the acute phase in those who become chronically infected. One way is through the development of viral escape mutations that are not recognized by the CD8+ T cell response [78]. Another mechanism involves upregulation of inhibitory T cell receptors such as programmed death factor 1 (PD-1) and mucin domain-containing molecule 3 (TIM3) resulting in functionally exhausted HCV-specific CD8 T cells. [79].
Treatment

Over the past decades HCV treatment options have evolved rapidly with cure rates of less than 10% increasing to 90% [80]. The infection is cured in more than 99% of patients who achieve a sustained virological response (SVR), which is defined as undetectable HCV RNA in serum 24 weeks after the end of treatment (EOT) [81]. Until DAA became available, standard antiviral treatment of chronic HCV infection consisted of weekly injections with PEG-IFN and daily RBV [82]. Standard treatment duration generally ranged from 24 weeks to 48 weeks and depended on HCV genotype. In general, treatment with PEG-IFN/RBV resulted in an SVR in 46%-60% of patients with a chronic genotype 1 and 4 infection, and 76%-80% of those with genotype 2 or 3 infection [83-85]. Individuals with HIV coinfection have considerably lower SVR rates than HCV-monoinfected individuals [86]. Treatment outcomes improved when treatment was started shortly after acute HCV infection in both HIV/HCV-coinfected and HCV-monoinfected individuals [87]. Treatment of prior non-responders was less effective and brought another round of side effects.

In 2012, two DAAs were approved in the Netherlands for HCV genotype 1 infection, namely telaprevir and boceprevir. These two NS3/4 protease inhibitors are given in combination with PEG-IFN/RBV and cannot be used as mono therapy due to emergence of resistant variants. This triple therapy leads to SVR rates of 70% in treatment-naïve individuals and 50% in previously treated individuals [88]. Although HCV treatment success rates improved, serious side effects during treatment were common and included flu-like symptoms, depression, and fatigue [89]. Treatment discontinuation occurred in 10%-15% of patients treated with PEG-IFN/RBV and 11%-25% [90-92] for patients on triple therapy. The addition of DAAs not only increased treatment discontinuation, but the side effects reported (skin rash and anemia) were more serious [93]. Fortunately, new and promising treatment regimens were approved in Europe, including the Netherlands, in 2014 and include an NS3/4A protease inhibitor (simeprevir ®) and an RNA-polymerase inhibitor (sofosbuvir®) [82,94]. These drugs will lead to shorter therapy duration, interferon-free regimens, increased SVR rates (up to 90%), and can be used for multiple HCV genotypes [95]. Unfortunately, for 2015 these treatment regimens are only reimbursed for patients with severe fibrosis, severe extra-hepatic manifestations, patients in work-up for liver transplantation, or patients post liver transplantation [96]. The decision to treat or to watchfully wait for more evidence or approval of other promising regimens should be guided by the HCV genotype and patients being treatment-naïve, treatment-experienced, or cirrhotic. The therapeutic landscape of HCV infection is moving ahead quickly, with new (non-nucleoside) protease inhibitors and RNA-polymerase inhibitors in the pipeline. The treatment regimens described here are limited to those included in the Dutch guidelines presented in October 2014 [94].

Relapse, reinfection, coinfection, and superinfection

One could speculate that patients who have cleared infection carry a protective immunity against reinfection as is observed in chimpanzees [97]. However, several groups have reported that reinfections or superinfections do occur among individuals with ongoing risk behavior, such as PWID [98]. Although HCV treatment among PWID can be as effective as treatment for non-PWID [99], physicians seem to be reluctant to offer HCV treatment to PWID. One of the reasons is the potential risk of reinfection following treatment [100]. There is limited data available on the risk of reinfection among PWID after successful treatment. In three relatively small studies (n=9, 27 and 35), the overall reinfection rate varied from 0.6/100 person-years to 3.2 cases/100 person-years [101,102,103].

It is not always clear whether a reinfection or viral relapse has occurred following HCV treatment, and from both a clinical and a research perspective it is necessary to clarify what we mean by relapse and reinfection. Classically, viral relapse following an EOT response - as indicated by the absence of HCV RNA in serum - is defined as the recurrence of HCV viremia with the same virus within 24 weeks of therapy cessation. Reinfection is defined as a case in which an initial infection is completely resolved prior to a subsequent infection [104]. This can be either a reinfection with a different genotype/subtype compared to the initial infection, or with the same subtype but a different strain. In individuals with high-risk behavior and frequent exposure to HCV, multiple viral strains can be detected at a single time point. This is referred to as a mixed infection. Two types of
mixed infections can be distinguished, namely coinfection and superinfection [104]. Coinfection can be defined as a simultaneous acquisition of two or more HCV viral strains. Superinfection occurs in individuals with chronic HCV infection who present with a new and different HCV viral strain(s) after reexposure to HCV.

Diagnosing reinfection is primarily based on ‘population’ sequencing, which generates a consensus sequence averaging the genomic variation present. Diagnosing mixed infections is more complicated as it involves analysis of variants that may be present as a minority population among a large population of different major variants. Population sequencing may still reveal a subpopulation of minor variants but only when they constitute 20%–30% of the virus population. To identify mixed infections with minority variants present at a frequency below 20%–30% additional laboratory tools are needed. Increasing our knowledge on the incidence of multiple infections and assessing the genetic relatedness of primary and successive viral strains will provide more insight into correlates of immunity against HCV and is crucial for vaccine development and targeted preventive strategies.

Prevention

Unfortunately no effective vaccine is available to date, although some progression has been made using T-cell vaccines [105]. Until an effective vaccine is available, several components are of key importance to reduce the burden of disease. First, prevention should be targeted at avoidance of infection. In high-income countries, screening of blood products significantly reduced the incidence of transfusion-associated transmission [9,10]. Comprehensive harm-reduction programs, including needle exchange and methadone substitution therapy, have likely contributed to a declining incidence of HCV infection among PWID [21-23]. Second, screening those who are at risk will lead to awareness of HCV status and early detection of infection. Third, among the infected population, treatment will reduce disease progression but also has the potential to prevent secondary transmission [106]. Unfortunately, PWID are often subject to marginalization and few receive HCV treatment. Although HCV treatment with PEG-IFN and RBV is cost-effective among PWID, these HCV treatment regimens are still expensive [107]. In order to answer to a necessary scale-up of HCV treatment, considerable investments by local and national governments need to be made.

Aims and outline of this thesis

The studies in this thesis were performed to improve our understanding of the risk, the natural history, and the long-term complications of HCV infection for PWID and HIV-infected MSM. Investigation of acute HCV infection can provide valuable insights into HCV pathogenesis, which could benefit vaccine design and treatment improvement. Development of successful preventive strategies is dependent on knowledge of factors that drive HCV transmission, especially with respect to marginalized populations such as PWID. Acute infection is usually asymptomatic and therefore rarely recognized. Importantly, longitudinal follow-up of HCV seroconverters provides insight into the full course of disease progression and HCV-associated morbidity and mortality. This might help improve treatment decision making. The data sources used in the studies are outlined in table 1.

Chapter 2 focuses on the risk of HCV infection among two risk groups: people who use drugs (PWUD), including PWID, and MSM. In 1978 the Netherlands introduced low threshold harm-reduction programs for PWUD that are still in use. Previous studies demonstrated a simultaneous decline in HIV and HCV incidence rates and found that full participation in these programs was associated with a lower risk of HIV and HCV infections [22]. In chapter 2.1 injection risk behavior and sexual risk behavior are investigated over a period of more than 25 years among PWUD from the Amsterdam Cohort Studies (ACS), in order to determine whether the PWUD in Amsterdam are still at risk for HIV and STI today. As HIV and HCV infections have overlapping modes of acquisition, a decline in injection risk behavior would likely lead to a decrease in both infections. With the prospect of increasing efficacy of HCV treatment, one would expect that increasing numbers of PWID with chronic HCV infection would be treated. However barriers to initiate treatment still exist. One of these barriers is a presumed high risk of HCV reinfection among PWID. To gain insight into this barrier we examined the reinfection risk among PWID in chapter 2.2.
To date, very few studies have been performed on HCV reinfection following successful treatment among HIV-infected MSM. In a relatively closed community with a high similarity of circulating viruses it can be difficult to determine the true incidence of new infections. In chapter 2.3 the incidence of reinfections is described among MSM attending the HIV treatment clinic of the AMC, one of the sites of the observational MOSAIC cohort study among MSM with acute HCV infection. In this study, genetic sequencing of the highly diverse fragment of the second envelope gene that includes the hypervariable region 1 was used to distinguish intra-host evolution from a new infection with the same genotype (clade-switch).

Chapter 2.4 reviews the available data from published studies among PWID examining HCV reinfection following treatment and tries to characterize those at risk for reinfection. In addition, strategies to distinguish reinfection from relapse or mixed infection are described.

Chapter 3 describes the natural history of HCV infection. Studying acute HCV infection can provide valuable insights into HCV pathogenesis. However, many of the early studies on spontaneous clearance were limited by the study population, including prevalent and symptomatic cases.

Chapter 3.1 describes factors associated with spontaneous clearance among HCV seroconverters from the ACS. This study includes HCV-negative PWID who were recruited in the community and seroconverted for HCV during follow-up. Higher HCV RNA levels during the first month of acute infection have been shown to be associated with spontaneous clearance. We gained more insight into the factors associated with these high RNA levels during acute HCV infection in a large collaborative study among PWID in chapter 3.2.

Several studies have been performed to investigate factors associated with HCV RNA levels. However these studies had a cross-sectional design, limited follow-up, included prevalent cases, and were often hospital-based. In chapter 3.3 the natural course of HCV RNA over time and factors associated with longitudinal HCV RNA levels up to 23 years from HCV seroconverters from the ACS were investigated. As PWID have a high risk for chronic infections such as HIV and HCV, they are also at risk for premature immune senescence. Chapter 3.4 examines whether PWID from the ACS with or without HCV and/or HIV infection are at increased risk for premature immune senescence.

It is clear that people can die from HCV infection, especially decades after infection. However, studies on the impact of chronic HCV infection on mortality rates in PWID are complicated by competing causes of mortality. Moreover, to determine the impact of chronic HCV on mortality in community-acquired HCV infection, PWID that resolve their HCV infection are the most appropriate control group as these two groups most likely share important characteristics, unlike a sample from the general population that is HCV-negative. In chapter 3.5 we compared the overall and cause-specific mortality following HCV seroconversion between PWID with a chronic HCV infection and PWID who spontaneously cleared their HCV infection.

In chapter 4, the general discussion, the main findings of this dissertation are discussed and related to the most recent literature. Furthermore, recommendations for future research and possible implications of the presented studies are shared.

Table 1. Data sources and study populations used in this thesis

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<tr>
<th>Data sources</th>
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<th>Study population</th>
<th>Study period</th>
<th>Chapter</th>
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<td>Amsterdam Cohort Studies (ACS)</td>
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<td>PWID</td>
<td>1985-2011</td>
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<td>The Netherlands</td>
<td>Chronically HCV infected PWID eligible for treatment</td>
<td>2004-2011</td>
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<td>The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC³) Study</td>
<td>Australia, Canada, the Netherlands, the United States</td>
<td>PWID with acute HCV infection</td>
<td>1986-2010</td>
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<td>MSM Observational Study of Acute Infection with hepatitis C (Mosaic)</td>
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<td>HIV infected men who have sex with men</td>
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<td>MSM attending the HIV-treatment clinic of the Academic Medical Center (AMC) Amsterdam</td>
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<td>HIV infected men who have sex with men</td>
<td>1994-2011</td>
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References


