Molecular epidemiology of hepatitis C virus
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Summary

In this thesis we describe how molecular epidemiological techniques were used to improve our understanding of the spread of hepatitis C virus (HCV) in the Netherlands. This thesis starts with an overview of the epidemiological, viral and clinical characteristics of HCV (Chapter 1). The research presented in this thesis was conducted among Dutch blood and plasma donors, injecting and non-injecting drug users, and men who have sex with men.

Dutch blood and plasma donors

In industrialised countries such as the Netherlands, introduction of routine donor screening in 1991 combined with indirect strategies such as donor selection, have drastically improved blood safety over the last decades. In order to further improve transfusion policy, and to increase our understanding of the spread of HCV in the general population, we examined HCV infections among Dutch blood and plasma donors (Chapter 2). The study population included all new and repeat donors in the Netherlands who, despite elaborate donor-selection methods, tested HCV seropositive during the period 1997-2002. In the Netherlands, donor-selection methods appear highly effective as both HCV prevalence and incidence among Dutch donors are extremely low. The risk of acquiring HCV through contaminated blood products in the Netherlands is less than 1 in 30 million donations, and is thereby negligible. HCV-seropositive new donors who were incidentally missed by donor-selection, mainly reported contaminated blood products received in the past, a history of injection drug use, or immigration from high endemic countries. HCV-positive repeat donors, however, reported only low-risk modes of HCV transmission that are impossible to detect by preliminary screening of risk-behaviour. As different routes of transmission were associated with certain HCV genotypes, molecular epidemiology proved to be a useful tool in elucidating risk profiles among individuals who (at first) did not report any recognisable risk factor for HCV.

Drug users

Drug users, in particular those who have shared needles in the past, have been heavily affected by the HCV epidemic. To investigate the changes in magnitude and characteristics of the HCV epidemic among drug users (DU) in Amsterdam over time, we compared HCV risk behaviour of young DU participating in the Amsterdam Cohort Studies (ACS) during the period 2000-2004 with data obtained from young DU in the period 1985-1989 (Chapter 3.1). Despite a sharp decline in injecting drug use and HCV prevalence over time, 44% of today’s
ever-injecting young DU still test HCV positive. However, in today’s DU duration of injection and age at interview were both independently associated with a positive HCV serostatus, suggesting that the majority of HCV infections was not acquired recently. HCV strains of genotype 1a and 3a still predominate among young DU, but HCV genotype 4d has recently entered the drug user population. After the year 2000, Amsterdam can be characterised as a low-prevalence injecting drug use area in which the high-risk population is ageing. Among the few recent HCV infections that still occur, the proportion of difficult-to-treat HCV genotypes 1a and 4d is increasing.

The HCV prevalence among self-declared never-injecting DU in the ACS is 6.3% (Chapter 3.2). Although never-injecting DU do not share needles and/or syringes, the HCV prevalence in this group is significantly higher than in the general population. The HCV incidence among never-injecting DU, on the other hand, is much lower than would be expected based on the HCV prevalence in this group. Both HIV status and start of injection during follow-up, both putative markers of past injection drug use, were independently associated with HCV prevalence among never-injecting DU. Most likely, the underreporting of past injecting drug use explains the elevated HCV prevalence in this group, but incidental HCV transmission through household or sexual contact with injecting DU cannot be excluded. Phylogenetic analysis revealed that indeed never-injecting DU harbour the same HCV strains as their injecting counterparts. Hence, all DU, injecting and non-injecting, should be routinely screened for the presence of HCV infection.

In Chapter 3.3 we studied the occurrence of HCV reinfection and HCV superinfection among 59 (injecting) DU that seroconverted for HCV during follow-up in the ACS. Depending on sample availability, seroconverters were tested for HCV RNA at to 2 to 10 different visits. Genetic typing showed 42% of HCV seroconverters experienced multiple HCV infections over time. Both HCV reinfection and HCV superinfection are common among actively injecting DU, suggesting that neither previously viral clearance nor ongoing HCV infection provides significant protective immunity against HCV. This finding will further complicate HCV vaccine development. Even with a vaccine based on multiple HCV epitopes, the question remains whether protective immunity for all different or even similar viral variants will be acquired. Moreover, health guidance should emphasise the risk of persistent HCV reinfection among those who continue risk behaviour after spontaneous or treatment-induced viral clearance.

**Men who have sex with men**

Sexual transmission of HCV rarely occurs by heterosexual intercourse, not even in the presence of HIV. Since 2000, however, HCV has emerged as a sexually transmitted infection (STI) among HIV-positive but not HIV-negative men who have sex with men (MSM)
in Amsterdam (Chapter 4.1). The HCV incidence among HIV-positive MSM participating in the ACS increased tenfold in the period 2000-2003 compared with previous years. Using a phylogenetic approach, we revealed the presence of an MSM-specific transmission network, probably sexual, in Amsterdam. Mucosal lesions and/or anal bleeding probably play an important role during sexual transmission of HCV as the majority of MSM in our study report high rates of concurrent ulcerative STI, rough sexual techniques, and all of them denied injection drug use. The emergence of HCV among HIV-positive MSM, especially of difficult-to-treat HCV genotypes 1a and 4d, might have major clinical implications. HIV/HCV coinfection has been associated with lower rates of spontaneous HCV clearance, accelerated liver disease, a less favourable HCV treatment outcome, delayed CD4 recovery after initiation of HAART, and increased HAART-associated hepatotoxicity. Regular HCV screening should be part of the routine care of HIV-positive MSM, and should be considered for MSM with negative or unknown HIV-status who report sexual high-risk behaviour. Early detection of HCV could mitigate HCV transmission by preventing further infections, and by improving treatment outcomes as results of early interferon treatment in HIV-positive MSM are promising.

Phylogenetic analysis of HCV strains circulating among HIV-positive MSM diagnosed with acute HCV in England, France, Germany and the Netherlands revealed 9 MSM specific HCV strains, mainly of difficult-to-treat genotypes 1a and 4d (Chapter 4.2). Once introduced, HCV rapidly spreads to neighbouring countries via a joint European MSM-specific transmission network. Evolutionary analysis determined that the majority of HCV transmission in Europe occurred after 1996, which coincides with the rise in sexual risk behaviour among MSM after the introduction of HAART. Risk factor evaluation suggests that indeed the majority of HCV infections among HIV-positive MSM in Europe relate to permcosual risk factors in the context of (traumatic) sexual practises. Sequences obtained from HIV-positive MSM in Australia lie in two distinct clusters, as might be expected given its geographic separation. In contrast to European cases, injecting drug use significantly contributes to the spread of HCV among HIV-positive MSM in Australia.

Finally, in Chapter 5 we discuss the main findings of our studies. Molecular epidemiology has proven to be a powerful tool to increase our understanding of the spread of HCV in the Netherlands. A better insight in the spread of HCV provides can be used to evaluate the impact of prevention measures taken in the past, and when necessary guide the development and implementation of new strategies to mitigate the further spread of HCV in the future. The latter especially applies to the recent spread of HCV among HIV-positive MSM. In order to curb this epidemic, we need to gain better insight in the risk factors responsible for HCV transmission in this population, and the MSM transmission networks via which HCV spreads.