Molecular epidemiology of hepatitis C virus

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CHAPTER 5

GENERAL DISCUSSION
General Discussion

Molecular epidemiology combines the genetic profile of a certain microorganism with epidemiological data of its host. Studies based solely on genetic typing of the microorganism often lack detailed patient information or suffer from selection bias caused by restricted sample availability or by choosing non-representative patient groups. Epidemiological studies that mainly focus on the host often experience trouble in assigning the mode of transmission. The two-sided approach used in molecular epidemiology proves to be very useful for investigating viral transmission through the community, as it can provide evidence for source tracing and improves our understanding of transmission networks. Insight in the current spread of HCV is needed to come up with targeted prevention campaigns that mitigate the further spread of HCV in the community.

Genetic typing

Molecular epidemiology requires the existence of a unified HCV classification system, and a commonly used HCV genomic consensus region that accurately reflects the epidemiological links between existing HCV isolates. The huge genetic diversity observed among circulating HCV strains interferes with both these prerequisites.

Over the years, HCV nomenclature has often changed as a result of the continuous characterisation of new HCV viral variants [1,2]. In this thesis, HCV strains were typed according to the HCV nomenclature consensus proposal of 2004 [3,4]. HCV was classified into 6 major genotypes (1 to 6) that are each further divided into 75 related subtypes (a,b,c ). Minimum genetic divergence among HCV genotypes is 30%, compared to a genetic divergence of at least 15% among HCV subtypes. As genetic divergence of former genotypes 6,7,8,9 and 11 was 21-29%, they were reassigned as different subtypes (6a-6l) of genotype 6. For the same reason, genotype 10a was reassigned as subtype 3k. However, in recent years new HCV subtypes [5], possibly a new HCV genotype [6], as well as intergenotypic [7,8] and intragenotypic [9] recombinants have been identified, suggesting that the current system again needs revision. Also in our lab we are currently characterising HCV strains of genotype 2 originating from Surinam [10] and genotype 4 originating from Egypt and Ethiopia [11] that do not fit the ranges of the current classification system.

Different genetic assays, each based on different parts of the HCV genome, are being used as each region might provide specific information on HCV epidemiology, pathogenesis, immunity or treatment. However, this severely hampers our understanding about the spread of HCV. Without recollection and retyping of samples, it often remains impossible to
determine whether outbreaks observed in e.g. different countries are interconnected [12-14]. In the absence of full genome sequencing, the NS5B region has the highest phylodynamic signal of all genes [15]. For this reason, the NS5B region (nucleotide 8276-8615) is one of the two genomic regions used for HCV classification [3]. To maintain the current HCV classification system and to facilitate data exchange in molecular epidemiological research, we highly recommend to characterise strains based on this NS5B region and to submit all sequence data to internationally available HCV databases.

How does HCV genetic diversity among different risk groups increase our understanding of the spread of HCV in the Netherlands?

The genetic diversity of HCV provides information on the mode of HCV transmission as well as on the time that a certain HCV strain has been circulating within a population. A significant proportion of patients with chronic HCV, also in the Netherlands, do not report any recognisable HCV risk factor [16-19]. Especially in these cases, genetic typing provides additional information on the most plausible route of transmission. Genetic diversity among HCV seropositive Dutch donors revealed three previously described risk profiles each associated with a certain genotype: (i) contaminated blood transfusions received in the past, mainly HCV genotype 1b, (ii) a history of injecting drug use, HCV genotypes 1a and 3a, and (iii) immigration from HCV endemic countries that results in the introduction of ‘exotic’ HCV genotypes uncommon for the Netherlands. The wide genetic diversity of HCV subtype 2, mainly 2b, within the Dutch population suggests longstanding nosocomial transmission. As associations between route of transmission and genotype exist, this implies that a changing genotype distribution reflects a shift in HCV epidemiology [20,21].

The emergence of HCV genotype 4d

Two separate introductions of HCV genotype 4d were identified in the Netherlands, one among injecting drug users (chapter 3) and one among HIV-positive MSM (chapter 4). The low genetic divergence among different HCV 4d isolates obtained suggests recent introduction and spread of this genotype, probably from Southern Europe [11,22-24]. The first known HCV 4d isolates among injecting drug users in Amsterdam stem from the late 1980s, whereas HCV 4d emerged only in the late 1990s among MSM (Chapter 3-4). The increasing prevalence of HCV 4d reflects the changing HCV epidemiology in the Netherlands, in which injecting drug use and high-risk sexual behaviour among MSM have become the two dominant modes of HCV transmission. Over time, the prevalence of especially HCV genotype 1b and 2, that mainly spread among recipients of contaminated blood products, haemophiliacs and dialysis patients, will likely decline [17,25].
HCV emerges as a sexually transmitted infection among HIV-positive MSM

HCV incidence among HIV-positive MSM in Amsterdam increased tenfold after the year 2000, compared with previous years (Chapter 4). Phylogenetic analysis confirmed that at least 5 MSM-specific HCV lineages of genotype 1a and 4d are currently circulating in Amsterdam. The low evolutionary distances among recent HCV isolates are typical of a recent introduction and a rapid spread of HCV in the MSM community. The fact that multiple introductions of different HCV genotypes took place suggests behavioural change in MSM rather than evolution of the virus into a variant that somehow is more easily transmitted. The high similarity of HCV strains circulating among HIV-positive MSM in different European countries implies that, once introduced, HCV rapidly spreads to MSM in neighbouring countries via a joint European transmission network (Chapter 4). Evolutionary analysis traced back the emergence of HCV in HIV-positive MSM to the late 1990s, which coincides with the rise in STI observed in HIV positive MSM after the introduction of HAART [26,27]. As MSM communities in Europe are large and interconnected, HCV transmission has probably become endemic among HIV positive MSM, similar to what has been observed for hepatitis A virus and hepatitis B virus [14,28].

The HCV epidemic among drug users

In addition to the recent introduction of HCV genotype 4d, two distinct strains of HCV genotype 1a, and one HCV 3a strain are currently circulating among (young) injecting drug users in Amsterdam (Chapter 3). Evolutionary analysis traced exponential growth of these strains back to the second half of the 20th century [29]. Although these traditional HCV strains of genotype 1a and 3a still predominate among drug users in Amsterdam, the genotype distribution has shifted. Concordant with data from other western European countries, the proportion of young drug users infected with HCV 3a is declining [29-31]. The epidemic of HCV 3a seems to have reached a steady state, while the 1a epidemic has not. HCV genotype 1a, and in a lesser extent the recently introduced strain of HCV genotype 4d, might therefore become the dominant subtypes among injecting drug users. Although in one study HCV genotype 3a has been associated with enhanced rates of viral clearance [32], no HCV intergenotypic differences in viral load, infectivity or natural course of infection have been described thus far, that might explain this shift in HCV genotype distribution. On the other hand, well-designed studies that include HCV seroconverters affected by a wide range of HCV genotypes are lacking. In drug users with HCV superinfection no particular pattern was observed, the dominant HCV strain switched from genotype 1a to 3a as often as from genotype 3a to 1a (Chapter 3). The fact that 1a and 3a sequences of both self-declared non-injecting DU and candidate donors intersperse with those circulating among injecting drug users suggests that these strains have bridged to other risk groups through household or sexual contact, occupation and/or health-care related exposure, or blood donations of former IDU (Chapter 2-3).
HCV reinfection and superinfection

In a cohort of drug users with a documented HCV seroconversion during follow-up, we demonstrated that 41% of participants experienced multiple HCV infections over time (Chapter 3). Due to the frequency of sampling and the fact that phylogenetic analysis might not be able to differentiate between intrahost viral evolution and reinfection by the same source (e.g. the steady injection partner), the true number of drug users who experience multiple infections will probably be even higher. Hence, in a high-risk population, HCV reinfection and HCV superinfection are far more common than was previously assumed [33-36]. Neither previous viral clearance nor ongoing infection seem to provide significant protective immunity against HCV. This has major implications for future research. First, it means that a future vaccine needs to be based on multiple HCV epitopes. Even then the question remains whether protective immunity will be acquired for different or even similar viral strains. Second, HCV incidence calculations based on the assumption that HCV seropositives are no longer susceptible underestimate the true HCV incidence and do not properly reflect HCV transmission dynamics within a network of high-risk individuals [37,38]. Molecular epidemiological HCV network studies among high-risk populations require frequent sampling and ongoing genetic typing. Third, health guidance should emphasise the risk of reinfection among those who might be re-exposed to HCV after spontaneous or treatment-induced viral clearance [39,40]. Potential risks related to HCV superinfection, on the other hand, still need to be confirmed. However, HCV superinfection might negatively affect HCV immune escape [41], natural course of infection [42-44] or treatment outcome [45,46]. Current laboratory are costly, time-consuming and cumbersome if one aims to detect minor variants in mixed infections [36,47], especially as the replacement of the initial HCV strain is often rapid and near complete [48]. This rapid replacement might again be a consequence of the inability of the adaptive immune system to recognise divergent viral strains. The high viral turnover of HCV might cause even the smallest fitness differences to result in a rapid decrease of the less fit viral strain over time [48,49].

Implications for HCV prevention

The introduction of the screening of donor blood in 1991, extended with nucleic acid amplification testing in 1999, drastically reduced the incidence of transfusion-acquired HCV. The residual risk of acquiring HCV through contaminated blood products in the Netherlands is estimated to be near zero (1:31.5 million donation) (Chapter 2). The contribution of healthcare related HCV transmission to the overall HCV-incidence in the Netherlands is assumed to be negligible. The big challenge is to trace the people that were previously infected in order to offer them treatment before irreversible liver damage occurs. First-generation migrants need special attention; they are estimated to be responsible for a large proportion
of chronic HCV infections in the Netherlands [50,51], and appear difficult to reach with the current health intervention campaigns [52]. Prevention of new infections should focus on the two major risk groups: men who have sex with men and drug users. In the absence of an HCV vaccine, prevention relies heavily on precautionary measures that mitigate the further spread of HCV.

**HIV-positive men who have sex with men**

Before the year 2000, sexual transmission of HCV among MSM in Amsterdam was rare (Chapter 4). During an anonymous survey in April 2008, 21% of HIV-positive MSM visiting the Amsterdam STI clinic tested positive for HCV [53]. During the last ten years, HCV has rapidly affected an increasing proportion of HIV-positive MSM in Europe, Australia and probably the US [54-56]. As early detection of HCV might prevent further HCV transmission, and success rates of interferon treatment initiated during the acute phase of HCV infection are promising also among those coinfected with HIV [57,58], regular HCV screening should be part of standard routine care in HIV-positive MSM. However, early HCV diagnosis and treatment will not stop the current epidemic without HIV-positive MSM altering their sexual risk behaviour. Increased awareness concerning the clinical consequences of HIV/HCV coinfection might help to reduce sexual risk taking of HIV positive MSM, in particular in the context of HIV seroconcordant sexual partnering (serosorting), and thereby curb the epidemic.

**HIV-negative men who have sex with men**

It is striking that HCV almost exclusively affects HIV-positive MSM [53]. This does not mean, however, that HIV-negative MSM are not a risk [59]. Both the fact that HIV-positive MSM diagnosed with acute HCV have relatively preserved CD4 counts [60,61], and high rates of HCV acquisition have been reported soon after primary HIV-infection [62], argue for behavioural risk factors rather than a role for HIV itself. HIV, however, might facilitate HCV transmission by increasing viral infectiousness due to higher HCV-RNA viral loads. Concurrent ulcerative STI, rough sexual techniques, a high number of sexual partners and sex in the context of drugs have been associated with HCV infection in MSM, suggesting an important role for mucosal lesions and/or anal bleeding [53,60,61,63]. As permucosal transmission of HIV is more efficient than permucosal transmission of HCV, and in MSM the background HIV prevalence exceeds the background HCV prevalence, acquisition of HIV will probably precede HCV infection among those MSM who engage in high-risk sexual behaviour [64]. To monitor possible bridging of HCV between HIV-positive and HIV-negative MSM, regular cross-sectional surveys that include molecular typing are needed, also among MSM with negative or unknown HIV status [53].
Drug users

In contrast to other countries [31,40,65-67], the HCV-incidence among injecting drug users in the Netherlands has drastically declined over the last 20 years [68]. Responsible for this decline are the low levels of injecting drug use and the Dutch harm reduction approach that combines methadone therapy and needle exchange programmes [69]. However, drug users do still remain a large reservoir of HCV in the Netherlands [50]. Treatment of drug users with chronic HCV infection will reduce the size of this reservoir and thereby will decrease the chance of new subjects to get infected. A high rate of HCV reinfection and superinfection (Chapter 3), as well as concerns regarding treatment adherence, argue against increased access of drug users to HCV treatment. Nonetheless, the improved success rates of HCV treatment and the low HCV incidence currently observed among drug users in the Netherlands, make that access of drug users to HCV treatment can no longer be denied. Especially as HCV treatment outcomes in drug users are comparable to that of non drug users when HCV care is given in an integrated multidisciplinary setting [70,71].

Concluding remarks

Using molecular epidemiological techniques we (i) obtained information about modes of HCV transmission in subjects without a recognisable risk factor (Chapter 2), (ii) identified recent sexual transmission of HCV among HIV-positive MSM (Chapter 4), (iii) characterised the HCV epidemic among injecting drug users (Chapter 3), and (iv) uncovered some of the complexities of the natural history of HCV infection among individuals who continue their high risk behaviour (Chapter 3). Hence, genetic typing has proven to be a useful tool to increase the understanding of the spread of HCV.

This thesis gives a detailed description of HCV epidemiology in Amsterdam. Although HCV incidence and prevalence in the rest of the country might not be as high as in Amsterdam, we have no reasons to believe that HCV strains circulating in other parts of the country differ from those obtained from Amsterdam [10,51,63,72]. The continuous characterisation of prevalent HCV infections among known risk groups in the Netherlands therefore seems redundant. However, genetic typing of incident HCV infections should be continued. Incident HCV infections provide evidence for source tracing and reveal possible changes in HCV epidemiology. Furthermore, HCV genotyping remains of major clinical importance. First to determine HCV treatment regimen and to predict treatment outcome, and second to exclude the possibility of reinfection or superinfection in patients who fail or relapse during or after HCV therapy [39,45,73]. In relation to HCV treatment, viral characterisation might even become more important in the near future. Future HCV treatment will probably consist of combination therapy with direct inhibitors targeting the life cycle of HCV combined with
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Current standard of care, i.e. pegylated interferon and ribavirin. Resistance mutations will likely develop, that require to be identified and monitored before and during treatment.

Major challenges lie ahead in both HCV prevention and treatment. The high STI prevalences and continuous outbreaks of newly introduced microorganism, especially among HIV positive MSM [74,75], raises an increasing demand for effective prevention strategies that specifically target HIV-positive MSM. The emergence of HCV genotypes 1a and 4d in both MSM and drug users, and the fact that (nearly) all HCV infected MSM are coinfected with HIV, will have a negative impact on HCV treatment results [11,31,76,77]. Future treatment of HCV will require individual treatment regimes and the new compounds to eradicate HCV that are currently under development [78,79]. In respect with the future burden of disease, more information is needed about the extent by which acquiring HCV on top of an existing HIV infection negatively affects the clinical course of HCV and possibly also HIV [80-83].

References


Discussion


Chapter 5


