Epidemiological studies among injecting drug users infected with HIV: highly active antiretroviral therapy, tuberculosis, hepatitis C, immunology
van Asten, L.C.H.I.

Citation for published version (APA):
Infection with concurrent multiple hepatitis C virus genotypes is associated with faster HIV disease progression

AIDS 2004, 18:2319-2324
Ze zijn dinkt ook of
zoek naar Druks winkel,
as ze enmaal verstwapt
zijn dan gaan ze trillen
en hebben ogen die
onder hand uit hun hoofd
vallen.

Maisha van Asten, 2001
Infection with concurrent multiple hepatitis C virus genotypes is associated with faster HIV disease progression

Liselotte van Asten and Maria Prins for the European Seroconverter Study among injecting drug users* and the Italian Seroconverter Study*

Objective: To elucidate the importance of hepatitis C Virus (HCV) genotype in HIV disease progression.

Design: This study was conducted among 126 HIV/HCV co-infected drug users with a known interval of HIV seroconversion whose HCV genotype was known early in HIV infection. Both clinical progression (to AIDS) and immunological progression (to a CD4+ T-cell count of 200 x 10^6 cells/l) by HCV genotype were studied using Cox proportional hazards analysis.

Results: The median duration of follow-up was 7.3 years (interquartile range (IQR), 4.6–10.1 years). The majority of the HCV infections concerned genotype 1 and genotype 3; The distribution was: HCV type 1: 48%, HCV type 3: 34%, HCV type 4: 13%, multiple HCV types: 5%. Concurrent multiple infections consisted of HCV genotypes 1b+3a, 1b+4 and 3a+4. HCV genotype 1 and multiple HCV genotype infections were associated with faster immunological progression [hazard ratio (HR), 2.02; 95% confidence interval (CI), 1.04–3.92 and HR, 2.74; 95% CI, 0.95–7.90, respectively]. Multiple HCV genotype infection was also associated with faster clinical progression (HR, 3.36; 95% CI, 0.82–13.79). These hazard ratios increased further and were all significant when analyses were limited to data in the pre-HAART era (HR, 3.92; 95% CI, 1.51–10.20; HR, 4.38; 95% CI, 1.04–18.40 and HR, 6.54; 95% CI, 1.39–30.76, respectively).

Conclusion: HIV disease progression differs by HCV genotype and is especially faster in individuals whose HCV infection involves more than one HCV genotype. The effect of HCV genotype on HIV progression was greater in the pre-highly active antiretroviral therapy (HAART) era, suggesting that the effectiveness of HAART may diminish the effect of HCV genotype on HIV disease progression.

© 2004 Lippincott Williams & Wilkins

AIDS 2004, 18:2319–2324

Keywords: HIV, disease progression, hepatitis C virus genotype, multiple hepatitis C virus genotype infections, injecting drug users, survival analysis, Europe

From the *Municipal Health Service, Cluster Infectious Diseases, Amsterdam, The Netherlands. *See Appendix.
Correspondence to Liselotte van Asten, Municipal Health Service Amsterdam, Cluster Infectious Diseases, Nieuwe Achtergracht 100, 1018 WT Amsterdam, The Netherlands.
E-mail: Liselotte.van.Asten@rivm.nl or: LiselottevanAsten@yahoo.com
Received: 7 May 2004; revised: 22 July 2004; accepted: 15 September 2004.
Introduction

The effect of hepatitis C virus (HCV) infection on HIV disease progression remains controversial. It has been suggested that discordant results from different studies may be due to HCV genetic heterogeneity [1], but studies of this issue are rare. Among haemophiliac men HCV genotype 1 appears to be associated with faster HIV progression [2–4]. The effect of infection with HCV type 4, a genotype on the rise among injecting drug users (IDU) [5,6], and the effect of infection with concurrent multiple HCV types has not been studied at all. Furthermore it is also unknown whether highly active antiretroviral therapy (HAART) changes an effect of HCV genotype on HIV disease progression. As HCV infection is almost universal among HIV-infected IDU we investigated HIVE disease progression among IDU for whom the HCV genotype was known early in HIV infection. Their interval of HIV seroconversion was also known, so that bias by unknown duration of HIV infection was not present [7].

Methods

The study population comprised 126 IDU co-infected with HIV and HCV, and receiving no treatment for HCV. For each IDU, dates of a seronegative and seropositive HIV test were documented. These HIV seroconverters originated from cohorts in seven European countries: the Netherlands, Spain, Austria, France, Switzerland, Italy and the UK (Scotland) [8,9].

For 104 of the 126 IDU, the HCV genotype was determined using a reverse transcriptase (RT)-polymerase chain reaction (PCR) targeting the core region of the HCV genome [5,10]. All such testing was conducted at the Municipal Health Service in Amsterdam. For the remaining 22, information on HCV genotype was determined by the line probe assay protocol [11] at each study centre. For the majority of all 126 IDU, the HCV genotype was determined early in HIV infection [median 1.4 years following HIV seroconversion, inter quartile range (IQR), 0.7–3.3].

The effect of HCV genotype on HIV disease progression (both clinical and immunological progression) was assessed in a Cox proportional hazards model. As pre-AIDS mortality (i.e. death occurring among individuals who have not yet progressed to an AIDS-defining diagnosis) due to natural causes is high among drug users and associated with HIV disease progression [8,12], clinical progression was defined as progression to AIDS (125 IDU) or pre-AIDS death (five IDU) for which we excluded non-natural causes of death such as accidents, suicide, overdose and homicide. Immunological progression was defined as progression to a CD4+ T-cell count of \(200 \times 10^6\) cells/l. For IDU who did not progress to an endpoint follow-up was censored at death (for clinical progression: pre-AIDS death from a non-natural cause), loss to follow up, or the censor date (maximally 1 January 2002). For the analyses of progression to the first CD4+ T-cell count of \(200 \times 10^6\) cells/l persons were also censored if the interval between two study visits was greater than 1 year. This was done to limit the risk of assigning inappropriately long follow-up to IDU whose CD4 cell count potentially may have dropped to \(200 \times 10^6\) cells/l in the time-period in which no CD4 measurements were available. IDU for whom no data were available on CD4+ measurements (13 IDU) or whose CD4+ measurement was already \(\leq 200 \times 10^6\) cells/l at study entry (five IDU) were excluded, limiting the analysis of immunological progression to 108 out of 126 IDU. In this subgroup the HCV genotype distribution did not differ from that in the total group. In line with previous studies [2,13], HCV genotype 3 was defined as the reference category. To study the effect of HCV genotype with HAART, analyses were repeated on data limited to the pre-HAART era. Data was censored when HAART became available, which was between March 1996 and September 1996 depending on the study site.

Results

The complete group of 126 HIV-positive IDU with a known HCV genotype was studied for a total follow-up time of 865 years since HIV seroconversion. Demographic characteristics and HCV genotype distribution are shown in Table 1. Of IDU for whom information on antiretroviral treatment was available and who were still AIDS-free and in active follow-up on the following dates: 60% (50 of 84) had initiated HAART by July 1997 and this had increased to 75% (58 of 77) by 1 January 1999.

Clinical progression

In the pre-HAART era, IDU infected with concurrent multiple HCV types showed a significantly elevated risk of clinical progression, compared to IDU infected with HCV genotype 3 [adjusted hazard ratio (HR) for all genotyping methods, 6.54; 95% confidence interval (CI), 1.39–30.76, Table 2]. Including data of the HAART era, the risk of progression was elevated to a lesser extent and not significant (adjusted HR, 3.36; 95% CI, 0.82–13.79). However, for this total period, when limiting the analyses to IDU for whom the genotype was determined by the same typing method (PCR of the core region) the hazard ratio increased and became significant (adjusted HR, 4.41; 95% CI, 1.05–18.57, not shown in table). No statistically sig-
**HIV progression varies by HCV genotype**

Table 1. Characteristics and hepatitis C virus (HCV) genotype distribution for 126 HIV-HCV co-infected injecting drug users.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Follow-up (median, IQR)</th>
<th>Age at HIV seroconversion (median, IQR)</th>
<th>Female (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%) of total</td>
<td>7.3 years (4.6-10.1)</td>
<td>27 years (24-33)</td>
<td>40 (32%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HCV genotype</th>
<th>n (% of total)</th>
<th>Subtype distribution</th>
<th>Determined by core PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV type 1</td>
<td>60 (48%)</td>
<td>subtype 1a (78%)</td>
<td>49 (82%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>subtype 1b (15%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>subtype 1 (4%)</td>
<td></td>
</tr>
<tr>
<td>HCV type 2</td>
<td>1 (1%)</td>
<td>subtype 2 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>HCV type 3</td>
<td>43 (34%)</td>
<td>subtype 3a (95%)</td>
<td>38 (88%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>subtype 3 (5%)</td>
<td></td>
</tr>
<tr>
<td>HCV type 4</td>
<td>16 (13%)</td>
<td>subtype 4 (100%)</td>
<td>11 (69%)</td>
</tr>
<tr>
<td>Multiple HCV types</td>
<td>6 (5%)</td>
<td>subtype 1b+3a (17%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>126 (100%)</td>
<td>subtype 1b+4 (67%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>subtype 3a+4 (17%)</td>
<td></td>
</tr>
</tbody>
</table>

*Genotype determined by polymerase chain reaction of the core region (n = 104) or Inno-LiPA assay (n = 22). Subtype not specified. For 11 IDU, sequencing of the NS5 region showed that 10 (91%) harboured subtype 4d and one (9%) harboured subtype 4a. IQR, inter quartile range.

Table 2. Hazard ratios for progression to AIDS or pre-AIDS death of a natural cause by hepatitis C virus genotype

<table>
<thead>
<tr>
<th>Hepatitis C genotype</th>
<th>No. IDU (cases)</th>
<th>Person years</th>
<th>Adjusted b HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 3</td>
<td>43 (11)</td>
<td>404</td>
<td>1</td>
<td>Overall: 0.35</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>60 (17)</td>
<td>310</td>
<td>1.23 (0.55-2.76)</td>
<td>0.62</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>16 (4)</td>
<td>120</td>
<td>0.83 (0.25-2.77)</td>
<td>0.76</td>
</tr>
<tr>
<td>Multiple types</td>
<td>6 (3)</td>
<td>31</td>
<td>3.36 (0.82-13.79)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pre-HAART era</th>
<th>No. IDU (cases)</th>
<th>Person years</th>
<th>Adjusted b HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall: 0.06</td>
<td>39 (6)</td>
<td>233</td>
<td>1</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>53 (7)</td>
<td>185</td>
<td>1.07 (0.35-3.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 (1)</td>
<td>71</td>
<td>0.49 (0.05-4.56)</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>6 (3)</td>
<td>21</td>
<td>6.34 (1.39-30.76)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

| *HCV type 2 excluded due to inadequate numbers for analysis (one injecting drug user [IDU]). Adjusted for age at HIV seroconversion, geographic region and setting of recruitment. Pre-highly active antiretroviral therapy (HAART) era: time period prior to the general availability of HAART for HIV. HAART became available between March 1996 and September 1996 depending on the study site. *Number of IDU who developed AIDS or died prior to AIDS (excluding non-natural causes of death such as overdose, suicide, accidents and homicide). HR, hazard ratio; 95% CI, 95% confidence interval.

Significant differences in clinical progression were seen between IDU infected with genotype 3 (the reference category) and genotypes 1 or 4 (adjusted HR, 1.23; 95% CI, 0.55-2.76 and HR, 0.83; 95% CI, 0.25-2.77, respectively, for the total study period).

**Immunologic progression**

Immunologic progression of HIV infection was faster among IDU infected with multiple HCV genotypes compared to IDU infected with HCV genotype 3 (adjusted HR, 2.74; 95% CI, 0.95-7.90; P = 0.06, Table 3). It was significantly increased among IDU infected with HCV genotype 1 (adjusted HR, 2.02; 95% CI, 1.04-3.92). Limiting analyses to IDU for whom routine CD4 + measurements were initiated within 1 year from HIV seroconversion (64% of the study population), showed no substantial change in the hazard ratios. Analyses limited to those IDU whose genotype was determined by PCR of the core region also yielded negligible change. However, as with clinical progression, limiting the analyses to the pre-HAART era greatly increased the risk for IDU infected with multiple HCV genotypes (adjusted HR, 4.38; 95% CI, 1.04-18.40 compared to genotype 3). For IDU infected with genotype 1 the risk also increased (adjusted HR, 3.92; 95% CI, 1.51-10.20). For the
Discussion

This study shows that among IDU, HIV disease progression differs with HCV genotype, being especially enhanced in those harbouring HCV infection involving more than one HCV genotype.

The effect of genotype on HIV progression appears to have been much greater in the pre-HAART era, suggesting that the effectiveness of HAART may diminish the effect of HCV genotype on HIV disease progression.

To our knowledge, only one other study on this issue included IDU as part of the study population (75%) [13] although with unknown duration of HIV infection. Unlike studies among haemophilic men [2-4], they found no difference in clinical progression of HIV between individuals infected with different HCV genotypes. We likewise found no effect of genotype 1 on clinical progression, but we did find genotype 1 to be associated with faster immunological progression than genotype 3. Interestingly, further analyses of our genotype 1 cases linked this effect to subtype la and not to subtype 1b.

The effect of infection with genotype 4 or with multiple HCV genotypes on HIV disease progression has not been analysed previously in any HIV risk group. Among our IDU the number with multiple HCV infections was small but showed HIV disease progression to be significantly enhanced in this group in the pre-HAART era. As a greater frequency of unsafe injecting might be expected to be associated with greater risk of multiple genotype infection, these infections may be a surrogate marker for exposure to other blood-borne infectious pathogens which may contribute to faster progression. However, this is not a likely explanation since no effect of lifestyle factors on HIV disease progression so far has been observed among IDU [14]. How multiple genotypes may cause faster HIV disease progression is not known. Sabin et al. [2] suggest two mechanisms which possibly play a role in the association between HCV genotype and HIV disease progression. Firstly, any direct interaction between HCV and HIV may differ by HCV genotype and further, CD4 cell proliferation within hepatic tissue might be HCV genotype-dependent and may affect HIV proliferation.

Different combinations of (sub)types 3a, 1b and 4 were found among IDU infected with multiple genotypes. In individuals infected with only one genotype, neither subtype 1b nor type 4 was associated with increased HIV progression compared to genotype 3, ruling out the possibility that a specific subtype was singly responsible for the increased risk associated with multiple genotypes. In our study, median CD4+ cell counts at the date of HCV sampling were lower in IDU with multiple HCV genotypes (233 x 10^3 cells/l; IQR, 201-738) than in others (500 x 10^3 cells/l; IQR, 308-720), but not significantly (P=0.40), although time since seroconversion did not differ (P=0.21). This finding may reflect a detrimental impact of multiple genotype infections on HIV disease progression, but conversely it might reflect a greater susceptibility to multiple genotype infections among faster progressors, or a faster outgrowth to detectable levels of multiple HCV genotypes that were either acquired early in HIV infection or even prior to HIV infection. The numbers in our study were small, and statistical methods taking

<table>
<thead>
<tr>
<th>Hepatitis C genotypea</th>
<th>Total study period</th>
<th>Pre-HAART erae</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. IDU (cases)</td>
<td>Person years</td>
<td>Adjustedb HR (95% CI)</td>
<td>P-value</td>
<td>No. IDU (cases)</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>38 (16)</td>
<td>231</td>
<td>1</td>
<td>0.04</td>
<td>34 (6)</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>51 (27)</td>
<td>226</td>
<td>2.02 (1.04-3.92)</td>
<td>0.04</td>
<td>43 (18)</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>13 (4)</td>
<td>89</td>
<td>0.66 (0.22-2.90)</td>
<td>0.47</td>
<td>12 (2)</td>
</tr>
<tr>
<td>Multiple types</td>
<td>6 (5)</td>
<td>30</td>
<td>2.74 (0.95-7.90)</td>
<td>0.06</td>
<td>6 (3)</td>
</tr>
</tbody>
</table>

Total study period, we analysed IDU infected with genotype 1 in whom the subtypes were known (la or 1b) finding that subtype la was associated with an elevated risk of immunologic progression whereas subtype 1b was not (adjusted HR, 2.10; 95% CI, 1.01-4.35 and HR, 0.86; 95% CI, 0.19-3.92, respectively) compared to genotype 3. Defining genotype 1 as the reference category instead of genotype 3 further revealed that for the total study period, immunologic progression of IDU infected with genotype 4 is significantly slower than that of IDU infected with genotype 1 (HR, 0.33; 95% CI, 0.11-0.96).

HCV type 2 excluded due to inadequate numbers for analysis [one injecting drug user (IDU)]. Pre-highly active antiretroviral therapy (HAART) era: time period prior to the general availability of HAART for HIV. HAART became available between March 1996 and September 1996 depending on the study site. Number of IDU who progressed to a CD4 T-cell count of 200 x 10^3 cells/l or less. HR, hazard ratio; 95% CI, 95% confidence interval.
into account small groups in a Cox regression model are not available; Cox regression with small numbers may yield confidence intervals that are too narrow [15]. Thus although a strong effect of multiple HCV genotype infection was found in the present study, this result needs confirmation in larger studies. The necessary data however, are sparse and difficult to obtain and for whom the HCV genotype was determined early in HIV infection. Studies that have longer follow-up in the HAART era are also necessary to determine whether any effect of HCV genotype on HIV disease progression remains now that HAART is available. How, biologically, HAART and HIV interact with HCV is not clear as studies report conflicting results [16].

Bias may also have been introduced into our study through technical aspects of HCV genotype determination. First, we used genotyping results from two different methods, although 83% were determined by the same in-house PCR, and limiting analyses to these results did not substantially change the hazard ratios, but for IDU infected with multiple HCV types statistical significance was reached. Second, both tests are able to pick up multiple genotype infections, but how much either test misclassifies multiple infections as single infections is unknown. If such misclassification is at random, it causes an underestimation of an association, meaning that possibly multiple HCV genotype infections are associated with an even greater risk of HIV progression than our study has revealed. However, multiple infections that are misclassified as a single genotype infection may possibly represent a select group of IDU in whom the predominant genotype is picked up and other genotypes are missed due to presence at very low levels. How this would affect results is not clear, but this study does show that for IDU in whom mixed infections are detected, HIV disease progression is enhanced.

A complicating factor when assessing the impact of HCV genotype on HIV disease progression is that HCV infection can be cleared and that infection with one genotype does not protect against infection with another. Therefore an individual who remains exposed to HCV (e.g., by risky injecting behaviour), may switch from one genotype to another over time or switch from one genotype to multiple genotypes and vice versa, or in due course clear HCV RNA altogether [17]. Notably, for the present study we assessed the effect of the HCV genotype that was present early in HIV infection. In future, due to the possibility of re-infection and clearance, HCV genotype should ideally be measured prior to HIV infection and longitudinally at different time-points within HIV-infected individuals to gain a better understanding of the effect of HCV genotype on HIV progression.

Acknowledgements

We thank Inge Verhaest, Saida Lamzira and M. Dierdorp (Municipal Health Service, Amsterdam) for HCV genotype determination and Ronald Geskus for critical review of the manuscript. We also thank the laboratories collaborating with the original studies for determining lymphocyte subsets and HCV genotype, the clinicians and health workers who contributed by collecting data, Lucy Phillips for editorial review and the participants for their ongoing participation.

Sponsorship: This study was supported by the Dutch AIDS Foundation (Stichting AIDS Fonds), as part of the Stimulation Programme on AIDS Research of the Dutch Programme Committee for AIDS Research (2172). It was also supported by the grants of the original studies and the Sarphati Foundation (Stichting Sarphati).

References

12. Prins M, Sabín CA, Lee CA, Devereux H, Coutinho RA. Pre-AIDS mortality and its association with HIV disease progression in

Appendix

The following investigators participate in the European Seroconverter among injecting drug users and in the Italian Seroconverter Study:

Ildefonso Hernandez-Aguado on behalf of the Valencian HIV Seroconversion Study, Department of Public Health, Miguel Hernandez University, Alicante, Spain; Robert Zangerle for the Innsbruck AIDS study, AIDS Unit, University of Innsbruck, Innsbruck; Faroudy Boufassa for the French SEROCO study group, Inserm U 292, Hôpital de Bicêtre, Le Kremlin Bicêtre, France; Barbara Broers for the Geneva HIV Cohort Study, Division of Infectious Diseases, Geneva University Hospital, Geneva, Switzerland; J. Roy Robertson for the Edinburgh Drug Addiction Study, Muirhouse Medical Group, Edinburgh, Scotland; Raymond P. Brettle for the Edinburgh City Hospital Cohort Study, Infectious Diseases Unit, Western General Hospital, Edinburgh, Scotland, Jim McMenamin for the Scottish National Collaborative HIV Testing Study, Scottish Center for Infection and Environmental Health, Glasgow, Scotland, Sylvia Bruisten and Roel A. Coutinho for the Amsterdam Cohort Study among drug users, Municipal Health Service, Cluster Infectious Diseases, Amsterdam, the Netherlands; Giovanni Rezza for the Italian Seroconverter Study, Instituto Superiore di Sanità, Roma, Italy.