Resistance-associated polymorphisms in Dutch hepatitis C genotype 1a patients with and without HIV infection

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ABSTRACT

Background and aim. Resistance-associated variants (RAVs) on the NS3 region of the hepatitis C virus (HCV) may be relevant for antiviral therapy, but data in human immunodeficiency virus (HIV) coinfected patients are scarce. We assessed frequencies of NS3 RAVs in patients infected with HCV genotype 1a with or without HIV coinfection. Material and methods. HCV NS3 amino acids 1-181 were sequenced by the Sanger method and analyzed for RAs. RAVs and their distribution between HCV genotype 1a clade I and II viruses were compared between HIV-infected versus HIV-uninfected patients. Results. 148 samples were available (n = 68 HIV and n = 80 non-HIV). Relative frequency of clade I and clade II was significantly different between HIV (85% and 15%) and non-HIV groups (49% and 51%). Overall, HIV infected patients exhibited significantly lower prevalence of RAVs than HIV-uninfected patients (62% vs. 79%, p = 0.03). However, Q80K prevalence was significantly higher in HIV-infected subjects (50% vs. 24%, p = 0.001), whereas prevalence of S122D/G/N/S (2% vs. 16%, p = 0.002) and N174G/N/S (10% vs. 55%, p < 0.0001) polymorphisms were significantly lower. Q80K was found exclusively in clade I viruses. S122 (3% vs. 22%, p=0.001) and N174 (13% vs. 75%, p<0.0001) polymorphisms had significantly lower prevalence in clade I than clade II viruses. Conclusions. In the Netherlands, prevalence of clade I viruses and Q80K was significantly higher in HCV genotype 1a infected patients with HIV coinfection than in those without HIV coinfection. Prevalence of N174 and S122 polymorphisms was significantly higher in clade II than clade I viruses.

Key words. Hepatitis C virus. HIV. Genotype 1a. Q80K. Drug resistance.

INTRODUCTION

With the introduction of interferon-free direct-acting antivirals (DAAs), sustained virologic response (SVR) rates increased substantially for hepatitis C virus (HCV) infected patients.1 As approximately 30% of patients with human immunodeficiency virus (HIV) are infected with HCV,2 coinfected patients form a significant proportion of patients eligible for treatment with DAAs. Nevertheless, only limited data are available on HCV genetic polymorphisms in HIV/HCV coinfected patients (summarized in table 1). The emergence of NS3 resistance-associated variants (RAVs) such as Q80K has been described in HCV patients, especially in those with genotype 1a.1 Successful therapy could be complicated by the high genetic diversity of HCV and its rapid mutation rate.3 Notably, occurrence rates of RAVs vary in different parts of the world, with a higher frequency of Q80K in North America than Europe, and a high variation in reported prevalence of Q80K (ranging from 9% to 18%) between individual European countries.4 This may be related to the high prevalence of genotype 1a in North America compared to Europe, where genotype 1b is more frequent.5 Moreover, within genotype 1a, two distinct clades (clade I and clade II) are distinguished, and Q80K presence appears to be restricted to clade I.6,7 Several studies reported reduced susceptibility to NS3/4A protease inhibitors in the presence of RAVs.8-10 Q80K exhibited a 10.9 fold reduced susceptibility...
<table>
<thead>
<tr>
<th>Study¹</th>
<th>Study specifics</th>
<th>Sample year</th>
<th>Patient characteristics</th>
<th>Previous treatment</th>
<th>Q80K HIV vs. non-HIV</th>
<th>Other RAVs HIV vs. non-HIV</th>
<th>No. of RAVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vicenti, et al.¹⁵</td>
<td>Archived samples; genotype 1a and 1b, whole NS3 genome</td>
<td>2007-2011</td>
<td>n = 50 HIV n = 17 non-HIV</td>
<td>Naive to PIs</td>
<td>6/50 (12%) vs. 5/19 (29%), p = NA</td>
<td>V36L: 2/50 (4%) vs. 2/17 (12%); T54S: 1/50 (2%) vs. 1/17 (6%); V55A: 1/50 (2%) vs. 0/17 (0%)</td>
<td>At least one RAV: 10/50 (20%) vs. 7/17 (41%), p = NA</td>
</tr>
<tr>
<td>Ehret, et al.¹⁴</td>
<td>CS: all genotype 1a; NS3 amino acids 1-181</td>
<td>All 2014</td>
<td>n = 23 HIV n = 19 non-HIV</td>
<td>NA</td>
<td>8/19 (42%) vs. 9/23 (39%), p = NS</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Jabara, et al.²³</td>
<td>Archived samples; all genotype 1a; NS3 amino acids 36-173</td>
<td>2000-2011</td>
<td>n = 20 HIV n = 20 non-HIV</td>
<td>Naive to all anti-HCV therapy</td>
<td>6/20 (30%) vs. 11/20 (55%), p = NA</td>
<td>V36ALM 38% vs.; 64% Q41R 24% vs. 4%; T54AS 24% vs. 29%; V55AI 16% vs. 29%; V107I 80% vs. 58%; A156SV 22% vs. 16%; V/170T 44% vs. 31%</td>
<td>Overall non-Q80K RAV cumulative frequency: 0.53% vs. 0.37%, p = NS</td>
</tr>
<tr>
<td>Lin, et al.²⁴</td>
<td>Archived samples; genotype 1a, 1b, 2, 4, 10 NS3 sites covering V36-V170,</td>
<td>1998-2010</td>
<td>n = 26 HIV n = 39 non-HIV</td>
<td>Naive to PIs</td>
<td>NA</td>
<td>NA</td>
<td>V36L, Q80K and R155K: 11/26 (42%) vs. 16/39 (41%), p = NA</td>
</tr>
<tr>
<td>Beloukas, et al.²⁵</td>
<td>Archived samples, genotype 1a, NS3 amino acids 1-181</td>
<td>2006-2014</td>
<td>n = 61 HIV n = 107 non-HIV</td>
<td>Naive to all anti-HCV therapy</td>
<td>7/61 (12%) vs. 18/107 (17%), p = 0.379</td>
<td>V36LM, T54S, V55A, Q80K, D168E, V/I170AT and N174S: 169/238 (63%)</td>
<td>NA</td>
</tr>
<tr>
<td>Applegate, et al.²⁶</td>
<td>Archived samples of a PS, genotype 1a, 11 NS3 sites covering V36-V170, NS5A, NS5B</td>
<td>2004-2008</td>
<td>n = 24 HIV n = 26 non-HIV</td>
<td>Naive to PIs</td>
<td>2/24 (8%) vs. 0/26 (0%), p = NA</td>
<td>V36M: 1/24 (4%) vs. 0/26 (0%); V55A: 0/24 (0%) vs. 2/26 (8%)</td>
<td>Median no. Of RAVs/subject: 6; Proportion subjects with 6 or more RAVs: 42% vs. 52%, p = 0.49</td>
</tr>
<tr>
<td>McCormick, et al.²⁷</td>
<td>CS, genotype 1a, 1b, 3 and 4, 181, NS5A, NS5B</td>
<td>Unknown</td>
<td>n = 57 HIV n = 23 non-HIV</td>
<td>27/57 HIV group Experienced with PEG-IFN/RBV Unknown for non-HIV</td>
<td>5/57 (9%) vs. 8/23 (35%), p = NA</td>
<td>T54ST: 0/57 (0%) vs. 2/23 (9%); V36L: 0/57 (0%) vs. 1/23 (4%); R155K: 0/57 (0%) vs. 1/23 (4%)</td>
<td>At least one RAV: 5/57 (9%) vs. 11/23 (48%), p &lt; 0.0001</td>
</tr>
</tbody>
</table>

¹ Abstracts or full articles from original studies found through a systematic search of the literature in the electronic database PubMed, conducted on August 4, 2015, with the following search terms used: “HIV” and synonyms combined with “hepatitis C” and synonyms combined with “NS3” and synonyms. Studies without genotype 1a NS3 RAV data in HIV vs. non-HIV infected subjects were excluded. ² Only genotype 1a data is given in the table. ³ Only NS3 data is given in the table. ⁴ Specific data only given in subgroup of 168 patients, with total study population consisting of 238 subjects. CS: cross-sectional study. RAV: resistance-associated variant. NA: not available. NS: not significant. PI: protease inhibitor. PS: prospective study.
to simeprevir in vitro\textsuperscript{8} and is reported to jeopardize achievement of SVR in the combination of the NS3/4A protease inhibitor simeprevir with pegylated-interferon and ribavirin.\textsuperscript{1} In “all oral” DAA regimens with NS3A, NS5A and/or NS5B inhibitors, effects of Q80K seem to be minor or non-existent.\textsuperscript{11,12} In vitro, for S122R, the sensitivity to simeprevir appears to be reduced by up to 20 fold.\textsuperscript{10} In addition, the N174 polymorphisms are reported to lead to 3.3-5.9 fold susceptibility reduction to telaprevir and boceprevir in vitro.\textsuperscript{9}

RAVs may form a high burden within the HIV-infected population because HCV subtype 1a is more prevalent in coinfected patients\textsuperscript{5} and more prone to NS3 drug resistance than other subtypes.\textsuperscript{7} Nevertheless, it remains to be determined to what extent NS3 RAVs impact treatment results in HCV patients with and without HIV infection, during treatment with interferon-free DAA regimens. While several studies have focused on RAVs in HIV-coinfected HCV patients, few studies assessed frequencies of NS3 RAVs in HIV/HCV coinfected patients with conflicting results.\textsuperscript{13-15}

In the current study we analyze the frequencies of NS3 RAVs in HCV genotype 1a patients with and without HIV. In addition, we assess the distribution of NS3 RAVs between clade I and II viruses.

**MATERIAL AND METHODS**

**Patients and samples**

A total of 148 samples from HCV genotype 1a patients were available from the University Medical Center Utrecht (UMCU) and the Academic Medical Center (AMC) in the Netherlands. Samples consisted of pretreatment archived samples from the CIRA study\textsuperscript{16} (all HIV uninfected) and samples from patients (previously) in regular care. Patients treated with NS3/4A protease inhibitors, NS5A or NS5B polymerase inhibitors, or patients treated with experimental therapy prior to sampling have been excluded. In addition, demographic and clinical data were collected.

**Sequencing procedure**

HCV RNA was isolated using the MagNA Pure 96 system (Roche Applied Sciences). A reverse transcription PCR was then performed using either the Superscript III One-Step Platinum Taq kit (Life Technologies, Inc., Gaithersburg, MD) with HCV genotype 1-specific primers (UMCU) or Superscript II (Life Technologies) with random hexamer primers (AMC). Subsequently, cDNA was amplified by nested PCR using either the Expand High Fidelity PCR System Kit (Roche Diagnostic) (UMCU) or Faststart Taq polymerase (Roche Applied Science) (AMC). Product length was confirmed on an agarose gel. When required, the product was purified using a QIAquick PCR purification kit (Qiagen Inc.). A cycle sequence reaction was run using the BigDye Terminator v3.1 sequencing kit (Technologies, Inc., Gaithersburg, MD) to prepare the samples for sequencing. After cycle sequence reactions, samples were sequenced using the 3730xl DNA analyzer (Applied Biosystems), covering NS3 amino acids 1-181. Both reverse transcriptase PCR protocols and nested PCR procedures were compared in 14 random samples and yielded similar results. The following amino acid positions were selected for RAV analyses (selected a-priori according to previous literature\textsuperscript{17}) by using geno2pheno [hcv] 0.92 with a fold change cutoff value of 1.2; V36, Q41, F43, T54, V55, Q80, V107, R109, S122, V132, R155, A156, V158, D168, V/I170, N174 and L/M175.

**Statistical analyses**

Presence of RAVs were compared between HIV-infected and HIV-uninfected groups, clade I and clade II viruses and samples taken before and after 2010. Additional sensitivity analyses were performed for RAV prevalences in samples taken before and after 2008, 2009 and 2011. Data were compared using non-parametric $\chi^2$, Fisher’s exact or Mann-Whitney U tests as appropriate. Qualitative data were summarized by numbers with percentages and quantitative data by medians with ranges. Statistical analyses were performed using SPSS statistics version 21.0.0 (IBM SPSS Statistics, Chicago, IL) and graphics were created using GraphPad Prism version 6.0 (GraphPad Software Inc., CA, USA). A two sided p-value of $< 0.05$ was considered statistically significant.

**RESULTS**

**Study population**

The NS3 region was sequenced in 148 unique samples from HCV genotype 1a infected patients ($n = 68$ HIV respectively $n = 80$ non-HIV). All samples were collected between 2000 and 2015. HIV status from all subjects was known, however, clinical data was incomplete in 19 subjects with sample dates between 2000 and 2004. Samples from HIV-infected patients were more frequently taken at later dates than those from HIV-uninfected (median 2011 (range 2003-2015) vs. 2008 (2000-2015), $p = 0.04$). HIV-infected patients were more frequently younger, male, acquired HCV more often through sexual contact (men who have sex with men (MSM)) and had higher baseline alanine aminotransferase levels (Table 2). HIV-uninfected patients were more likely to have acquired HCV through injection drug use or by unknown causes and had more
frequently cirrhosis at baseline (diagnosis by FibroScan®, liver biopsy or imaging modalities). Of the 50 HIV-infected subjects treated with combined antiretroviral therapy, 41 (82%) had undetectable HIV RNA (<50 copies/mL).

### RAVs in the study population

Polymorphisms at positions F43, R109, V132, S138, R155, V158, D168 and L/M175 were not observed. While samples from HIV-infected were more frequently of recent dates than those from HIV-uninfected patients, presence of at least one RAV did not differ between samples taken before or after 2010, neither in the HIV-infected group (62% vs. 62%, p = 1.0), nor in the HIV-uninfected group (78% vs. 80%, p = 0.74), nor in the whole group (72% vs. 70%, p = 1.0). In sensitivity analyses, no differences were seen in samples taken before and after 2008, 2009 and 2011. In sensitivity analyses, no differences were seen in samples taken before and after 2008, 2009 and 2011. As a proxy of duration of HCV infection, presence of RAVs between patients with and without cirrhosis was assessed, with no significant differences found in those with HIV (67% vs. 63%, p = 1.0), those without HIV (74% vs. 80%, p = 0.74) or in the whole group (72% vs. 69%, p = 1.0).

### RAVs in HIV infected vs. HIV uninfected groups

Overall, HIV-infected patients had lower RAV frequencies than HIV-uninfected patients, with 42 (62%) exhibiting at least one RAV vs. 63 patients (79%, p = 0.03) in the HIV-uninfected group (Figure 1). In addition, total number of RAVs per subject was significantly lower in the HIV-infected group (Figure 2). Prevalence of Q80K was higher in HIV-infected patients (50% vs. 24%, p = 0.001), whereas S122D/G/N/S polymorphisms (2% vs. 16%, p = 0.002) and N174G/N/S polymorphisms (10% vs. 55%, p < 0.0001) had lower prevalences (Table 3). Within the HIV-infected group, CD4 cell count ≥350 or treatment with combined antiretroviral therapy did not affect prevalence of the RAVs.

### RAVs distribution among HCV transmission modes

Distribution of RAVs among the various modes of HCV acquisition in the total study population are given in Table 4. Significantly higher rates of Q80K were observed in HCV transmission by MSM vs. other modes of transmission (29/58 (50%) vs. 24/90 (27%), p = 0.005). Lower frequencies of Q80K were observed among injection drug users vs. other modes of HCV transmission (4/25 (16%) vs.
S122 and N174 polymorphisms displayed lower frequencies in MSM than in other modes of transmission (For S122 1/58 (2%) vs. 13/90 (14%) with p = 0.009, and for N174 45/90 (50%) vs. 6/58 (10%) with p < 0.0001). For all other polymorphisms, no differences in mode of HCV transmission were observed.

**DISCUSSION**

In the current study, significant higher frequencies of Q80K and lower frequencies of polymorphisms on the S122 and N174 loci were observed in NS3/4A protease inhibitor treatment-naive HIV-infected patients than in HIV-uninfected patients with HCV genotype 1a. These results may be explained by differences in HCV acquisition between the groups, as a subset of HCV genotype 1a strains may circulate among the MSM community,18 whereas a
broad variety in strains may be acquired through other transmission routes. Indeed, several studies among HIV-positive MSM in European studies, including the Netherlands, revealed the existence of MSM-specific HCV transmission networks in which HCV co-circulating strains were identified.19,20 This may have been exhibited in the current study by higher Q80K frequencies in the MSM group whereas the other RAVs displayed a more even distribution among the other transmission modes. Another explanation may be the high prevalence of genotype 1a clade I viruses in HIV-infected patients, which may lead to subsequent higher Q80K rates.7 In line with our observations, a recent study on NS3 RAVs in HIV-infected patients (n = 40 HCV genotype 1a and n = 19 genotype 1b) observed 10/23 vs. 4/17 RAVs in genotype 1a clade I respectively clade II, of which 8 out of 10 RAVs in clade I were Q80K.21

Q80K reduces susceptibility to simeprevir in vitro, with lower SVR rates especially in pegylated-interferon con-

### Table 3. Distribution of resistance-associated variants in 148 NS3/4A protease inhibitor treatment-naive patients with and without HIV infection.

<table>
<thead>
<tr>
<th>Variant</th>
<th>All patients n = 148</th>
<th>HIV-infected n = 68</th>
<th>HIV-uninfected n = 80</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V36L</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Q41H</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>0.46</td>
</tr>
<tr>
<td>T54ST</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (4%)</td>
<td>0.25</td>
</tr>
<tr>
<td>V55AI</td>
<td>3 (2%)</td>
<td>3 (4%)</td>
<td>3 (4%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Q80K</td>
<td>53 (36%)</td>
<td>34 (50%)</td>
<td>19 (24%)</td>
<td>0.001</td>
</tr>
<tr>
<td>V107GI</td>
<td>3 (2%)</td>
<td>3 (4%)</td>
<td>0 (0%)</td>
<td>0.10</td>
</tr>
<tr>
<td>S122DGNS</td>
<td>14 (10%)</td>
<td>1 (2%)</td>
<td>13 (16%)</td>
<td>0.002</td>
</tr>
<tr>
<td>A156V</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>0.46</td>
</tr>
<tr>
<td>V1I70VT</td>
<td>8 (5%)</td>
<td>3 (4%)</td>
<td>5 (6%)</td>
<td>0.73</td>
</tr>
<tr>
<td>N174GNS</td>
<td>51 (35%)</td>
<td>7 (10%)</td>
<td>44 (55%)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Data are presented as n (%). Data on F43, R109, V132, S138, R155, A156, V158, D168 and L/M175 polymorphisms prevalence are not given as they did not occur in the study population.

### Table 4. Distribution of resistance-associated variants in 148 NS3/4A protease inhibitor treatment-naive patients according to modes of HCV transmission.

<table>
<thead>
<tr>
<th>Variant</th>
<th>MSM n = 58</th>
<th>Injection drugs n = 25</th>
<th>Transfusion n = 14</th>
<th>Other/Unknown n = 51</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V36L</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Q41H</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>T54ST</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (6%)</td>
<td>0.24</td>
</tr>
<tr>
<td>V55AI</td>
<td>0 (0%)</td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td>2 (4%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Q80K</td>
<td>29 (50%)</td>
<td>4 (16%)</td>
<td>6 (43%)</td>
<td>14 (28%)</td>
<td>0.01</td>
</tr>
<tr>
<td>V107GI</td>
<td>3 (5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.35</td>
</tr>
<tr>
<td>S122DGNS</td>
<td>1 (2%)</td>
<td>5 (20%)</td>
<td>2 (14%)</td>
<td>6 (12%)</td>
<td>0.02</td>
</tr>
<tr>
<td>A156V</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (7%)</td>
<td>0 (0%)</td>
<td>0.10</td>
</tr>
<tr>
<td>V1I70VT</td>
<td>2 (3%)</td>
<td>2 (8%)</td>
<td>1 (7%)</td>
<td>3 (6%)</td>
<td>0.68</td>
</tr>
<tr>
<td>N174GNS</td>
<td>6 (10%)</td>
<td>10 (40%)</td>
<td>6 (43%)</td>
<td>29 (57%)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Data are presented as n (%). Data on F43, R109, V132, S138, R155, A156, V158, D168 and L/M175 polymorphisms prevalence are not given as they did not occur in the study population. MSM: men who have sex with men.
In our study we observed lower number of RAVs per subject in the HIV-infected population, which is in line with another study on this subject.28 Another instructive observation from our study is higher prevalence of clade I than clade II viruses in HIV-infected with an equal distribution of clade I and clade II viruses in HIV-uninfected subjects. This is contrary to the findings of another study7 that found HIV-infected subjects to have a trend towards higher prevalence rates of clade II viruses. In this latter study however, both HIV-infected and HIV-uninfected subjects were most frequently infected by HCV through intravenous drug use or by unknown means as opposed to homosexual contact in our HIV-infected study population. In line with other reports, Q80K was exclusively seen in clade I viruses in our study.5,7 However, we are the first to describe higher prevalence of S122 and N174 polymorphisms in clade II viruses.

Our study has several limitations, including the retrospective design and availability of limited clinical data in some patients. Second, we assessed RAVs by population-based sequencing and do not have deep sequencing data (i.e. mutations expressed in ≥20% of the HCV quasispecies population).32 However, a previous study on NS5A RAVs investigating several sequencing cut off values from 1-20%, described a reduction in SVR only in high level resistant polymorphisms.31 Thus, the widely used population-based sequencing method is highly likely to be sufficient in detecting clinically relevant RAVs. The strengths of this study are that we analyzed a relatively large subset of NS3 RAVs throughout 15 years of time in both HIV-infected and HIV-uninfected patients and the relatively large sample size compared to the available literature.

In conclusion, the current study demonstrated increased prevalence of Q80K and reduced prevalence of S122 and N174 polymorphisms in HIV-infected patients compared to HIV-uninfected patients with HCV genotype 1a. Although testing for RAVs prior to treatment with NS3/4A protease inhibitors does not constitute standard of care with currently available DAA regimens, it could be useful in the near future when treating DAA-failures or difficult-to-treat patients.

**ABBREVIATIONS**

- AMC: Academic Medical Center.
- DAA: direct-acting antiviral.
- HCV: hepatitis C virus.
- HIV: human immunodeficiency virus.
- MSM: men who have sex with men.
- RAV: resistance-associated variant.
- SVR: sustained virologic response.
- UMCU: University Medical Center Utrecht.
CONFLICT OF INTEREST

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