Epidemiology of HCV mixed infection and reinfection in the treatment setting

From a virologists’ Perspective...

Janke schinkel, MD PhD
Academic Medical Center, Amsterdam
INHSU 2015
Content

• Mixed infection
  – Definition
  – Detection of mixed infection
  – Epidemiology among PWID

• Reinfection
  – Adaptive Immunity to HCV
Mixed infection (1)

Messina, Hepatology, 2014
Mixed infection

- Presence of different variants from the same genotype at the same time
  - Virus exists within patient many (closely related) different variants (“quasispecies”)
  - Distribution of pairwise genetic distances in a mix of viral variants provides the answer
  - \textit{Cut off} needed for genetic distance between variants to distinguish mono- from mixed infection
Genetic distance in mixed infection

Pham et al, hepatology 2010

Variant A  ACTGACTGA
Variant B  GCTGACTGA
Variant C  ACGGACTGA

<table>
<thead>
<tr>
<th></th>
<th>variant A</th>
<th>variant B</th>
<th>variant C</th>
</tr>
</thead>
<tbody>
<tr>
<td>variant A</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>variant B</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>variant C</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

A > C  2
B > C  3
A > B  1

Pairwise genetic distance

![Graph showing genetic distance distribution]
HCV genetic variability across the genome

Selection of genomic fragment for detection of mixed infection depends on the characteristics of the epidemic
Relevance of detecting mixed infections

EASL treatment guidelines 2015

<table>
<thead>
<tr>
<th>Patients</th>
<th>PegIFN-α, RBV and sofosbuvir</th>
<th>PegIFN-α, RBV and simeprevir</th>
<th>Sofosbuvir and RBV</th>
<th>Sofosbuvir and ledipasvir</th>
<th>Ritonavir-boosted paritaprevir, ombitasvir and dasabuvir</th>
<th>Ritonavir-boosted paritaprevir, ombitasvir and simeprevir</th>
<th>Sofosbuvir and simeprevir</th>
<th>Sofosbuvir and daclatasvir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1a</td>
<td>12 wk, then PegIFN-α and RBV 12 wk (treatment-naïve or relapers) or 36 wk (partial or null responders)</td>
<td>No</td>
<td>12 wk</td>
<td>8-12 wk, without RBV</td>
<td>12 wk with RBV</td>
<td>No</td>
<td>12 wk without RBV</td>
<td>12 wk without RBV</td>
</tr>
<tr>
<td>Genotype 1b</td>
<td>12 wk</td>
<td>No</td>
<td>12 wk, without RBV</td>
<td>12 wk without RBV</td>
<td>No</td>
<td>No</td>
<td>12 wk without RBV</td>
<td>12 wk without RBV</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>12 wk</td>
<td>No</td>
<td>12 wk</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>12 wk without RBV</td>
<td>12 wk without RBV</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>12 wk</td>
<td>No</td>
<td>24 wk</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>12 wk without RBV</td>
<td>12 wk without RBV</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>12 wk, then PegIFN-α and RBV 12 wk (treatment-naïve or relapers) or 36 wk (partial or null responders)</td>
<td>No</td>
<td>12 wk, without RBV</td>
<td>No</td>
<td>No</td>
<td>12 wk with RBV</td>
<td>12 wk without RBV</td>
<td>12 wk without RBV</td>
</tr>
<tr>
<td>Genotype 5 or 6</td>
<td>12 wk</td>
<td>No</td>
<td>12 wk, without RBV</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>12 weeks</td>
<td>12 weeks without RBV</td>
</tr>
</tbody>
</table>

DAA Treatment without interferon is (still) genotype specific

Detection of mixed infections with different subtypes / variants not relevant for treatment
Mixed infections: dynamics of treatment failure

Cunningham et al, Nature reviews in Gastroenterology and Hepatology, 2015

Adapted from Abdelrahman, Hepatology 2015
multiple infections over time

Grebely, Hepatology et al 2012
How to detect mixed infections
wish list

- Sensitive assay
- “Unbiased” PCR
  - Ability to pick up all genotype
- Adequate genotype assignment
- Easy to apply in clinical settings
- Cheap
# Methods for detecting mixed infection

<table>
<thead>
<tr>
<th>technique</th>
<th>advantages</th>
<th>disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR + Sanger sequencing (core / NS5B)</td>
<td>easy, cheap</td>
<td>Not sensitive, interpretation of mixed bp difficult</td>
</tr>
<tr>
<td>PCR, cloning, sequencing</td>
<td>sensitive</td>
<td>More expensive, time consuming</td>
</tr>
<tr>
<td>Genotype specific nested PCR</td>
<td>sensitive</td>
<td>Risk of cross-contamination, time consuming</td>
</tr>
<tr>
<td>PCR + NGS</td>
<td>(very) sensitive</td>
<td>No standardized pipeline available yet, expensive</td>
</tr>
</tbody>
</table>
### commercial assays for genotyping

<table>
<thead>
<tr>
<th>assay</th>
<th>technique</th>
<th>genotyping</th>
<th>disadvantages</th>
<th>Performance of detecting mixed infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott m2000 RealTime HCV Genotype II assay</td>
<td>genotype-specific real-time PCR (specific primer / probes)</td>
<td>1 – 6, Subtype 1a, 1b</td>
<td>Not always resolved (10%)</td>
<td>??, false positive mixed infection reported</td>
</tr>
<tr>
<td>Versant HCV genotype assay (LiPA) 2.0</td>
<td>PCR, hybridisation (5’ end, core)</td>
<td>Detection of genotype 1 – 6, subtypes 1a, 1b and some 6</td>
<td>Misclassifies genotype 6 as 1, incomplete assignment,</td>
<td>??, false positive mixed infections</td>
</tr>
</tbody>
</table>
NextGen genotyping

- No PCR, random priming for cDNA synthesis
- Identification of (short) genome fragments for accurate genotyping
- 'simple' pipeline without haplotype reconstruction
- Proof of concept: mixed infection (90%/10%) accurately identified
Epidemiology of mixed infection among PWID

• Observed prevalence depends on
  – Characteristics of population (risk behavior)
  – Persistence of mixed infection
  – Method used
Epidemiology of mixed infections in pWID

Cunningham et al, Nature reviews in Gastroenterology and Hepatology, 2015
Multiple infections in 23/59 (39%) seroconverters
Incident mixed infection: 9/89 (10%)
NextGen sequencing

- Amsterdam Cohort Studies among PWID, founded 1985
- 12 participants chronically infected followed from seroconversion
- Median follow up 12 years
- Total follow up: 143 years
- Number of samples: 156, median 13 per subject
- Gene: NS5B fragment (389 bp) according to Murphy et al*. (1 primer pair, second set for genotype 6)

![Graph showing % of samples from different dates]

Murphy et al, J Clinical Microbiology 2007
subjects without mixed infections with multiple genotypes (n = 4)
subjects with mixed infections with multiple genotypes, low prevalence of minor variants (< 1%) (n = 4)
subjects with mixed infections with prevalence of minor variants above 1% (n = 4)
What did we learn?

• 1/3 of subjects no evidence of mixed infection despite long follow up
• 1/3 of subjects evidence for mixed infection with different genotype present < 1%
• 1/3 of subjects evidence for mixed infection with minor variant > 1%
• *Mixed infections do not persist*
Quantitative summary of NGS study ACS

<table>
<thead>
<tr>
<th>Category</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N of Persons with multiple consecutive infections</td>
<td>8/12 (67%)</td>
</tr>
<tr>
<td>Incidence of superinfections</td>
<td>11/100 PY</td>
</tr>
<tr>
<td>N of persons with (ever) a mixed infection</td>
<td>8/12 (67%)</td>
</tr>
<tr>
<td>Percentage of samples with mixed infections</td>
<td>7%</td>
</tr>
</tbody>
</table>
Reinfection following SVR in PWID

• Yes.... occurs...

Table 1.  Overview of Studies on Hepatitis C Virus Reinfection Following Treatment Among People Who Inject Drugs

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Study Design</th>
<th>Genotyping</th>
<th>Sequence Analysis</th>
<th>No.</th>
<th>Median Age at Treatment Start, y</th>
<th>% Male</th>
<th>IDU Pretreatment &lt;6 mo</th>
<th>IDU Post treatment</th>
<th>Follow-up, Median (IQR)</th>
<th>PY Ever PWID/PWID Who Continue</th>
<th>No. of Re-infections</th>
<th>Reinfection Rate (95% CI) per 100 PY Ever PWID/PWID Who Continue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Backmund et al., 2004</td>
<td>Germany</td>
<td>Pros</td>
<td>Yes</td>
<td>No</td>
<td>18</td>
<td>32</td>
<td>61</td>
<td>NA</td>
<td>9</td>
<td>Mean 2.8 (SD 0.8–5.1)</td>
<td>50.8/23.8</td>
<td>2</td>
<td>3.94 (0.48–14.22)/8.4 (1.02–30.36)</td>
</tr>
<tr>
<td>Daigard et al., 2002</td>
<td>Norway</td>
<td>Pros</td>
<td>Yes</td>
<td>No</td>
<td>27</td>
<td>30</td>
<td>66</td>
<td>0</td>
<td>9</td>
<td>5.4 (1.1–6.8)</td>
<td>125.0/40.0</td>
<td>1</td>
<td>0.8 (0–5)/2.5 (0–14)</td>
</tr>
<tr>
<td>Currie et al., 2008</td>
<td>US</td>
<td>Pros</td>
<td>No</td>
<td>No</td>
<td>9</td>
<td>46 (mean)</td>
<td>88</td>
<td>NA</td>
<td>2</td>
<td>3.6 (3.2–6.0)</td>
<td>38.0/3.5</td>
<td>1</td>
<td>2.63 (0.07–14.66)/28.57 (0.72–159.19)</td>
</tr>
<tr>
<td>Grebely et al., 2010</td>
<td>Canada</td>
<td>Pros</td>
<td>Yes</td>
<td>Yes</td>
<td>35</td>
<td>44 (mean)</td>
<td>86</td>
<td>19</td>
<td>16</td>
<td>2.0 (0.4–5.0)</td>
<td>62.5/37.7</td>
<td>2</td>
<td>3.20 (0.39–11.56)/5.30 (0,64–19.18)</td>
</tr>
<tr>
<td>Bata et al., 2010</td>
<td>Australia</td>
<td>Pros</td>
<td>Yes</td>
<td>No</td>
<td>57</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>Grady et al., 2012</td>
<td>Netherlands</td>
<td>Pros</td>
<td>Yes</td>
<td>Yes</td>
<td>42</td>
<td>51</td>
<td>73</td>
<td>5^</td>
<td>11</td>
<td>2.5 (1.5–3.7)</td>
<td>131.6/32.3</td>
<td>1</td>
<td>0.76 (0.04–3.73)/3.42 (0.17–16.90)</td>
</tr>
<tr>
<td>Grebely, 2012</td>
<td>Australia</td>
<td>Pros</td>
<td>Yes</td>
<td>Yes</td>
<td>68</td>
<td>36</td>
<td>72</td>
<td>33^</td>
<td>NA</td>
<td>1.2 (0.1–3.0)</td>
<td>108</td>
<td>5</td>
<td>4.7 (1.9–11.2)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; IDU, injection drug use; IQR, interquartile range; NA, specific information was not available; Pros, prospective; PWID, people who inject drugs; PY, person-years.

^ During treatment.

^ Follow-up from end of treatment.

Grady et al, CID, 2013
But...

Secondary infection following spontaneous clearance have:

• Higher clearance rates
• Lower peak viremia
• Shorter duration of viremia upon reclearance
  (Osburn 2010, Sacks-Davis 2015)

*Adaptive immune responses are generated following spontaneous clearance of primary infection*
Incidence of reinfection following SVR in HIV+ MSM with acute HCV

Adaptive responses genotype specific following treatment induced clearance?

Reinfection with different genotype

Reinfection with same genotype

Thomas et al, AIDS 2015
Role of neutralizing antibodies

Functional study

- HIV + MSM (MOSAIC)
- Treatment induced clearance of acute HCV-1a infection
- Neutralizing responses in sera were more potent against genotype 1a viruses
- Protection against subsequent HCV-1a infections following SVR

Thomas et al, submitted
conclusions

• Mixed infections occur frequently among PWID (7% mixed infections in ACS, 10% in HITS-p..)
• They tend not to persist
• They are therefore not an obstacle for current treatment regimens
• Reinfection do occur among PWID following SVR with a reported incidence 1 – 8 per 100 PY
• (partial) protective immune responses are generated, even in the HIV-infected population
• Allow more time before treating a secondary infection?
• More data are needed on outcome of DAA-treatment in PWID, risk of reinfection, and the likelihood of spontaneous clearance of reinfections
Thanks to..

- Participants of the ACS and MOSAIC
- My co-workers at the AMC and the Public Health Service in Amsterdam
- (former) PhD students: Xiomara Thomas, Cynthia Ho, Joost vanhommerig, Sabrina Merat