Relapse or reinfection of hepatitis C after DAA treatment: unraveled by phylogenetic analysis.

Results from the Spanish GEHEP-004 cohort.

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Thursday 22nd June 2017
A cure for hepatitis C

• HCV: no latent forms <-> HIV
  → curable infection: spontaneous clearance or by antiviral treatment

• DAA regimens: viral cure rates 90-95% for all combinations (NS3 – NS5A – NS5B inhibitors)

<table>
<thead>
<tr>
<th>Combination regimen</th>
<th>Genotype 1</th>
<th>Genotype 2</th>
<th>Genotype 3</th>
<th>Genotype 4</th>
<th>Genotypes 5 and 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sofosbuvir + ribavirin</td>
<td>No</td>
<td>Suboptimal</td>
<td>Suboptimal</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Sofosbuvir/ledipasvir ± ribavirin</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sofosbuvir/velpatasvir ± ribavirin</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ombitasvir/paritaprevir/ritonavir + dasabuvir ± ribavirin</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ombitasvir/paritaprevir/ritonavir ± ribavirin</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Grazoprevir/elbasvir ± ribavirin</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sofosbuvir + daclatasvir ± ribavirin</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sofosbuvir + simeprevir ± ribavirin</td>
<td>Suboptimal</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

From EASL guidelines 2017
Reinfection of hepatitis C

- No protective immunity: reinfection possible after cure → further transmission

- High reinfection and transmission rates among patients with persistent risk behavior: PWID and HIV-positive MSM

- HIV-negative MSM in pre-exposure prophylaxis studies (Hoornenberg et al. CROI 2017, #519)

From Simmons et al. CID 2015
Relapse or reinfection?

• Distinguishing between virologic relapse and infection with a new viral strain:
  o True efficacy of current DAA therapies
  o Define the most appropriate retreatment if needed

• Wide variety of reinfection rates reported for patients with persistent risk behavior → more accurate estimation to support prevention strategies
Spanish GEHEP cohort

• GEHEP-004: 7189 HCV patients treated with IFN-free DAA regimens from 54 Spanish centers

• Around 450 patients failed standard DAA therapies
  → 53 patients: at baseline and time of SVR12

• Sequencing of 2 or 3 regions (NS3 – NS5A – NS5B)

• Determination HCV genotype and recombination

• NGS to rule out a mixed infection for reinfections with ≠ HCV subtype
Phylogenetic analysis

- **BLAST**: ten most similar sequences to each taxon

- **Concatenated alignments** → **NJ** and **ML trees**
  
  (GTR gamma model – 1000 bootstrap replicates)

- **Reinfection**: ≠ HCV geno/subtype or ≠ clustering

- **Relapse**: clustering in same clade (bootstrap >70%)

Methods

53 patients

**Viral sequencing:**

- before therapy
- at time of failure

**HCV**

**DAA**

**Virological relapse**

**REINFECTION**

NS3 – NS5A – NS5B
Dominance of HCV1a infection and transmission in PWID

<table>
<thead>
<tr>
<th>HCV1a</th>
<th>HCV1b</th>
<th>HCV3a</th>
<th>HCV4</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.5%</td>
<td>24.5%</td>
<td>15.1%</td>
<td>18.9%</td>
</tr>
</tbody>
</table>

• Transmission route of infection (known for 79%):
  - PWID: 78.6% → 81.8% HIV – 51.5% HCV1a
  - Sexual contact: 7 patients (5 MSM – 2 hetero)
  - Blood transfusion: 1 – hemodialysis: 1
HCV genotype misclassifications

- Assignment of baseline samples by phylogenetic analysis and subtyping tools (COMET and Oxford) in agreement with commercial assays: 66.0%
  - Majority due to lack of information on subtype level
  - Six discordant cases: wrongly classified on genotype and/or subtype level
- No evidence of recombination detected using software packages Simplot and RDP4
Phylogenetic analysis: need for long genomic stretches

• Poor phylogenetic signal of single fragments: inconsistencies between different genes

**NS5A**: reinfection  
**NS5B**: relapse
Phylogenetic analysis: multiple genes

• Only conclusions drawn for concatenated alignments (51/53 patients)

• Patient 6: late relapse or potential reinfection?
Relapse versus reinfection

Virological relapse
45 patients

Reinfection
5 patients

3 ≠ HCV subtype
4 PWID – 1 MSM
4 HIV/HCV co-infected
Discussion

• Reinfection in high-risk patients: multifactorial risk behavior <-> one dominant route of transmission

• Patients with unknown route of transmission = HCV mono-infected

• High number of HCV1a and HCV4 cases ( <-> Aguilera et al. 2017): large share of HIV co-infected patients

• Phylogenetics of single fragments: insufficient phylogenetic signal -> need for longer fragments
Conclusions

• Majority of patients: virological relapse

• 10% of DAA failures reinfected: PWID + HIV co-infected

• 2/5 reinfections: same HCV subtype as baseline => need for sequencing and phylogenetic analysis:
  o Determine correct HCV genotype
  o Discriminate relapse and intra-subtype reinfection
  o Additional information about the presence of RASs

• Only confident conclusions in case of long genomic fragments => full-genome or multiple regions
**Acknowledgments**

- Clinical and Evolutionary Virology – KU Leuven, Belgium  
  Anne-Mieke Vandamme
- San Cecilio University Hospital, Granada, Spain  
  Féderico García – Ana Belen Perez – Natalia Chueca - Francisco Javier Salmerón
- Hospital Gregorio Marañón, Madrid, Spain  
  Teresa Aldamiz-Echevarría
- University Hospital Jerez, Cadiz, Spain  
  Juan Carlos Alados
- Hospital Miguel Servet, Zaragoza, Spain  
  Ana María Martínez-Sapiña
- Hospital Infanta Elena, Huelva, Spain  
  Dolores Merino
- University Hospital de Valme, Sevilla, Spain  
  Juan Antonio Pineda
- Hospital de La Linea, Cadiz, Spain  
  Francisco Téllez
- Hospital Virgen del Rocío, Sevilla, Spain  
  Pompeyo Viciana
- Hospital Reina Sofía, Cordoba, Spain  
  Antonio Rivero-Juarez
- Hospital Ramón y Cajal, Madrid, Spain  
  María Jesús Vivanco
- University Hospital La Paz, Madrid, Spain  
  Víctor Hontañón