Hepatitis C: Latest Update on Diagnosis and Treatment

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Disclosures

- I will discuss products that are not FDA approved.
- Financial Disclosures
  » Speaking honoraria
    - Abbott Molecular*
    - BioFire (Idaho Technology)
    - bioMerieux
    - Cepheid
  » Scientific Advisory Board
    - Cepheid
    - BioFire
  » Research support
    - GenMark
    - BioFire
    - Luminex
    - Qiagen
  » Consultant
    - Becton Dickinson

Objectives

- Appreciate the impact that molecular testing has on monitoring viral response to therapy.
- State the roles of genotyping and IL28B polymorphism testing in HCV treatment regimens.
- Cite the different therapeutic approaches used for the most common genotypes in the U.S.

Introduction

- Hepatitis C virus is the most common chronic blood-borne viral infection in North America.
- Major cause of chronic hepatitis
- Causes progressive hepatic fibrosis which leads to cirrhosis and an increased risk of hepatocellular carcinoma
- Chronic Hepatitis C is the leading indication for liver transplantation in U.S.

Acute Viral Hepatitis by Type, U.S.

Source: CDC Sentinel Counties Study on Viral Hepatitis

Global prevalence of hepatitis C infection
HCV Epidemiology

- 3.9 million Americans infected with HCV (1.8% of pop.) according to National Health and Nutrition examination Survey (NHANES III)
  - 2.7 million have chronic HCV (HCV RNA detected in serum)
- Worldwide, ~200 million infected with HCV (3% of pop.)
- Although annual incidence of new cases is decreasing, chronic HCV-associated liver disease is on rise

![HCV Epidemiology Diagram](image)

Estimates of Acute and Chronic Disease Burden for Viral Hepatitis, U.S.

<table>
<thead>
<tr>
<th></th>
<th>HAV</th>
<th>HBV</th>
<th>HCV</th>
<th>HDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute infections (x 1000)/year</td>
<td>125-200</td>
<td>140-320</td>
<td>35-180</td>
<td>6-13</td>
</tr>
<tr>
<td>Fulminant deaths/year</td>
<td>100</td>
<td>150</td>
<td>?</td>
<td>35</td>
</tr>
<tr>
<td>Chronic infections</td>
<td>0</td>
<td>1-1.25 million</td>
<td>3.5 million</td>
<td>70,000</td>
</tr>
<tr>
<td>Chronic liver disease deaths/year</td>
<td>0</td>
<td>5,000</td>
<td>8-10,000</td>
<td>1,000</td>
</tr>
</tbody>
</table>


Natural History of HCV

- Family Flaviviridae
- Enveloped, RNA virus
  - Highly mutable genome
  - Rapid mutation in a hypervariable region of the genome coding for the envelope proteins and escapes immune surveillance by the host
- Occurs in vivo as a group of “quasispecies”

![Natural History of HCV Diagram](image)

HCV Laboratory Diagnosis

- The average time from exposure to antibody to HCV (anti-HCV) seroconversion is 8–9 weeks.
  - Anti-HCV can be detected in >97% of persons by 6 months after exposure.
- HCV RNA can be detected in blood within 1–3 weeks after exposure.

![HCV Laboratory Diagnosis Diagram](image)

HCV: Typical Serologic Course

- Symptoms
- Anti-HCV
- ALT

![HCV: Typical Serologic Course Diagram](image)
Diagnostic Tests for HCV

- **HCV antibody**
  - Used to diagnose hepatitis C infection
  - Not useful in the acute phase as it takes at least 4 weeks after infection before antibody appears
  - May be falsely negative in patients on hemodialysis or with immunodeficiency, or falsely positive in patients with autoimmune disorder
  - Confirmatory test: recombinant immunoblot assay (RIBA)*
  - OraSure Technologies - OraQuick HCV Rapid Antibody Test
- **HCV RNA**
  - Often used to diagnose HCV infection in the acute phase
  - Main use is in monitoring the response to antiviral therapy

HCV Laboratory Diagnosis

- **EIA**
  - Negative
  - Positive
    - Measure HCV RNA by PCR
      - Negative
        - High risk
        - RIBA*
          - Negative
            - Consider treatment for HCV infection
          - Positive
            - Previous infection and clearance
      - Low risk
        - No further workup

Diagnostic Tests for HCV: Liver biopsy

- Provides helpful information on the current status of the liver injury
  - May reveal advanced fibrosis or cirrhosis that necessitates surveillance for hepatocellular carcinoma
- Good predictor of outcome/severity of disease based on degree of fibrosis
  - May affect treatment decisions
- Not required to assess response to therapy
- Repeat biopsy every 4-5 years recommended to follow untreated patients

QUALitative Molecular tests for HCV RNA

- Diagnosis of HCV infection
- Measure success of therapy (sustained virologic response, SVR)
- Target the 5'UTR of the HCV genome
  - 5'UTR is the most conserved region (better sensitivity)
QUANTitative assays for HCV

- Predict efficacy of therapy
- Monitor response to therapy
- Early generations had problems quantifying certain genotypes
- Moderate correlation between different assays
- Use of International units (IU) provides some standardization

Recommendations for quantitative HCV RNA

- Initial determination of baseline viremia
- 4 weeks into treatment to assess rapid virologic response (RVR)
- 12 weeks into treatment to assess early virologic response (EVR)
- After 24/48 weeks of therapy to assess end of treatment response (ETR)
- 24 weeks after completion of therapy to determine sustained virologic response (SVR)

Virologic response to therapy

- RVR (rapid virologic response)
  - HCV RNA undetectable after 4 weeks of therapy
  - HCV G2/G3: may be able to shorten treatment duration to 12–16 weeks
  - HCV G1: may be used as an indicator for both shortened and extended treatment durations
- EVR (early virologic response)
  - A ≥2 log₁₀ reduction in HCV RNA after 12 weeks of therapy (partial EVR) or HCV RNA not detected (complete EVR)
  - Good predictor of sustained viral response (SVR)

Sustained Virologic Response

- Testing 6 months after cessation of treatment
  - Normalization of liver enzyme levels, AND
  - Negative tests for HCV RNA (Sensitivity 50 IU/mL)
- Has been associated with resolution of liver injury, reduction in fibrosis, low likelihood of relapse and improved survival

Lack of SVR

- Breakthrough: Reappearance of HCV RNA while still on therapy
- Relapse: Reappearance of HCV RNA after therapy discontinued
- Non-responder: Failure to clear HCV RNA after 24w of therapy
- Null responder: Failure to decrease HCV RNA by 2 log₁₀ after 24w of therapy
- Partial responder: 2 log₁₀ decrease in HCV RNA but still positive at 24w

Virologic Responses

- Ghany et al., Hepatology 2009
**FDA-approved Quantitative HCV RNA Tests**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Distributor</th>
<th>Technology</th>
<th>Sensitivity (IU/ml)</th>
<th>Linear Range (IU/ml)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative HCV RNA detection assays</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplicor™ HCV Monitor 2.0</td>
<td>Roche Molecular Systems</td>
<td>RT-PCR</td>
<td>680</td>
<td>500,000</td>
<td></td>
</tr>
<tr>
<td>Versant™ HCV RNA 3.0</td>
<td>Siemens Healthcare</td>
<td>ddNTP</td>
<td>615</td>
<td>7.7 million</td>
<td></td>
</tr>
<tr>
<td>Cobas Ampliprep/Cobas AmpliPrep HCV</td>
<td>Roche Molecular Systems</td>
<td>Real-time RT-PCR</td>
<td>43</td>
<td>100,000</td>
<td></td>
</tr>
<tr>
<td>Versant™ HCV 2.0, for use with High Pure System</td>
<td>Siemens Healthcare</td>
<td>Real-time RT-PCR</td>
<td>35</td>
<td>1 million</td>
<td></td>
</tr>
<tr>
<td>Abbott RealTime™ HCV</td>
<td>Abbott Molecular Diagnostics</td>
<td>Real-time RT-PCR</td>
<td>12</td>
<td>150 million</td>
<td></td>
</tr>
</tbody>
</table>

**HCV viral genome (9.5 kb RNA)**

- **Envelope Glycoproteins**: E1, E2
- **Core Protein**: NS5a, NS5b
- **Integral Membrane Protein**: NS4a, NS4b
- **RNA Polymerase**: NS3

![HCV viral genome diagram]

- Shown in numbers below is the percentage nucleotide divergence between different HCV isolates.

**Quantitative HCV RT-PCR Verification**

- Abbott RealTime HCV vs. Roche COBAS AmpliPrep/Cobas TaqMan HCV Test
  - Patient sample comparison
  - Scatter Plot Correlation
    - Correlation Coefficient: 0.9788
    - Excellent correlation was observed between the two real-time RT-PCR assays.

- Patient sample comparison: Below linear range
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**Quantitative HCV RT-PCR Verification**

- Abbott RealTime HCV vs. Roche COBAS AmpliPrep/Cobas TaqMan HCV Test
  - Limit of detection (PI: 12 IU/ml; UNC: 10 IU/ml) is sufficient to eliminate the need for a separate qualitative test.
**LOD/LOQ and New Therapy**

<table>
<thead>
<tr>
<th>Test</th>
<th>Limit of Detection</th>
<th>Limit of Quantification*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HCV m2000 (Automated)</td>
<td>12 IU/mL</td>
<td>12 IU/mL</td>
</tr>
<tr>
<td>Roche TaqMan HCV CAP/CTM v.1 (Automated)</td>
<td>18 IU/mL</td>
<td>43 IU/mL</td>
</tr>
<tr>
<td>Roche TaqMan HCV HighPure (Manual)</td>
<td>15 IU/mL</td>
<td>25 IU/mL</td>
</tr>
</tbody>
</table>

*LOQ needed for monitoring new protease inhibitors is 25 IU/mL.

**HCV Genotyping**

**Predictors of better response to anti-viral therapy for HCV**

- Age < 40 years old
- Female gender
- No fibrosis or only portal fibrosis of the liver
- Baseline viral load < 6 x 10^5 copies/mL
- Infection with genotype 2 or 3
  - best predictor of treatment response (70-80% vs. genotype 1 40-50%)

**HCV: genotype distribution**

**Approaches to HCV Genotyping**

- Direct DNA sequencing following RT-PCR

![Diagram](image)

**Commercial Genotyping Assays**

- LiPA (Line probe assay; Bayer)
- Trugene 5'NC (Sequencing assay; Siemens)
- Abbott HCV (Real-time PCR/probe assay)
  - Genotype 1a and 1b detected by NS5b
  - Genotypes 2-6 using 5'UTR
- Most are directed to the 5'UTR (5'NC) which may not give accurate subtype information for GT1
- **ALL** are labeled for Research Use Only
Implications of inability to subtype HCV

- Type-level information largely acceptable for clinical use (subtype not integrated in therapeutic decisions...yet)
- Not suitable for epidemiologic studies or studies of clinical differences between subtypes
- Companies market assays to determine subtype, resulting in reporting of subtype and publication of inaccurate subtype information

Standard of Care Therapy

- Combination of peginterferon (PegIFN) and ribavirin (RBV)
  - administered for either 48 weeks (HCV genotypes 1, 4, 5, and 6) or for 24 weeks (HCV genotypes 2 and 3)
  - SVR is associated with long-term clearance of HCV infection, which is regarded as a virologic "cure"

<table>
<thead>
<tr>
<th>Genotype</th>
<th>24w</th>
<th>48w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26-36%</td>
<td>41-57%</td>
</tr>
<tr>
<td>2 or 3</td>
<td>74-78%</td>
<td>70-83%</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>50-77%</td>
</tr>
</tbody>
</table>

Limitations of IFN-based therapy

- Efficacy (HCV GT 1)

Beyond interferon/ribavirin

- New directly acting antivirals (DAA) or protease inhibitors (PI)
  - Approved for use in combination with peginterferonα-2/ribavirin
  - Improvement in SVR rates for patients with genotype 1

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Boceprevir (VICTRELIS)</th>
<th>Telaprevir (INCIVEK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS3/4A protease inhibitor</td>
<td>NS3 protease inhibitor</td>
<td></td>
</tr>
<tr>
<td>Clinical Trials</td>
<td>SPRING-2: treatment naive responders</td>
<td>ADVANCE: treatment naive responders</td>
</tr>
<tr>
<td></td>
<td>RESPOND-2: previous non-responders</td>
<td>REALIZE: partial responders</td>
</tr>
<tr>
<td></td>
<td>ILLUMINATE: treatment naive</td>
<td>HIKI: non-responders</td>
</tr>
<tr>
<td></td>
<td>REALIZE: partial responders</td>
<td>RISE: non-responders</td>
</tr>
</tbody>
</table>
Phase 3 Clinical Trials in Genotype 1

- **Treatment-Naïve Patients**
  - Boceprevir: SPRINT-2 trial
  - Telaprevir: ADVANCE trial

- **Patients with prior non response or relapse**
  - Boceprevir: RESPOND-2 trial
  - Telaprevir: REALIZE trial

**Boceprevir**

- 79% (285/363) of treatment-naïve patients achieved SVR vs 46% (166/361) with pegIFN-RBV alone
- Treatment-naïve patients with cirrhosis who have eRVR (extended RVR = HCV ND at 4 and 12w) may benefit from a total treatment duration of 48 weeks

**Telaprevir**

- 86% (246/286) of prior relapsers achieved SVR vs 22% (15/68) with pegIFN-RBV alone
- 59% (57/97) of prior partial responders achieved SVR vs 15% (4/27) with pegIFN-RBV alone
- 32% (47/147) of prior null responders achieved SVR vs 5% (2/37) with pegIFN-RBV alone

**Boceprevir**

- Indicated for Genotype 1 patients only in combination with peginterferon α-2a + ribavirin
- 4 week RVR lead-in with dual therapy followed by triple therapy
- Treatment naïve patients can finish therapy in 24-48 weeks
- Previous partial responders/relapsers can finish in 36-48 weeks
- Treatment can be stopped at 12 or 24 weeks due to futility
- Recommended HCV RNA quantification* at baseline and weeks 4, 8, 12, 24, end of therapy and 24 weeks post-therapy

*LLQ ≥25 IU/ml

**Telaprevir**

- Indicated for Genotype 1 patients only in combination with peginterferon α-2a + ribavirin
- No lead-in – patients begin triple therapy immediately for 12 weeks
- Treatment naïve patients and relapsers can effectively finish therapy in 24-48 weeks
- Previous partial responders/null responders can effectively finish in 48 weeks
- Treatment can be stopped at 4, 12 or 24 weeks due to futility
- Recommended HCV RNA quantification at baseline and weeks 4, 12, 24, end of therapy and 24 weeks post-therapy
**Viral load monitoring: boceprevir**

<table>
<thead>
<tr>
<th>Treatment Week</th>
<th>Clinical Rationale</th>
<th>Clinical Decision</th>
</tr>
</thead>
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<tr>
<td>Baseline</td>
<td>Predictor of response</td>
<td>SVR higher when ≥ 800,000 IU/ml</td>
</tr>
<tr>
<td>4</td>
<td>Indicates treatment length</td>
<td>Undetectable: 28-36 week treatment, Detectable: 48 week regimen</td>
</tr>
<tr>
<td>12</td>
<td>Indicates treatment futility</td>
<td>Undetectable: if ND at 12 weeks: 12w triple + 12w dual (24w), Detectable: &gt;100 IU/ml: futility, d/c</td>
</tr>
<tr>
<td>End of treatment</td>
<td>SVR assessment</td>
<td>Undetectable: assess SVR at 24 weeks post-treatment, Detectable: partial response</td>
</tr>
<tr>
<td>24 week post-treatment</td>
<td>SVR assessment</td>
<td>&lt;25 IU/ml: SVR achieved, &gt;25 IU/ml: relapse, SVR not achieved</td>
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**Viral load monitoring: telaprevir**

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</tr>
<tr>
<td>12</td>
<td>Indicates treatment futility</td>
<td>Undetectable: if ND at 4 weeks: &gt;100 IU/ml: futility, d/c</td>
</tr>
<tr>
<td>24</td>
<td>Indicates EOT response or futility</td>
<td>Undetectable: if ND at 4 weeks: +12w dual (24w), Detectable: treatment futility, d/c</td>
</tr>
<tr>
<td>End of treatment</td>
<td>SVR assessment</td>
<td>Undetectable: assess SVR at 24 weeks post-treatment, Detectable: partial response</td>
</tr>
<tr>
<td>24 week post-treatment</td>
<td>SVR assessment</td>
<td>&lt;25 IU/ml: SVR achieved, &gt;25 IU/ml: relapse, SVR not achieved</td>
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</tbody>
</table>

**Protease Inhibitor Resistance**

- Mutations that confer either high or low level resistance to boceprevir and telaprevir cluster around the catalytic site of the NS3/4A serine protease.
  - Rapidly selected with monotherapy
  - ~12-16% during combination therapy of treatment naive patients for both drugs
- 53% (295/543) from SPRINT-2 and RESPOND-2 (boceprevir) trials who did not achieve SVR
  - more often among black patients and poor interferon responders
- No (or low dose) RBV arms increased resistance (40-45%)
- HCV subtype matters (1a vs. 1b) – more common with subtype 1a

**Boceprevir and Telaprevir Genotypic Resistance**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Telaprevir</th>
<th>Boceprevir</th>
</tr>
</thead>
<tbody>
<tr>
<td>V36A/M</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T54S/A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V55A</td>
<td>In vitro</td>
<td>+</td>
</tr>
<tr>
<td>Q80R/K</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R155K/T/Q</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A156S</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A156T/V</td>
<td>+</td>
<td>In vitro</td>
</tr>
<tr>
<td>D168A/V/I/H</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V170A/T</td>
<td>In vitro</td>
<td>+</td>
</tr>
</tbody>
</table>

**Treatment Emergent NS3 Protease Domain Mutations in Boceprevir-Treated Patients who Did Not Achieve SVR**

<table>
<thead>
<tr>
<th>Subjects with HCV Genotype 1a</th>
<th>Subjects with HCV Genotype 1b</th>
</tr>
</thead>
</table>

*Data taken from SPRINT-2 and RESPOND-2 Trials*

**IL28B polymorphism testing**

- Interleukin 28 (interferon γ3) on chromosome 19
- More likely to achieve SVR with PegIFN and RBV depending on the nucleotide sequence
  - Polymorphism of C/T allele at position rs12979860: CC genotype is found more than twice as frequently in persons who have spontaneously cleared HCV infection than in those who had progressed chronic hepatitis
  - Polymorphism of G/T allele at position rs8099917, where T is the favorable genotype
- Predictive value of IL28B genotype testing for SVR is superior to that of the pretreatment HCV RNA level, fibrosis stage, age, and sex, and is higher for HCV genotype 1 than for genotypes 2 and 3.
Predicting IFN response: IL28B

- Nefdhal N. Hepatol 2011

**IL28B polymorphism testing**

- Consideration should be given to ordering the test when it is likely to influence either the physician’s or patient’s decision to initiate therapy.
- There are insufficient data to determine whether IL28B testing can be used to recommend selection of SOC over a PI-based regimen with a favorable genotype (CC) and in deciding upon the duration of therapy with either regimen.

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**Summary: Major Recent Developments**

- OraQuick HCV Rapid Antibody Test
- Quantitative tests have become more automated, more sensitive and more reproducible
  - Eliminate the practical need for qualitative tests?
- HCV genotyping to include GT1 subtypes are limited
  - Need FDA-approved and accurate platforms
- New DAA/PI therapies increase SVR percentages in both treatment naïve patients and previous non/null responders
  - New quantitative measurement guidance
  - Role of resistance detection?
- IL28B polymorphism testing for GT1 is an excellent predictor of SVR
  - Up to 50% of T/T patients still respond- how does IL28B affect decision to treat?