Laboratory and Clinical Diagnosis of HCV Infection

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I

Nonspecific Liver Tests
Biological Markers of Liver Disease

- Serum ALT and AST activity:
  - Not specific,
  - No prognostic value,
  - Marker of the response to antiviral therapy.

- Serum bilirubin and alkaline phosphatase activity:
  - Marker of associated cholestasis.
  - No prognostic value.

- Serum $\gamma$-glutamyl transpeptidase activity:
  - Not specific.
Liver Biopsy Examination

- **Necro-inflammatory activity**:  
  - Reflects the degree of necrosis and inflammation,  
  - Important predictor of liver disease outcome,  
  - Important to assess the indication for antiviral therapy in certain instances.

- **Fibrosis**:  
  - Major prognostic significance.
Screening for Hepatocellular Carcinoma

- $\alpha$-fetoprotein levels.

- Regular ultrasonographic evaluations (every 6 months?).

- Confirmation:
  - Computerized Tomography (CT),
  - Spiral CT,
  - Magnetic Resonance Imaging,
  - Lipiodol CT,
  - Hepatic angiography.
II

HCV Virological Tests
Anti-HCV Antibody Detection

- **ELISA tests**:
  - Very sensitive in immunocompetent patients,
  - Sensitive in hemodialysis and immunodepressed patients (except profound immunodepression),
  - Specificity: 99%.

- **“Immunoblot“ tests**:
  - No utility in the diagnostic context.
Qualitative HCV RNA Detection

- “Polymerase chain reaction“ (PCR)
  - Amplicor HCV v2.0 (Roche),
  - Lower limit of detection: 50 IU/ml.

- “Transcription-mediated amplification“ (TMA)
  - Versant HCV RNA Qualitative Assay (Bayer),
  - Lower limit of detection: 10 IU/ml.
HCV RNA Quantification

- “Polymerase chain reaction“ (PCR)
  - (Cobas) Amplicor HCV Monitor v2.0 (Roche),
  - Range of quantification: 600 to 500,000 IU/ml.

- “branched DNA“ signal amplification
  - Versant HCV RNA Quantitative Assay (Bayer),
  - Range of quantification: 615 to 7,700,000 IU/ml.

- Other tests: SuperQuant (NGI), LCx (Abbott), real-time PCR...
Molecular Determination of HCV Genotype ("Genotyping")

- **Direct sequencing**:
  - Home-made: NS5B, E1 regions,
  - 5' noncoding region (Trugene HCV 5’NC Genotyping Kit, Visible Genetics).

- **Reverse hybridization**:
  - INNO-LiPA HCV II (Innogenetics).

*NB*: Typing errors in 5’ NC: exceptional.
Subtyping errors in 5’ NC: 10%-25%, without clinical incidence.
Serological Determination of HCV Genotype

• Serological determination of HCV genotype ("serotyping"):
  - Identifies the 6 types, but not the subtypes,
  - Interpretable in approximately 90% of patients with chronic hepatitis C,
  - Concordance with molecular tests: 95%.
III

Practical Use of HCV Virological Tests
### Diagnosis of Acute Hepatitis C

<table>
<thead>
<tr>
<th>Anti-HCV Ab</th>
<th>HCV RNA*</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>Not acute hepatitis C</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>Acute hepatitis C</td>
</tr>
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<td>-</td>
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</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Hard to say...</td>
</tr>
</tbody>
</table>

*HCV RNA assay with LLD $\leq$ 50 IU/ml
Diagnosis of Chronic Hepatitis C

- ELISA detection of anti-HCV antibodies,

- Confirmation by positive HCV RNA detection* if ELISA (+),

- HCV RNA detection* if ELISA (-) in hemodialysis and immunodepressed patients.

*HCV RNA assay with LLD ≤ 50 IU/ml
PEG-IFN-α QW + ribavirin QD

Naive Patients (48 weeks)

PEG-IFN-α 2a + ribavirin: 46% Type 1, 42% Types 2/3

PEG-IFN-α 2b + ribavirin: 76% Type 1, 82% Types 2/3

(Manns et al., Lancet 2001;358:958-65; Fried et al., submitted)
Summary

- The outcome of antiviral therapy significantly varies according to the HCV genotype (genotype 1 vs genotypes 2/3).

=> The therapeutic strategy will be different for genotype 1 and genotypes 2/3, respectively.

=> HCV genotype determination must be performed after the diagnosis of chronic hepatitis C is made if antiviral therapy is envisaged.
PEG-IFN-α QW + ribavirin QD
Naive Patients (48 weeks)

(Manns et al., Lancet 2001;358:958-65 ; Fried et al., submitted)
“Sustained Virological Response”

- Persistence of response (RNA -) : 385/395 (97.5%),
- 10 “late” relapses, all before 2 years.

(N = 395)

(McHutchison et al., AASLD 2001)
Summary

• Approximately 80% of patients infected with HCV genotypes 2 and 3 treated with PEG-IFN-α plus ribavirin achieve a sustained virological response.

• The sustained virological response appears to be associated with a definitive cure of infection in the vast majority of cases.

=> It is reasonable to propose antiviral treatment to all of the patients with detectable HCV RNA and an HCV genotype 2 or 3, whatever their liver histology score.
PEG-IFN-α 2a QW + ribavirin QD

Treatment Duration: Genotypes 2/3

- 24 weeks: 78% + 78%
- 48 weeks: 73% + 77%

(Hadziyannis et al., EASL 2002)
Summary

- In the patients infected with HCV genotypes 2 or 3, 48 weeks of treatment do not do better than 24 weeks of treatment.

=> The patients infected with HCV genotypes 2 and 3 must be treated with the combination of PEG-IFN-α plus ribavirin for 24 weeks.
Assessment of Virological Response

Genotypes 2 and 3

PEG-IFN-α QW + ribavirin QD

W0  W24  W48  W72

HCV RNA  HCV RNA  HCV RNA  = Sustained virological response
PEG-IFN-α QW + ribavirin QD

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(Manns et al., Lancet 2001;358:958-65; Fried et al., submitted)
Benefits from Therapy

- **YES**... if moderate to severe necro-inflammatory activity and/or fibrosis.

- **NO**... if “mild” lesions.

*(International Consensus Conference on Hepatitis C, Paris, Février 1999)*
Résumé

- The administration of PEG-IFN-α and ribavirin for 48 weeks allows for a sustained virological response in less than half of the patients infected with HCV genotype 1.

- In this context, the potential benefits of therapy must be evaluated according to baseline liver biopsy.

=> in the patients infected with HCV genotype 1 (and, by extension, 4, 5 or 6):

  - Liver biopsy helps in making a decision to treat,
  - Antiviral treatment must be proposed if the natural prognosis is pejorative.
PEG-IFN-α 2a QW + ribavirin DQD

Treatment Duration: Genotype 1

(Hadziyannis et al., EASL 2002)
Optimal Treatment Duration

- 48 weeks of treatment do significantly better than 24 weeks.

=> The patients with HCV genotype 1 (and 4, 5, 6) infection and an indication for treatment should receive the combination of PEG-IFN-α and ribavirin for 48 weeks.
PEG-IFN-α2a QW + ribavirin QD
All genotypes

Week 12 (N = 453)

- 2 log reduction or HCV RNA (-)
  - YES
    - n = 390 (86%)
      - SVR
        - n = 253 (65%)
      - No SVR
        - n = 137 (35%)
  - NO
    - n = 63 (14%)
      - SVR
        - n = 2 (3%)
      - No SVR
        - n = 61 (97%)

(Fried et al., DDW 2001)
Week 12 (N = 298)

PEG-IFN-α2a QW + ribavirin QD

Genotype 1

2 log reduction or HCV RNA (-)

YES

n = 240 (81%)

SVR

n = 137 (57%)

No SVR

n = 103 (43%)

NO

n = 58 (19%)

SVR

n = 1 (2%)

No SVR

n = 57 (98%)

(Ferenci et al., AASLD 2001)
PEG-IFN-2b QW + ribavirin QD

All genotypes

Week 12 (N = 174)

Yes

2 log reduction or HCV RNA (-)

n = 143 (82%)

SVR

n = 114 (78%)

No SVR

n = 29 (22%)

No

n = 31 (18%)

SVR

n = 0 (0%)

No SVR

n = 31 (100%)

(T. Poynard, personal communication)
Assessment of Virological Response

Genotypes 1 (and 4, 5, 6)

PEG-IFN-α QW + ribavirin QD

W0 | W24 | W48 | W72

HCV RNA

viral load

HCV RNA

HCV RNA
IV

Treatment Algorithm
**CHRONIC HEPATITIS C**

**HCV Genotype determination**

**GENOTYPE 2 OR 3**
- **PEG-IFN-α + ribavirin**
- **24 weeks**

- **HCV RNA detection**
  (sensitive qualitative assay)
  **at the end of treatment and 24 weeks later**

- End-of-treatment virological response
  Sustained virological response

**GENOTYPE 1 (and 4, 5 or 6)**

- **Liver biopsy**
- **Bad prognosis**
- **Good prognosis**

- **Viral load quantification**
  at baseline and week 12

- **> 2 log decrease**
  or HCV RNA (-) at week 12

- **< 2 log decrease**
  at week 12

  - Stop treatment
  - or continue in order to slow evolution of liver disease

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