Assessment of Liver Fibrosis

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Fibrosis staging in chronic HCV infection: Why?

1. Identify persons with cirrhosis (Metavir F4)
   - Screening for hepatocellular carcinoma and gastroesophageal varices
2. Identify persons with significant fibrosis (> Metavir F2)
   - Determine treatment need
   - Identify persons who can safely wait for treatment
3. Identify persons at risk for disease progression
4. Characterize response to therapy

Fibrosis staging in chronic HCV infection: How?

- Proteomics
- Glycomics
- SNPs
- Gene expression

Stellate cell activation

Mild fibrosis (F1)

Significant advanced fibrosis(F2-F3)

Cirrhosis (F4)

Martinez 2011
Liver biopsy: A flawed gold standard

Strengths
- History / dogma
- Established semi-quantitative fibrosis staging systems: Knodell, Scheuer, Metavir, Ishak modified HAI
- Provides simultaneous evaluation of necroinflammation, steatosis, steatohepatitis, iron overload etc.

Weaknesses
- Expensive
- Invasive
- Sampling error – 1/50,000 of the liver
- Adequate size: >15 mm with > 5 portal tracts
- Intraobserver and interobserver variation
- Morbidity – pain in 20%
- Major complications in 0.5%
- Patient reluctance

Serum markers

Direct (class I)
- Intended to detect extracellular matrix (ECM) turnover and/or fibrogenic cell changes

<table>
<thead>
<tr>
<th>Collagen fragments</th>
<th>Cellular adhesion</th>
<th>Glycoproteins</th>
<th>Glycosaminoglycans</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-terminal procollagen peptides VN</td>
<td>Leptin</td>
<td>Fibronectin</td>
<td>Hyaluronic Acid</td>
</tr>
<tr>
<td>Type II collagen</td>
<td>Viscerin</td>
<td>YSL-40</td>
<td>Hyaluronic Acid</td>
</tr>
<tr>
<td>Urine-hydroxyprolyl pyridinoline</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Indirect (class II)
- Reflect functional alterations in liver function
- Do not necessarily reflect ECM turnover or fibrogenic cell changes

<table>
<thead>
<tr>
<th>ALT</th>
<th>Total cholesterol</th>
<th>AST</th>
<th>LDL cholesterol</th>
<th>GGT</th>
<th>Bilirubin</th>
<th>Prothrombin time</th>
<th>Albumin</th>
<th>Platelets</th>
<th>Immunoglobulins</th>
<th>Apolipoproteins</th>
<th>Macroglobulin</th>
<th>Ferritin</th>
<th>C-reactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

Serum markers have been tested in A LOT of combinations!!!

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Panel</th>
<th>Markers in Panel</th>
<th>N</th>
<th>AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>Williams</td>
<td>AAR, AAR</td>
<td>177</td>
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<tr>
<td>1997</td>
<td>Bonacini</td>
<td>CDS index</td>
<td>PLT, AAR, PT</td>
<td>75</td>
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<tr>
<td>2001</td>
<td>Fortunato</td>
<td>PLT, AST, age, sex</td>
<td>302</td>
<td>0.78-0.80</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>Fortunato</td>
<td>Fibrotest</td>
<td>AAR, Hpt, GGT, APoA1, bilirubin</td>
<td>339</td>
<td>0.84-0.87</td>
</tr>
<tr>
<td>2003</td>
<td>Kaul</td>
<td>PLT, AST, age, sex, spider nevi</td>
<td>264</td>
<td>N/A</td>
<td></td>
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<tr>
<td>2004</td>
<td>Kaul</td>
<td>FIB4</td>
<td>PLT, AST, age, sex</td>
<td>270</td>
<td>0.80-0.88</td>
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<tr>
<td>2005</td>
<td>Poynard</td>
<td>AP index</td>
<td>Age, PLT</td>
<td>620</td>
<td>0.76-0.80</td>
</tr>
<tr>
<td>2006</td>
<td>Poynard</td>
<td>ELF-score</td>
<td>Age, AST, Hb, ferritin</td>
<td>130</td>
<td>0.87-0.80</td>
</tr>
<tr>
<td>2007</td>
<td>Poynard</td>
<td>FIB4</td>
<td>Age, AST, Hb, ferritin</td>
<td>130</td>
<td>0.87-0.80</td>
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<td>2008</td>
<td>Poynard</td>
<td>FIB4</td>
<td>Age, AST, Hb, ferritin</td>
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<td>0.87-0.80</td>
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<tr>
<td>2009</td>
<td>Poynard</td>
<td>FIB4</td>
<td>Age, AST, Hb, ferritin</td>
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<td>0.87-0.80</td>
</tr>
<tr>
<td>2010</td>
<td>Poynard</td>
<td>FIB4</td>
<td>Age, AST, Hb, ferritin</td>
<td>130</td>
<td>0.87-0.80</td>
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<tr>
<td>2011</td>
<td>Poynard</td>
<td>FIB4</td>
<td>Age, AST, Hb, ferritin</td>
<td>130</td>
<td>0.87-0.80</td>
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Froehlich 1993; Perrault 1978; Bedossa 2003; Holund 1980
Performance of select serum marker panels to assess liver fibrosis/cirrhosis in chronic HCV

<table>
<thead>
<tr>
<th>Class</th>
<th>Score</th>
<th>Markers</th>
<th>N</th>
<th>% &gt; F2</th>
<th>AUC &gt; F2</th>
<th>% F4</th>
<th>AUC F4</th>
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</thead>
<tbody>
<tr>
<td>II</td>
<td>FibroTest</td>
<td>GGT, haptoglobin, bilirubin, apolipoprotein A1, alpha-2-macroglobulin, AST, platelets</td>
<td>2342</td>
<td>33-74</td>
<td>0.74-0.89</td>
<td>15-20</td>
<td>0.76-0.87</td>
</tr>
<tr>
<td>II</td>
<td>APRI</td>
<td>Age, AST, platelets</td>
<td>3160</td>
<td>27-74</td>
<td>0.69-0.88</td>
<td>3-25</td>
<td>0.61-0.94</td>
</tr>
<tr>
<td>II</td>
<td>FIB-4</td>
<td>Age, AST, platelets</td>
<td>1778</td>
<td>21-36</td>
<td>0.74-0.85</td>
<td>7</td>
<td>0.91</td>
</tr>
<tr>
<td>II</td>
<td>Forns</td>
<td>Age, AST, platelets</td>
<td>1982</td>
<td>32-59</td>
<td>0.75-0.91</td>
<td>3-20</td>
<td>-</td>
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<tr>
<td>I/II</td>
<td>ELF</td>
<td>Terminal peptide of collagen type III, TIMP-1, TIMP-2, age</td>
<td>1146</td>
<td>27-64</td>
<td>0.77-0.87</td>
<td>12-16</td>
<td>0.87-0.90</td>
</tr>
<tr>
<td>I/II</td>
<td>Hepascore</td>
<td>Age, AST, platelets, prothrombin time, macroglobulin, AST, hyaluronate, age, sex</td>
<td>1660</td>
<td>39-79</td>
<td>0.74-0.86</td>
<td>6-34</td>
<td>0.80-0.94</td>
</tr>
<tr>
<td>I/II</td>
<td>Fibrometer</td>
<td>Platelets, prothrombin time, macroglobulin, AST, hyaluronate, age, sex</td>
<td>1039</td>
<td>41-56</td>
<td>0.78-0.89</td>
<td>4-15</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*13 biomarkers with at least two published validations in patients with chronic HCV (5 patented)
*ELF, Fibrotest, Fibrometer and Hepascore are patented

Martinez 2011; Shaheen 2007; Poynard 2007; Manning 2008

APRI vs. FibroTest

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<tr>
<th>Class</th>
<th>Score</th>
<th>Markers</th>
<th>N</th>
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APRI
- Ease of performance/cost: Routinely performed, inexpensive markers
- Liver specific: Platelet count reflects other injury (e.g., HIV)
- External validation: Lower accuracy in validation studies than original report

FibroTest
- Primary strengths:
  - Exclusion of fibrosis in low/average prevalence settings
  - Exclusion of cirrhosis in high prevalence settings
- Ability to distinguish between different stages of fibrosis: Better than APRI but still has lower diagnostic value to discriminate between two adjacent stages

Serum markers of fibrosis: Are we there yet?

**Strengths**
- Easy to handle
- Highly reproducible

**Limitations**
- Direct markers are not generally available in hospital laboratories
- Some are non-specific (e.g., affected by renal function, HIV)
- Difficulty in distinguishing between mild/moderate disease
- Correlation with clinical outcomes?
- Intraindividual changes not examined

Martinez 2008; Parker 2006
Transient elastography (Fibroscan)

- Liver stiffness measured by ultrasound (Fibroscan®, Echosens, France)
- Evaluates the velocity of propagation of a shock wave within liver tissue
- Velocity of wave relates to tissue stiffness
  - Harder tissue → faster propagation
  - Normal liver is viscous: not favorable to wave propagation
  - Fibrosis hardens tissue: favors more rapid propagation

Denzer 2009

<table>
<thead>
<tr>
<th>Class</th>
<th>Score</th>
<th>Method</th>
<th>N</th>
<th>% ≥ F2</th>
<th>AUC ≥ F2</th>
<th>% F4</th>
<th>AUC F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fibroscan</td>
<td>Transient elastography</td>
<td>2052</td>
<td>37</td>
<td>0.72-0.91</td>
<td>8.25</td>
<td>0.87-0.98</td>
</tr>
</tbody>
</table>

Strengths
- Ease of use: quick, noninvasive, inexpensive, high patient acceptance, reproducibility
- Reduced sampling error (volume assessed is 100x the liver biopsy)
- Wide range of values for cirrhosis

Limitations
- Unreliable in morbid obesity
- Acute inflammation, cholestasis and steatosis have been shown to elevate liver stiffness values
- Lack of standardized cutpoints
- Recent meta-analysis of 50 studies (23 HCV-infected)
  - Cutpoints of 4.5 – 11.2 for ≥ F2 fibrosis and 10.1-19 for cirrhosis

Martinez 2011; Friedrich-Rust 2008

Non-invasive diagnosis: Where do we stand?

- **AASLD practice guidelines (2009)**
  - A liver biopsy should be considered in patients with chronic HCV if the patient and health care provider wish information regarding fibrosis stage for prognostic purposes or to make a decision regarding treatment
  - Currently available noninvasive tests may be useful in defining the presence or absence of advanced fibrosis in persons with chronic hepatitis C infection, but should not replace the liver biopsy in routine clinical practice

- **EASL practice guidelines (2011)**
  - Liver biopsy is still regarded as the reference method to assess the grade of inflammation and the stage of fibrosis
  - Transient elastography (TE) can be used to assess liver fibrosis in patients with chronic hepatitis C
  - Non-invasive serum markers can be recommended for the detection of significant fibrosis (METAVIR F2-F4)
  - The combination of blood tests or the combination of TE and a blood test improve accuracy and reduce the necessity of using liver biopsy to resolve uncertainty

How do we move forward?

1. Recognize that liver biopsy is not an ideal gold standard

   - No more new surrogates!!
   - Focus on external validation of existing surrogates
   - Validate existing surrogates against clinical outcomes

Liver biopsy is not an ideal gold standard

- Prevalence of significant fibrosis: 40%
- Sensitivity/specificity of biopsy: 90%
- True accuracy of surrogate: 99%
- Estimated accuracy of surrogate: 90%

Because of the limitations of the biopsy, a perfect surrogate cannot be distinguished from a less valid surrogate. We may already have the perfect surrogate!

Mehta SH et al, J Hepatology 2009
Validation against clinical outcomes (survival)

Liver stiffness and FibroTest performed better than the liver biopsy in predicting survival

Vergnia, Gastroenterology 2011

How do we move forward?

1. Recognize that liver biopsy is not an ideal gold standard
   - No more new surrogates!!
   - Focus on external validation of existing surrogates
   - Validate existing surrogates against clinical outcomes
   - Incorporate metrics of calibration (assesses the ability of the marker to distinguish between different fibrosis stages)

Are we using the right metrics for validation?

- Most validation has been based on discrimination statistics
  - Test most commonly used (AUC) implies a dichotomous comparison
    - Significant vs. non-significant fibrosis
    - Cirrhosis vs. no cirrhosis
- Calibration is also important
  - Comparison of predicted stage vs. actual stage
- Recalibration is potentially more important
  - Use when a score systematically over or underestimates risk when applied to a new population
  - Account for differences in mean values of markers in different populations

Leroy J Hepatology 2007
How do we move forward?

1. **Recognize that liver biopsy is not an ideal gold standard**
   - No more new surrogates!!
   - Focus on external validation of existing surrogates
   - Validate existing surrogates against *clinical* outcomes
   - Incorporate metrics of calibration (assesses the ability of the marker to distinguish between different fibrosis stages)

2. **Assess reliability of the measurements**

---

**Multiple measurements may improve accuracy**

- 591 persons with four semi-annual LSM assessments
- LSM were stable in the majority
- A single measurement \( \geq 12.3 \) kPa appears valid for identifying cirrhosis
- Requirement of two measurements \(<8\)kPa significantly improves accuracy for ruling out significant fibrosis

---

How do we move forward?

1. **Recognize that liver biopsy is not an ideal gold standard**
   - No more new surrogates!!
   - Focus on external validation of existing surrogates
   - Validate existing surrogates against *clinical* outcomes
   - Incorporate metrics of calibration (assesses the ability of the marker to distinguish between different fibrosis stages)

2. **Assess reliability of the measurements**

3. **Consider combination algorithms**
Combination algorithms improve accuracy (vs. biopsy)

- Reduction in need for biopsy:
  - FIBROSIS: Castera: 72%; SAFE: 48%
  - CIRRHOSIS: Castera: 78%; SAFE: 74%

SAFE biopsy (Sebastiani) Castera algorithm (Castera)

Combination algorithms improve accuracy (vs clinical outcomes)

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM</td>
<td>3.1</td>
<td>1.5-6.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Fibro Test</td>
<td>55</td>
<td>4.1-736</td>
<td>0.003</td>
</tr>
<tr>
<td>Acti Test</td>
<td>0.07</td>
<td>0.01-0.47</td>
<td>0.006</td>
</tr>
<tr>
<td>HCV treatment</td>
<td>0.19</td>
<td>0.09-0.39</td>
<td>0.14</td>
</tr>
<tr>
<td>Age</td>
<td>1.03</td>
<td>0.99-1.06</td>
<td>0.14</td>
</tr>
</tbody>
</table>

AUC of combination 0.91 0.83-0.95

- Also considered as predictors: Liver biopsy, APRI, FIB-4

Conclusions

- Even with the anticipated FDA approval of Boceprevir and Telaprevir, liver disease staging remains important
  - Some may want to wait for interferon-free regimens
  - Not everyone may be ready for treatment
  - Treating everyone is not the reality for the developing world
- Liver biopsy remains the gold standard for staging
- BUT, the tide is changing......
  - Validation against clinical outcomes may improve acceptability of surrogate markers
  - Fibroscan and FibroTest are the most promising candidates
  - The final word will likely involve some combination of surrogates and the biopsy