Identifying the Cirrhotic Patient-Whose Job Is It Now?
Identifying the Cirrhotic Patient—Whose Job Is It Now?

- Gold Standard: Laparoscopy and Liver biopsy
- History and PE
- US
- Blood markers
- Stiffness measurement:
  - Fibroscan
  - ARFI
  - SSI
  - MRE
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Progression of Fibrosis
In chronic viral hepatitis

Asymptomatic process that may take years or decades to fully develop
Continuous with heterogeneous speed in the same liver

Staging of hepatic fibrosis
• assess prognosis (ie risk of disease progression)
• decide the need for therapy
Staging of liver disease: implications

- **Mild Fibrosis**
  - Watchful waiting Strategy (in HIV ?)

- **Significant fibrosis**
  - Treatment

- **Advanced fibrosis**
  - Screening x OV and HCC

- **Stages**:
  - F0
  - F1
  - F2
  - F3
  - F4

- Categorizations:
  - Cirrhosis with Portal Hypertension
  - Decompensated Cirrhosis
Laparoscopy
Proportion of concordant and discordant results between 3 pathologists; n = 234

![Bar chart showing the proportion of concordant and discordant results for different Central Metavir Biopsy Scores (F0, F1, F2, F3, F4). The chart displays the percentage of concordant and discordant results across each score level.]
<table>
<thead>
<tr>
<th>Factors affecting liver tissue morphologic assessment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy site selection in liver tissues</td>
<td>It can be completely non-representative site, such as subcapsular</td>
</tr>
<tr>
<td>Selection of certain section out of the whole sample</td>
<td>If the whole sample is used, then the error theoretically is not possible</td>
</tr>
<tr>
<td>Quality of biopsy specimen sections or microtomy</td>
<td>Thick or disrupted tissue sections can hinder the pathology from the observer. The thickness should not exceed 3-4 micrometres.</td>
</tr>
<tr>
<td>Number of viewable visual fields</td>
<td>Inaccuracies can occur if only some separate visual fields are examined</td>
</tr>
</tbody>
</table>
Liver Biopsy in Cirrhotic Patients

Kenneth E. Sherman, M.D., Ph.D., Zachary D. Goodman, M.D., Ph.D., Sara T. Sullivan, M.D., and Sima Faris-Young, M.D., for the GILF Study Group

University of Cincinnati College of Medicine, Division of Digestive Diseases, Cincinnati, Ohio; The Armed Forces Institute of Pathology, Washington, DC; and Internune, San Francisco, California

Figure 1. Presence of fragmentation of liver tissue by the method of biopsy. Striped line indicates the presence of fragmentation, gray line indicates no fragmentation.

Figure 2. Histogram of liver biopsy length.

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Published by Blackwell Publishing
Liver biopsy: the gold standard

- Liver biopsy remains the gold standard for diagnosis of cirrhosis
- High decreeee of concordancy between pathologists
- Technical issue:
  - Sampling site (subcapsula site: oversetimation)
  - Sampling dimension (bigger is better) → 16 gauge needle
  - Disrupted or thick session must be avoided (thickness < 3.4 μm)
  - Automated cutting needles provide superior quality liver biopsies in subjects with cirrhosis.
Identifying the Cirrhotic Patient-Whose Job Is It Now?

- Laparoscopy and Liver Biopsy: gold standard with many limitations
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  - ARFI
  - SSI
  - MRE
Cirrhosis: complications

- **Esophageal Varices**
  - F1 +/- red signs
  - F2 +/- red signs
  - F3 +/- red signs

- **Ascites**
  - Mild
  - Moderate
  - Severe Refractory
  - Complicated
    - Hepatorenal syndrome
    - Spontaneous Bacterial Peritonitis

- **Hepatic Encephalopathy**
  - Grade 1
  - Grade 2
  - Grade 3

- **Hepatic Failure**
  - Low PT
  - Low Albumin
  - High Bilirubin

- **Hepatocellular Carcinoma**
T2 FAT SAT Hyper intense lesion

T1 Hypointense lesion. PV right branch with canalized thrombosis
Nad hypotrophy 2\textsuperscript{nd} segment

T1: no steatossi

Diffusion:
no indirect signs of hypercellularity
Late arterial phase right branch re-canalized with thrombosis of portal branches
Peripheral contrast enhancement typical of NRI
Fig. 1. HIV-associated obliterator portopathy. (a) CT portography of the liver explant of patient one. The image shows a drastic reduction in the caliber of the portal branches (normal caliber of the hepatic branches is close to that of the hepatic veins and never comparable to the diameter of the hepatic arteries of the liver (arrow)); an irregular and lacunal aspect of the portal branches with mural defect filling (the portal branch in square B is to be compared to the portal branch in square A which has a normal aspect); complete interruption of distal portal vein branches (square C) with areas of devascularization of the liver-parenchyma (star). GB, gallbladder; LHV, left hepatic vein; MHV, median hepatic vein; RHV, right hepatic vein. (b) Nodular regenerative hyperplasia of the liver. Nodular architecture in the absence of significant fibrosis. Reticulin argentation (Gomori's method); original magnification 5×. (c) Portal vein obliteration. Parietal thickness of the portal vein reducing the vascular lumen (arrow); Hematein-eosin-safran; original magnification 20×.
Liver Ultrasound Surface Nodularity and diagnosis of cirrhosis

Liver surface appeared as a dotted or irregular line and/or the liver parenchyma was not homogeneous, with areas of different echogenicity, reflecting an underlying nodularity.

Paggi S et al J Hepatology 2008 49: 564-71;
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  - ARFI
  - SSI
  - MRE
Biology of liver injury, inflammation and fibrogenesis

Inciting Injury

Acute phase proteins (↑α2MG, ↓Haptoglobin)

Hepatocyte

Injured Hepatocyte

Recruitment of inflammatory cells

NK cells

T-cells

Cytokines

Kupffer cell

Stellate cell

Injured hepatocytes

Injured endothelial cells

Cell-cell interaction

Activated stellate cells

Stellate cell activation

Rockey D and Bissel M Hepatology 2006
The pathway of Extra Cellular Matrix (ECM) production and degradation

MMP: Matrix Metallo Proteinase
TIMP: Tissue Inhibitor of Metallo Proteinase

Rockey D and Bissel M Hepatology 2006
Direct noninvasive markers of liver fibrosis

Component of Extra Cellular Matrix (ECM)
Regulatory Enzymes of ECM
Acute phase protein

- Single markers
  - Hyaluronic acid
  - Laminin
  - YKL-40
  - Type IV collagen/7S domain
  - (N terminal) Propetide of Procollagen III (PIIIP and PIIINP)
  - MMP-2
  - TIMP-1
  - $\alpha_2$ Macroglobulin ($\alpha_2$M)
  - Haptoglobin

- PANELS
  - Fibrospect II (HA, TIMP-1, $\alpha_2$M)\(^1\)
  - MP3 (PIIINP, MMP-1)\(^2\)
  - ELF (PIIIP, HA, TIMP-1)\(^3\)

Impact of HIV or ART not established

**Proprietary algorithm**

1 Patel K et al J Hepatol 2004
2 Leroy V et al Am J Gastroenterol 2004
3 Rosenberg WM et al Gastroenterol 2004
<table>
<thead>
<tr>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST/ALT ratio</td>
<td>PLT count</td>
<td>γGT</td>
<td>Cholesterol or N glycans</td>
<td></td>
</tr>
<tr>
<td>INR or ApoA1</td>
<td>Insulin Sensit. (HOMA)</td>
<td>Age</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**INDIRECT MARKERS OF FIBROSIS**
- Cirrhosis with Portal Hypertension
- Decompensated Cirrhosis
Indirect markers of Fibrosis

- **Single parameter**
  - Platelet count $< 150 \times 10^9$/L
  - AST/ALT $> 1$

- **Combined scores**
  - Forns$^1$:
    - Age, plt, $\gamma$GT, cholesterol
  - APRI$^2$:
    - AST, plt
  - Fibroindex$^3$:
    - plt, AST, $\gamma$GT
  - FPI$^4$:
    - AST, cholesterol, past alcohol intake, HOMA, age
  - FIB-4$^5$:
    - plt, AST, ALT, age
  - Bonacini$^6$:
    - ALT, AST, INR, plt
  - Pohl$^7$:
    - ALT, AST, plt
  - AP$^8$:
    - age, plt
  - Glycocirrho test$^9$:
    - profile of serum proteins N-glycans

Affected by:
HIV per se
ART

---

Combined indirect and direct serum markers of hepatic fibrosis

Component of Extra Cellular Matrix (ECM)
Regulatory Enzymes of ECM
Acute phase protein
Indirect markers *

- **Fibrotest**¹:
  - Haptoglobin, α2M, apoA-1, γGT*, bilirubin*, γ-globulin*.

- **SHASTA**²:
  - HA, AST*, albumin

- **Fibrometer**³:
  - Plt*, PT%, AST*, HA, α2M, gender, age

- **Hepascore**⁴:
  - HA, α2M, γGT*, age, gender

* Affected by HIV &/or ART

Proprietary algorithm

### Clinical Studies on Fibrosis markers panels in HIV/HCV

<table>
<thead>
<tr>
<th>Author (yr)</th>
<th>Tests(s)</th>
<th>Adequacy of biopsy</th>
<th>N</th>
<th>On ART</th>
<th>F4</th>
<th>F&lt;2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myers (2003)</td>
<td>Fibrotest</td>
<td>Unclear</td>
<td>130</td>
<td>N/A</td>
<td>13%</td>
<td>55%</td>
</tr>
<tr>
<td>Kelleher (2005)</td>
<td>APRI SHASTA</td>
<td>&gt;10 mm</td>
<td>95</td>
<td>100%</td>
<td>16%</td>
<td>72%</td>
</tr>
<tr>
<td>Al-Mohri (2005)</td>
<td>APRI</td>
<td>Unclear</td>
<td>46</td>
<td>65%</td>
<td>20%</td>
<td>28%</td>
</tr>
<tr>
<td>Nunes (2005)</td>
<td>APRI Forns</td>
<td>Median 15 mm</td>
<td>40</td>
<td>83%</td>
<td>33%</td>
<td>52%</td>
</tr>
<tr>
<td>Macias (2006)</td>
<td>APRI Forns</td>
<td>≥ 15 mm</td>
<td>263</td>
<td>100%</td>
<td>15%</td>
<td>42%</td>
</tr>
<tr>
<td>Trang (2008)</td>
<td>APRI FIB-4</td>
<td>22.5 ± 2.5 mm (M+SD)</td>
<td>81</td>
<td>N/A</td>
<td>23%</td>
<td>38%</td>
</tr>
<tr>
<td>Loko (2008)</td>
<td>APRI Forns FIB-4</td>
<td>81% &gt; 10 mm</td>
<td>200</td>
<td>87%</td>
<td>20%</td>
<td>32%</td>
</tr>
<tr>
<td>Cacoub (2008)</td>
<td>Fibrometer Hepascore Fibrotest FIB-4 SHASTA APRI</td>
<td>18.6 ± 7.9 (M+SD)</td>
<td>272</td>
<td>?</td>
<td>10%</td>
<td>25%</td>
</tr>
</tbody>
</table>
Diagnostic accuracy of Fibrosis markers panel in HIV/HCV: F0F1F3F3 vs F4

* P < 0.05 vs. APRI & Forns
Surrogate Fibrosis markers: NPV and PPV for diagnosis of cirrhosis

Myers 2003
Macias 2006
Loko 2008
Comparison of non-invasive liver fibrosis biomarkers for diagnosis of cirrhosis in 200 HIV/HCV patients

Proportion of patients unclassified or with correct or uncorrect * classification

* p<0.007 vs. Forns’, APRI and FIB-4

Correct = Cirrhosis yes or not

Loko Am J Gastroenterol 2008
Summary of Diagnostic Odds Ratio of fibrosis marker panels for the prediction of cirrhosis in HIV/HCV

DOR = $+LR/-LR$

Reasonable test performance

<table>
<thead>
<tr>
<th>Test</th>
<th>DOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FibroTest (Myers 2003)</td>
<td>66.0 (3.9-1127)</td>
</tr>
<tr>
<td>APRI (Nunes 2005)</td>
<td>19.1 (2.7-133.5)</td>
</tr>
<tr>
<td>APRI (Macias 2006)</td>
<td>24.0 (4.4-131.4)</td>
</tr>
<tr>
<td>Forns' (Nunes 2005)</td>
<td>10.6 (2.2-51.6)</td>
</tr>
<tr>
<td>Summary</td>
<td>11.0 (4.6-26.2)</td>
</tr>
</tbody>
</table>

Shaheen AAM and Myers RP HIV Clin Trials 2008
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- US
- Blood markers
- **Stiffness measurement:**
  - Fibroscan
  - ARFI
  - SSI
  - MRE
FIBROSCAN®

Vibration controlled Transient Elastometry

Vibrating Probe: elastic wave propagates into liver tissue

US probe: measures the displacements induced by the propagation of the wave
FibroScan

The probe induces an elastic wave through the liver.

The velocity of the wave is evaluated in a region located from 2.5 to 6.5 cm below the skin surface.

LB: 1/50,000 of the liver
FibroScan: 1/500 of the liver
Liver Stiffness Measurement by Transient Elastometry (FibroScan®)

- Stiffness in KPa median of 5-10 determination with IQR
- Measurement with IQR > 30% lower validity
- No reference “normal” range
- High intra and interobserver agreement (0.98) between trained physicians or nurses (training=100 tests) \(^1\)
- Feasibility is influenced by BMI and steatosis \(^1\)
- Results could be biased by:
  - The extent of necroinflammatory activity \(^2,3\)
  - Macronodular pattern of cirrhosis and perisinusoidal fibrosis \(^4\)
- Reflects the elevation of portal pressure: correlation with LSM if HVPG < 10 mmHg \(^4\)

Relationship between Fibroscan and liver fibrotic area

Kawamoto, 2006

30 liver resection specimens

Fibrotic area stained blue with Azan-Mallory
Performance of Transient Elastometry in HIV+

NPV: Negative Predictive value %; PPV: positive predictive value%; PCC: patients correctly classified

<table>
<thead>
<tr>
<th>Reference</th>
<th>Test</th>
<th>N</th>
<th>%</th>
<th>AUC</th>
<th>%</th>
<th>AUC</th>
<th>%</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanchez-Conde et al. [13*]</td>
<td>TE</td>
<td>100</td>
<td>0.80</td>
<td></td>
<td></td>
<td>0.93</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>APRI</td>
<td>100</td>
<td>43</td>
<td></td>
<td></td>
<td>0.77</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FIB-4</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Forns</td>
<td>97</td>
<td></td>
<td></td>
<td></td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HGM-2</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degos et al. [28*]</td>
<td>TE</td>
<td>110</td>
<td>60</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
</tr>
</tbody>
</table>

TE, transient elastography.
Comparison of non-invasive liver fibrosis biomarkers and Transient Elastometry

Proportion of patients unclassified or with correct or uncorrect * classification

* p<0.007 vs. Forns’, APRI and FIB-4

Cacoub P et al J Hepatol 2008
Macias et al J Hepatol 2008 in press

Correct = difference < 2 stages from METAVIR classification
Transient elastography in chronic viral hepatitis: a critical appraisal

Ana-Carolina Cardoso,¹ Roberto J Carvalho-Filho,² Patrick Marcellin¹

**Table 1** Proposed indications for the use of TE in patients with compensated chronic hepatitis C and B

<table>
<thead>
<tr>
<th>Purposes</th>
<th>Indications</th>
</tr>
</thead>
</table>
| Baseline assessment and therapeutic decisions* | Alternative to liver biopsy for subjects with contraindications to the procedure or for patients who refuse to be biopsied. Alternative to liver biopsy for patients without conditions with potential impact on the accuracy of TE measurement and/or on the outcome of viral infection (alcohol abuse, overweight, insulin resistance, HIV infection) if:  
(a) TE ≥ 14.6 kPa in HCV patients†; or  
(b) TE ≥ 12.0 kPa in HBV patients with normal ALT‡. |
| Follow-up§                              | Annual TE measurements for patients without indication for antiviral therapy. Consider earlier liver biopsy in case of unexplained TE elevation.  
Annual TE measurements for all patients during (HBV) or after (HBV or HCV) antiviral therapy. Consider earlier liver biopsy in case of unexplained TE elevation. |

*Appropriate screening for oesophageal varices and hepatocellular carcinoma is recommended.  
†The impact of ALT levels on the accuracy of TE in HCV infection remains to be defined.  
‡Increased ALT levels seem to increase TE values.  
§After baseline assessment of liver fibrosis with liver biopsy and TE measurement.  
HBV, hepatitis B virus; HCV, hepatitis C virus; TE, transient elastography.
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- Blood markers:
  - Non patented: poor sensitivity
  - Patented: many limitations
- Stiffness measurements:
  - Stiffness is not only fibrosis
  - Several pros few cons
## Diagnosis of Cirrhosis

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Liver Biopsy</th>
<th>Blood Tests</th>
<th>Transient Elastometry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not Patented</td>
<td>Patented</td>
<td>Fibroscan</td>
</tr>
<tr>
<td></td>
<td>Direct 1/50,000 of the liver</td>
<td>Indirect and Global</td>
<td>Indirect 1/500 of the liver</td>
</tr>
<tr>
<td></td>
<td>Indirect and Global</td>
<td>Indirect and global</td>
<td>1/1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MRE: Indirect global</td>
</tr>
</tbody>
</table>

**ART: COMBINATION OF DRUGS**

**FIBROSIS ASSESSMENT: COMBINATIONS OF TESTS**

<table>
<thead>
<tr>
<th>Cost</th>
<th>High</th>
<th>Low</th>
<th>High</th>
<th>Medium</th>
<th>Low</th>
<th>Very High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediacy</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>Limitations</td>
<td>Poorly Accepted Technical issues</td>
<td>Low PPV High n. unclassified &lt;5% (Gilbert, Haemolysis, Inflamm.)</td>
<td>&lt;20% (steatosis, obesity,) dedicated device, false positive: congestion, inflammation, cholestasis</td>
<td>Limited volume Narrow range of values Frequency not predet. Absence of control</td>
<td>Time consuming Siderosis</td>
<td></td>
</tr>
</tbody>
</table>
Staging of Liver disease: the pieces of the puzzle
Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C

Saved biopsies 51%
PPV 91.3
NPV 100
Accuracy 94%
+ LR 6.17
-LR 0
Under diagnosed 0
Over diagnosed 6

Saved biopsies 56%
PPV 90
NPV 100
Accuracy 94%
+ LR 7.6
-LR 0
Under diagnosed 0
Over diagnosed 5

Saved biopsies 71%
PPV 58
NPV 99
Accuracy 93%
+ LR 12.9
-LR 0.13
Under diagnosed 1
Over diagnosed 6

Sebastiani G et al. J Hepatol 2006
Fig. 4  Suggested diagnostic flow chart for the detection of advanced fibrosis in CHC patients with elevated ALT.

Silvia Paggi, Agostino Colli, Mirella Fraquelli, Mauro Viganò, Paolo Del Poggio, Corinna Facciotto, Massimo ...

A non-invasive algorithm accurately predicts advanced fibrosis in hepatitis C: A comparison using histology with internal/external validation

Journal of Hepatology Volume 49, Issue 4 2008 564 - 571

http://dx.doi.org/10.1016/j.jhep.2008.07.007
Combining FibroScan and Fibrotest in HCV patients

<table>
<thead>
<tr>
<th>AUROC</th>
<th>F≥2</th>
<th>F≥3</th>
<th>F=4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FibroScan</strong></td>
<td>0.83</td>
<td>0.90</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>(0.76-0.88)</td>
<td>(0.85-0.94)</td>
<td>(0.91-0.98)</td>
</tr>
<tr>
<td><strong>Fibrotest</strong></td>
<td>0.85</td>
<td>0.90</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>(0.78-0.90)</td>
<td>(0.85-0.94)</td>
<td>(0.81-0.91)</td>
</tr>
<tr>
<td><strong>FibroScan + Fibrotest</strong></td>
<td>0.88</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>(0.82-0.92)</td>
<td>(0.91-0.97)</td>
<td>(0.91-0.97)</td>
</tr>
</tbody>
</table>

Clinical History
Physiscal examination
US → LSN
Blood tests including: PLT AST ALT
(APRI and FIB-4)

Agreement

Disagreement or gray zones

Liver stiffness evaluation

Fibrotest or Fibrospect

Agreement with other non invasive markers &
No conditions with potential impact on stiffness evaluation
Or markers algorithm performance

Cirrhosis

No Cirrhosis

Disagreement or Gray zone

Liver Biopsy with 16G automatic cutting needle

OV screening (ev. 2-3 yrs)
US HCC screening 6 monthly
HVPG measure ? → liver & spleen stiffness evaluation

No Cirrhosis