Fatty liver-to biopsy? CON

Ran Oren, MD
Dept Gastroenterology and Liver Disease
Hadassah University Hospital
Jerusalem, Israel
Disclosure

• Redhill biopharma Ltd-Scientific Advisory Board

• JetPrep Ltd- Founder/Consultant

• Galmed pharmaceuticals- Scientific Advisory Board

• Microtech Medical Technologies Ltd- Consultant

• Hadasit – Scientific Advisory Board

• I am against liver biopsy for patients with NAFLD
Relevant issues

• Should we perform liver biopsy at all?

• The advantages/ disadvantages of liver biopsy

• NAFLD vs NASH

• Are there alternatives to liver biopsy?

• Guidelines recommendations

• Conclusions
0-1999
DON'T WORRY
IT IS JUST
FATTY LIVER

1999-
DO WORRY-
FATTY LIVER
DISEASE

2004-
MOREOVER-
THE
METABOLIC
SYNDROME

2006-
CARDIO-
VASCULAR
DISEASE

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Liver Biopsy for patients with NAFLD

• Is it the gold standard for the diagnosis?

• Who should undergo biopsy?

• When should we biopsy?

• Can we biopsy everybody?
Figure 2. Paired liver biopsy specimens from a single patient (A and C are from the first biopsy; B and D are from the second biopsy). (A) Bland macrovesicular steatosis with no hepatocyte ballooning. (B) Macrovesicular steatosis with moderate/obvious hepatocyte ballooning. (C) Focal perisinusoidal fibrosis and focal periportal fibrosis without bridging fibrosis. (D) Bridging fibrosis (arrow). (A and B: hematoxylin-eosin saffron staining, magnification ×200; C and D: picro-sirius red-hematein staining, magnification ×25.)
Liver Biopsy

Don C. Rockey,1 Stephen H. Caldwell,2 Zachary D. Goodman,3 Rendon C. Nelson,4 and Alastair D. Smith5

This position paper has been approved by the AASLD and represents the position of the association.

College of Cardiology and the American Heart Association Practice Guidelines3).4

Pitfalls of Liver Biopsy—Sampling Error

Although liver biopsy clearly provides important diagnostic and prognostic information and helps define treatment plans, it must be recognized that liver biopsy may be associated with sampling variability. For example, in a study of 124 patients with chronic HCV infection who underwent laparoscopy-guided left and right lobe liver biopsies,196 33% of cases had discordant results by at least one histologic stage (modified Scheuer system). A smaller, but substantial proportion of biopsies were discordant by
Disadvantages of Liver Biopsy

- Invasive
- 10%-20% inter/intra-observer variability
- Samples only 1/50,000 of organ
- Mortality 1-3/10,000
- Morbidity 3/1000
- Pain in 20%-30%
- Contraindications
- Obstacle to treatment
- Expense
  - Direct cost $1500-$2000
  - Indirect costs (time off work, hospital stay)

NonAlcoholic Fatty Liver Disease

- Steatosis (NAFL)
- Steatohepatitis (NASH)

Complications after 2,084 Liver Biopsies

- Moderate pain 20%
- Severe pain requiring IV analgesia, narcotics 3%
- Vasovagal episode 2%
- Severe complications 0.57%
  - Hemoperitoneum in (1)
  - Bile peritonitis (3)
  - Pneumothorax (1)
  - Punctured viscera (3)
- Death 0%
Need for Alternative Fibrosis Markers

- Biopsy determines static mass of fibrosis
- Indirect marker of liver injury
- May not be reflective of a drug-induced change in ECM remodelling
- Ideal test
  - Detect dynamic changes
  - Specific for liver disease
  - Able to detect small changes widely applicable to multiple liver diseases
  - Reproducible

NASH and Cirrhosis
Non-invasive markers of liver injury

- Fibroscan- elastometry
- MRI
- BioMarkers
- FibroTest-ActiTes-FibroMax
  - Other markers published
    - Hyaluronic acid
    - SpectroTest: HA, A2M, TIMP1
    - GlycoCirrhotest
    - APRI: AST, platelets
    - Forns: Age, GGT, Platelets
    - Rosenberg: HA, PIIIP, TIMP1
    - Leroy: TIMP1, MMP2
    - FPI: Age, CT, AST, Insulin, OH
    - Laine: HA, CarbohydrateDeficT, Transferrin
    - AP: Age-platelets
Correlation with histological parameters

- **Univariate** (Kendall coefficient)
  
<table>
<thead>
<tr>
<th></th>
<th>Fibrosis</th>
<th>Activity</th>
<th>Steatosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stiffness</td>
<td>$r$=0.55</td>
<td>$p&lt;0.0001$</td>
<td>$r=0.21$</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>$r=$ ---</td>
<td></td>
<td>$r=0.36$</td>
</tr>
</tbody>
</table>

- **Multivariate**
  Only fibrosis is significantly correlated to stiffness.

*ZioI et al. Hepatology 2005*
Reproducibility (1)

- Patients
  15 patients without ascites and fibrosis stages ranging from F0 to F4

- Results
  ✓ 3 examinations with the same operator:
    Mean coefficient of variation: 3.2%
    → liver stiffness measurement is reproducible
  ✓ 3 examinations with three different operators:
    Mean coefficient of variation: 3.3 %
    → changing the operator does not add variability to liver stiffness measurements

Sandrin et al. Ultrasound in Medicine and Biology 2003
NASH (1)

129 patients with biopsy proven NASH or cryptogenic cirrhosis with risk factors of obesity and diabetes

- **prediction of significant fibrosis (modified Brunt score ≥ stage 2)**
  - Logistic regression
    - Age, diabetes, AST/ALT ratio and stiffness
  - Stepwise multivariate analysis
    - Stiffness (OR 1.15, p = 0.002) and age (OR 1.05, p 0.03)

- **ROC analysis**
  - Cut-off of 10 kPa
    - Sensibility 88% and specificity 72%

De Ledinghen et al. EASL 2006
27 patients with NAFLD and NASH (Brunt classification)

Takeda et al. AASLD 2006
Discussion

- **Good correlation** between stiffness and fibrosis stage in patients with chronic liver diseases of various etiology: HCV, HBV, ASH, NASH, biliary disease.

- **Very accurate** for the diagnosis of severe fibrosis (F3) and cirrhoses (F4) especially in patient with excessive alcohol intake and normal biological parameters.

- For the diagnosis of patients with **significant fibrosis** (F2), accuracy is **equivalent** to those of the **best blood markers**.

- **Stiffness** appears closely correlated with HVPG measurements.

- Within **cirrhotic patients**, liver stiffness is related to the risk of developing complications such as grade II or III oesophageal varices, Child B or C, ascites, carcinoma or varices bleeding.

- Liver stiffness is helpful to follow **HCV patients under treatment** and to detect patient with **drug induced liver fibrosis**.
Biochemical Markers
FibroTest  ActiTest+ age & gender=Fibromax

• Serologic markers based algorithm
• Assess the degree of fibrosis and necro-inflammatory histological activity & steatosis

In Situ
Liver Injury

Scar Matrix-Activated Stellate Cells

In Serum: 5-Marker Panel

Alpha2Macroglobulin
Total Bilirubin
Gamma GT
Apolipoprotein A1
Haptoglobin

FibroTest  ActiTest

FibroTest

Score: 0.42
(F1-F2)

ActiTest

Score: 0.50
(A1-A2)
FibroTest: from blood donors to cirrhotics (n=1570)
Clin Chem 2004, Comp Hepatol 2004
Recommendations

• Perform fibroscan with fibromax

• Good correlation-accurate

• No correlation- liver biopsy
Liver Biopsy

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Recommendations

1. Liver biopsy should be considered in patients in whom diagnosis is in question, and when knowledge of a specific diagnosis is likely to alter the management plan (Class I, Level B).

2. Liver histology is an important adjunct in the management of patients with known liver disease, particularly in situations where (prognostic) information about fibrosis stage may guide subsequent treatment; the decision to perform liver biopsy in these situations should be closely tied to consideration of the risks and benefits of the procedure (Class I, Level B).
15. Liver biopsy should be considered in patients with suspected NAFLD in whom competing etiologies for hepatic steatosis and co-existing chronic liver diseases cannot be excluded without a liver biopsy.
Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis

June 2012
Prognosis and complications

- Disease progression from NAFLD to NASH to cirrhosis/liver failure and HCC.
- **NAFLD does not exacerbate hepatotoxicity**, and side effects of pharmacologic agents, including HMG-CoA reductase inhibitors, are not more likely to occur.
- NAFLD and coexistent obesity and related metabolic factors may exacerbate other liver diseases—e.g., alcoholic liver disease.
- Concurrence of NAFLD with hepatitis C or human immunodeficiency virus (HIV) worsens their prognoses and decreases their responses to therapy.
- **Hepatitis C, genotype 3**, is commonly associated with hepatic steatosis, which may confuse a diagnosis of hepatitis C vs. NASH vs. both together.
- Liver biopsy may indicate the severity of disease, but only fibrosis, and not inflammation or necrosis, has been confirmed to predict the disease prognosis.
- Histologic progression to end-stage liver disease may occur: NASH + bridging fibrosis or cirrhosis.
- End-stage NASH is an often under-recognized cause of cryptogenic cirrhosis; progressive fibrosis may be obscured by stable or improving steatosis and serologic features, especially in older NASH patients.
- NASH-related (cryptogenic) cirrhosis increases the risk of hepatocellular carcinoma (HCC).
- Causes of mortality in cirrhotic NASH patients:
  - Liver failure
  - Sepsis
  - Variceal hemorrhage
  - HCC
  - Cardiovascular disease
Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease

Stuart McPherson,1 Stephen F Stewart,1 Elsbeth Henderson,1 Alastair D Burt,2 Christopher P Day2


Fatty Liver Clinic from 2003 to 2009. The AST/ALT ratio, AST to platelet ratio index, BARD (weighted sum of BMI > 28 = 1 point, AST/ALT ratio > 0.8 = 2 points, diabetes = 1 point), FIB-4 (age × AST (IU/l)/platelet count (×10⁹/litre) × √ALT (IU/l)) and NAFLD fibrosis scores were calculated from blood tests taken at time of biopsy.

What are the new findings?

► AST/ALT ratio, FIB-4 score, NAFLD fibrosis score and BARD score can reliably exclude advanced fibrosis in patients with NAFLD.

► Using non-invasive scores to exclude advanced fibrosis liver biopsy can be avoided in more than two thirds of patients with NAFLD.
Figure 1  Receiver operating characteristic (ROC) curves for the non-invasive scores for a diagnosis of advanced fibrosis (Kleiner fibrosis stage 3–4). ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; NAFLD, non-alcoholic fatty liver disease.
Non-invasive diagnosis of non-alcoholic fatty liver disease.  
A critical appraisal

Mariana V. Machado, Helena Cortez-Pinto*

Departamento de Gastenterologia, Hospital Santa Maria, CHLN, Unidade de Nutrição e Metabolismo, Faculdade de Medicina de Lisboa, IMM, Lisbon, Portugal

Summary

Non-alcoholic fatty liver disease (NAFLD) affects one in every three subjects in the occidental world. The vast majority will not progress, but a relevant minority will develop liver cirrhosis and its complications. The classical gold standard for diagnosing and staging NAFLD and assessing fibrosis is liver biopsy (LB). However, it has important sample error issues and subjectivity in the interpretation, apart from a small but real risk of complications. The decision to perform an LB is even harder in a condition so prevalent such as NAFLD, in which the probability of finding severe liver injury is low. In an attempt to overcome LB and to subcategorize patients with NAFLD in different stages, non-invasive methods...
Principles of screening

World Health Organization guidelines were published in 1968, but are still applicable today

• The condition should be an important health problem.
• There should be a treatment for the condition.
• Facilities for diagnosis and treatment should be available.
• There should be a latent stage of the disease.
• There should be a test or examination for the condition.
• The test should be acceptable to the population.
• The natural history of the disease should be adequately understood.
• There should be an agreed policy on whom to treat.
• The total cost of finding a case should be economically balanced in relation to medical expenditure as a whole.
• Case-finding should be a continuous process, not just a "once and for all" project.
Summary

• NAFLD is most prevalent

• NASH is an important issue

• Currently the precise treatment of both is undefined

• Liver biopsy is not only invasive but also has a significant sampling error
Liver Biopsy: the Gold Standard is neither Gold nor Standard

JAZZ STANDARDS
And the winner is...
The Nature of the Problem

• In a growing epidemic of obesity how do we identify patients at risk of liver disease
• Who has NAFLD?
• Who has NASH?
• Who has fibrosis?
• Once detected how do we monitor NAFLD
  – Natural history
  – Response to interventions
Fibroscan-take home massage

- A promising non invasive method for diagnosis and follow up

- Equivalent to serum markers for The diagnosis of significant fibrosis

- Combined Fibroscan/Fibromarkers could avoid liver biopsy on most cases
Serum markers take home massage

- Measure indirect markers of liver function
- Influenced by inflammation, co-morbidity or medications
- Combination of markers are better than a single marker
- Efficient in follow up
- Are compatible or better than liver biopsy for fibrosis
- Less dependence on professional expertise and lower cost
Liver Biopsy—take home massage

- May identify various lesions other than histology
  - iron/ductal reaction

- Serum markers as well as fibroscan will never replace the range and complexity of information that can be obtained by liver biopsy
And the winner is...
Non-Invasive: Test of Fibrosis

- **Blood tests**
  - Fibrotest
  - APRI
  - ELF
  - Forn’s
  - FIBROspect
  - Fibrometer
  - Hepascore
  - FIB-4 (coinfected patients)

- **Liver Imaging**
  - Transient elastrography
  - MR spectoscopy
  - Diffuse-weighted MRI

Halfon *Am J Gastro*. 2006; 101: 547-55
Wai *Hepatol*. 2003; 38: 518-26

Cales, *J Hepatol*. 20054; 42: 1373-1383
Overview

- Biopsy
- Imaging
- Indirect markers
- Direct markers
- The Future
Importance of Determining Stage of Fibrosis

• Assessment of disease
  - Detect fibrosis
  - Current status
  - Prognosis
• Treatment decisions
  - Monitoring disease
  - Natural history
  - Treatment effects
  - Drug development

Single Test
Cross-sectional Data

Multiple Tests
Dynamic changes over time
Liver Biopsy: The Gold Standard

• Provides assessment of fibrosis severity (stage)
  - Guides discussions regarding prognosis and timing of antiviral therapy and other intervention
• Provides assessment of necroinflammation activity (grade)
  - Predictor of risk of progression
• Determines presence/absence of concurrent diseases
  - Steatosis, iron overload
Liver Biopsy: The Bronze Standard

• **Sampling error**: 1/50,000 of liver

• **Size and # portal tracts matters**
  - Correct classification of stage if 15 mm = 65% and if 25 mm = 75%
  - If ≤ 10 complete portal tracts, reduced accuracy in classification of grade and stage

• **Variation between biopsies**
  - 30% variation in stage in Left vs. Right lobe

• **Measurement error**
  - Experts vs. “Routine Pathology” $\kappa$ = 0.48 vs. 0.15
  - Senior vs. Junior experts $\kappa$ = 0.6 vs. 0.5 respectively

Bedossa P. *Hepatology*; 2005 38:1356-8
Rousselet MC. *Hepatol*; 2005 41: 252-64
Colleredo G. J. *Hepatol*; 2003:239-244
Regev A. *Am J Gastro* 2002; 197: 2614-18
• Ordinal categorical variables

• Categories are not evenly distributed
Moderate prediction of Outcome For Three Categories of Biopsy Stage

Ishak Stage

0 - 1 Mild
2 - 4 Moderate
4 - 6 Severe
Liver Biopsy: Limitation

• Invasive procedure with associated morbidity
  - Major complications = 0.5%
  - Contraindicated in some patient populations
  - Or requires trans-jugular route

• Cost and inconvenience
  - Does not easily lend itself to repeat testing
# Prediction of Future Liver Related Outcomes

<table>
<thead>
<tr>
<th>Test</th>
<th>(^1\text{AUC Fibrotest (95% CI)}) n=243</th>
<th>(^2\text{AUC ELF (95% CI)}) N=447</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>0.66 (0.52, 0.78)</td>
<td>0.82 (0.77, 0.89)</td>
</tr>
<tr>
<td>Serum Marker</td>
<td>0.76 (0.63, 0.84)</td>
<td>0.86 (0.81, 0.92)</td>
</tr>
</tbody>
</table>

\(^1\text{Ngo Y. et al, Clin Chem. 2006; 52(10): 1887-1896}\)

\(^2\text{Parkes et al. manuscript in preparation}\)

- prognostic value (AUROC) of FibroTest vs. biopsy fibrosis staging for overall survival.
Range in healthy patients

- Patients
  544 patients from social medical center
  - self declared alcohol intake < 30g per day
  - BMI < 30 kg/m²
  - Negative HBV and HCV serologies
  - AST and ALT < 40 UI/L, GGT < 45 UI/L, ferritin < 350 µg/L, platelets > 150 10³/mm³, mean globular volume < 98 µm³

- Results
  ✓ 318 patients considered without risk factor for liver diseases
    → Mean: 5.3 ± 1.5 kPa from 1.5 to 10.9 kPa
    → 90th percentile = 7.3 (6.8-7.6) kPa
    → 95th percentile = 7.9 (7.6-8.8) kPa
  ✓ Higher in men than in women (5.4 versus 4.6, p<0.001)
  ✓ No correlation with age

Roulot et al. AASLD 2006
Table 11  NASH Clinical Research Network histological scoring system

<table>
<thead>
<tr>
<th>Steatosis</th>
<th>S score</th>
<th>Lobular inflammation</th>
<th>L score</th>
<th>Hepatocyte ballooning</th>
<th>B score</th>
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<tbody>
<tr>
<td>&lt; 5%</td>
<td>0</td>
<td>None</td>
<td>0</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>5–33%</td>
<td>1</td>
<td>&lt; 2</td>
<td>1</td>
<td>Few ballooned cells</td>
<td>1</td>
</tr>
<tr>
<td>34–66%</td>
<td>2</td>
<td>2–4</td>
<td>2</td>
<td>Many ballooned cells</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 66%</td>
<td>3</td>
<td>&gt; 4</td>
<td>3</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>NASH fibrosis stage</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Mild, zone 3 perisinusoidal fibrosis</td>
<td>1a</td>
</tr>
<tr>
<td>Moderate, zone 3 perisinusoidal fibrosis</td>
<td>1b</td>
</tr>
</tbody>
</table>

Portal/periportal fibrosis only 1c
Zone 3 perisinusoidal and portal/periportal fibrosis 2
Bridging fibrosis 3
Cirrhosis 4

Source: Kleiner et al., Hepatology 2005;41:1313–21 [35].