Update in Non-Alcoholic Steatohepatitis

Zobair Younossi MD, MPH, FACG, AGAF, FAASLD

Chairman, Department of Medicine, Inova Fairfax Hospital
Vice President for Research, Inova Health System
Professor of Medicine, VCU-Inova Campus
Affiliate Professor of Biomedical Sciences,
George Mason University
Falls Church, Virginia

No Conflict related to this presentation

Non-alcoholic Fatty Liver Disease

The Diagnosis and Management of Non-Alcoholic Fatty Liver Disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association

Systematic review with meta-analysis: non-alcoholic steatohepatitis - a case for personalised treatment based on pathogenic targets

ACG 2014 Annual Postgraduate Course • October 18-19, 2014
The Spectrum of NAFLD

- Exclusion of liver diseases
- NASH requires specific pathologic criteria
- Important for prognosis

NAFLD is the Hepatic Manifestation of Visceral Obesity

In order to understand the full impact and the complexity of NAFLD, it is important to consider its close association with obesity
Lets Examine The Epidemiologic Evidence Supporting the Association of NAFLD with Obesity and Metabolic Syndrome

NAFLD and NASH

NAFLD is Closely Associated with Visceral Obesity and Insulin Resistance

- Most patients with NAFLD are obese or have metabolic syndrome
- NAFLD patients with MS (especially T2D) have higher mortality
- A smaller proportion of patients have lean NAFLD with different clinical profile
Most Patients with Non-alcoholic Fatty Liver Disease are Obese

- Cohort: 11,613 NHANES-III participants
- NAFLD was defined as the presence moderate-severe hepatic steatosis (by ultrasound), absence of excessive alcohol use and other chronic liver diseases
- Prevalence of NAFLD in obese and overweight: 17.7% (N=2,061)
- Prevalence of NAFLD in lean individuals (BMI<25): 3.7% (N=431)

Clinical and laboratory profile of lean NAFLD is different

<table>
<thead>
<tr>
<th></th>
<th>Lean (BMI&lt;25)</th>
<th>Overweight or obese (BMI&gt;25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAFLD (N=431)</td>
<td>Control (N=4026)</td>
<td>NAFLD (N=2061)</td>
</tr>
<tr>
<td>White, %</td>
<td>72.48 ± 4.51</td>
<td>79.17 ± 1.99</td>
<td>76.33 ± 2.58</td>
</tr>
<tr>
<td>African-American, %</td>
<td>10.25 ± 2.08</td>
<td>8.34 ± 0.92</td>
<td>8.19 ± 1.26</td>
</tr>
<tr>
<td>Hispanic, %</td>
<td>6.97 ± 1.64</td>
<td>4.15 ± 0.68</td>
<td>7.34 ± 1.39</td>
</tr>
<tr>
<td>Other ethnicity, %</td>
<td>10.30 ± 3.10</td>
<td>8.33 ± 1.24</td>
<td>7.82 ± 1.71</td>
</tr>
<tr>
<td>Male, %</td>
<td>43.57 ± 4.03</td>
<td>42.24 ± 1.14</td>
<td>54.78 ± 1.76</td>
</tr>
<tr>
<td>Visceral obesity, %</td>
<td>5.05 ± 1.49</td>
<td>4.45 ± 0.40</td>
<td>7.08 ± 1.64</td>
</tr>
<tr>
<td>Insulin resistance, %</td>
<td>13.35 ± 2.41</td>
<td>6.03 ± 0.48</td>
<td>63.62 ± 1.77</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>6.72 ± 1.41</td>
<td>1.34 ± 0.25</td>
<td>16.13 ± 1.06</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>62.65 ± 3.80</td>
<td>53.77 ± 1.44</td>
<td>87.19 ± 1.53</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>17.83 ± 2.39</td>
<td>10.46 ± 0.56</td>
<td>39.10 ± 1.91</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>41.94 ± 1.15</td>
<td>39.61 ± 0.43</td>
<td>48.10 ± 0.61</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>22.17 ± 0.16</td>
<td>22.09 ± 0.04</td>
<td>23.62 ± 0.97</td>
</tr>
<tr>
<td>ALT: SI (U/L)</td>
<td>17.96 ± 0.09</td>
<td>14.29 ± 0.27</td>
<td>25.63 ± 0.80</td>
</tr>
<tr>
<td>AST: SI (U/L)</td>
<td>21.50 ± 0.40</td>
<td>19.57 ± 0.16</td>
<td>24.82 ± 0.53</td>
</tr>
</tbody>
</table>

Younossi Z et al. Medicine 2012
NAFLD and NASH: An Update

NAFLD in the Setting of Metabolic Syndrome is Associated with Adverse Outcomes

Metabolic Syndrome and NAFLD

- NHANES III-NDI linked files (1988-94) (N=6709)
- NAFLD defined by US and clinical data (N=1448)
- Follow-up and mortality: 377 deaths over 26,064 person-years of follow-up
- All-cause mortality, cardiovascular mortality and liver-related mortality were higher in patients with metabolic syndrome and NAFLD (P < .001)

Younossi Z et al. Metabolism 2012
Metabolic Syndrome and NAFLD

- Overall Mortality
  - Participants with Metabolic Syndrome and NAFLD
  - Participants with Metabolic Syndrome and without NAFLD
  - Logrank P-value < .0001

- Cardiac-specific mortality
  - Logrank P-value < .0001
  - Time to Event (years)

- Liver-specific mortality
  - Logrank P-value = 0.006
  - Time to Event (years)

Adjusted Relative Risk of Death In NAFLD

Having NAFLD in Patients with Metabolic Syndrome is Independently Associated with Mortality

<table>
<thead>
<tr>
<th>Factors</th>
<th>Overall</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.10 (1.03-1.18)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.22 (1.30-1.31)</td>
<td></td>
</tr>
<tr>
<td>Liver-specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of deaths</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>1.13 (1.12-1.14)</td>
<td></td>
</tr>
<tr>
<td>Diagnosis of NAFLD with metabolic syndrome</td>
<td>2.33 (1.74-7.13)</td>
<td></td>
</tr>
</tbody>
</table>

Liver-specific Mortality

Cardiac Mortality

- Number of deaths
- BMI
- Diagnosis of NAFLD with metabolic syndrome
- Age, years

Younossi Z et al. Metabolism 2012
The epidemiology of NAFLD parallels that of obesity and metabolic syndrome.

The pathogenesis of NAFLD is similar to the pathogenesis of obesity and metabolic syndrome.

Therefore, one of the important therapeutic strategies for NAFLD should focus on dealing with metabolic syndrome.

Prevalence of NAFLD is increasing with the growing epidemic of obesity.
Epidemiologic impact of NAFLD

Worldwide Prevalence of NAFLD

AASLD Guideline:
• NAFLD: 6-33% (Med 20%)
• NASH: 3-5%

Obese: 75% NAFLD and 19% NASH
Morbidly Obese:
• NAFLD: 93%
• NASH: 26-49%
• Diabetes: 49.5-87% NAFLD

Prevalence of NAFLD in Increasing in Children

Using surrogate makers, prevalence of NAFLD in children is 2.6-17.3%
Autopsy study from UCSD (N=742)
• Prevalence: 9.6%; rates increasing with age
• More common in boys
• Highest rate in Hispanics

Schwimmer JB 2006, Argo C 2009
Because of the Epidemic of Obesity, NAFLD is Becoming the Most Common Cause of Chronic Liver Disease in the U.S.

NHANES Cycles from 1988 to 2008 (N=39,750)

Because of the Epidemic of Obesity, NAFLD is Becoming the Most Common Cause of Chronic Liver Disease in the U.S.

Because of the increasing wave of obesity in children, the disease burden from NAFLD will continue to increase.
Non-alcoholic Fatty Liver Disease

Natural History for the Spectrum of NAFLD

Natural History of NAFLD
Simple Steatosis or NAFL

NAFLD and Liver Related Mortality

- Biopsy-proven NAFLD patients (N=289)
- Clinico-demographic information from the time of biopsy were available.
  - 59.2% had NASH whereas 40.8% had non-NASH NAFLD
  - NASH patients were predominantly female, had higher AST, ALT and higher fasting serum glucose
- Mortality data were obtained from NDI-Plus
  - During median follow-up of 150 months (max 342 months)
  - NASH patients had higher probability of liver-related mortality than non-NASH NAFLD patients (p-value = 0.0026).

### NAFLD and Liver Related Mortality

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Overall mortality aHR (95% CI)</th>
<th>Liver-related mortality aHR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NASH</td>
<td>1.13 (0.74 - 1.71)</td>
<td>9.16 (2.10 - 9.88)</td>
</tr>
<tr>
<td>Age</td>
<td>1.07 (1.05 - 1.10)</td>
<td>1.06 (1.02 - 1.10)</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.95 (0.62 - 1.47)</td>
<td>1.44 (0.62 - 3.34)</td>
</tr>
<tr>
<td>Caucasian race</td>
<td>1.67 (0.92 - 3.06)</td>
<td>1.85 (0.62 - 5.47)</td>
</tr>
<tr>
<td>Obesity</td>
<td>0.91 (0.60 - 1.40)</td>
<td>0.88 (0.38 - 2.04)</td>
</tr>
<tr>
<td>Type II diabetes</td>
<td>2.09 (1.39 - 3.14)</td>
<td>2.19 (1.00 - 4.81)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.01 (0.68 - 1.52)</td>
<td>0.48 (0.19 - 1.23)</td>
</tr>
</tbody>
</table>

There is clear evidence that NASH patients are at higher risk for LRM.
Several case reports and case series of well documented HCC in patients with NAFLD/NASH

Two population-based cohort studies of NAFLD
- One study suggested 0.3% over 6 years

Three clinic based cohort studies of NAFLD or NASH (not restricted to cirrhosis)
- Up to 6% absolute risk of HCC in approximately 20 year follow up
- Lower relative risk compared to alcohol or HCV

Summary of Outcomes of NASH
Non-alcoholic Fatty Liver Disease

Can We Predict Outcomes such as Advanced Fibrosis and Liver-related Mortality in Patients with NAFLD?

Non-alcoholic Fatty Liver Disease

Can Clinical Data Predict Advanced Fibrosis and Mortality?
NAFLD patients with components of MS are at highest risk for advanced fibrosis

NAFLD with liver biopsy (N=432) in multivariate analysis, elevated AST and ALT, presence of diabetes mellitus, male gender and Caucasian ethnicity were associated with moderate to severe fibrosis (p<0.0001)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Advanced Fibrosis OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>1.61 (1.21-2.01)</td>
<td>0.0374</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.64 (1.13-2.17)</td>
<td>0.0258</td>
</tr>
<tr>
<td>HTN and DM</td>
<td>1.69 (1.11-2.28)</td>
<td>0.0246</td>
</tr>
<tr>
<td>HTN+DM+VD</td>
<td>1.72 (1.13-2.31)</td>
<td>0.0205</td>
</tr>
</tbody>
</table>


Non-alcoholic Fatty Liver Disease

Can Clinical Data Predict Advanced Fibrosis and Mortality?
Predicting Mortality in NAFLD
The Role of Metabolic Syndrome

NHANES III-NDI Linked Mortality Files (N=15,866)
- NAFLD (N=991) and No Liver Disease (13,004)

Clinical data and over 160 months of follow up

Independent risks for liver-related mortality in NAFLD:
- IR: AHR = 53.55 (9.22 - 344.29), p < 0.0001
- Obesity: AHR = 11.19 (2.43 - 51.56), p = 0.003
- Metabolic syndrome: AHR = 12.08 (1.10 - 132.22), p = 0.042
- Older age: AHR = 1.10 (1.08 - 1.12), p = 0.020
- Male gender: AHR = 9.53 (1.36 - 66.55), p = 0.024

Long-term Outcomes of Diabetics with NAFLD

- NAFLD & DM (n=44) vs. NAFLD alone (n=88)
- Patients with NAFLD and DM have:
  - Higher rate of cirrhosis (25% vs. 10.2, p=0.04)
  - Higher liver-related mortality (RR=22.83, p=0.003)
  - Higher mortality (RR=3.3, p=0.002)
Long-term Outcomes of Diabetics with NAFLD

- NAFLD & DM (n=44) vs. NAFLD alone (n=88)
- Patients with NAFLD and DM have:
  - Higher rate of cirrhosis (25% vs. 10.2, p=0.04)
  - Higher liver-related mortality (RR=22.83, p=0.003)

Having clinical DM, elevated liver enzymes and male gender are associated with advanced fibrosis and LRM

Younossi et al. Clin Gastro and Hepatology 2004

Non-alcoholic Fatty Liver Disease

Can Pathologic Variables on the Liver Biopsy Predict Adverse Outcomes Such as Fibrosis and LRM?
Non-alcoholic Fatty Liver Disease

Can Pathologic Variables on the Liver Biopsy Predict Fibrosis?

NAFLD patients with evidence hepatocyte ballooning at risk for fibrosis

- 132 Liver biopsy specimens from NAFLD patients were read two hepatopathologists (TG and DK)
- Clinical and pathological data were available
  - 48% male, 88% white, and 33% diabetic
  - 21.2% had advanced fibrosis and 20.3% sinusoidal fibrosis
- Ballooning degeneration and Mallory bodies were independently associated with sinusoidal fibrosis
- AST/ALT ratio and ballooning degeneration were also independently associated with periportal portal fibrosis
- Therefore, ballooning degeneration is important for development of progressive NAFLD

Non-alcoholic Fatty Liver Disease

Can Pathologic Variables on the Liver Biopsy Predict LRM?

- 209 NAFLD patients with liver biopsy slides, clinical data and mortality data were included.
- All biopsies were read centrally using the original criteria for NAFLD subtypes, NAFLD Activity Score (NAS), Brunt’s criteria and ZG criteria
- Median follow up = 146 months (max 342 months)
- During follow-up, 31% of patients died with 9% dying of LRM
- Regardless of the pathologic criteria used, NASH patients had higher LRM than non-NASH NAFLD
  - (13.0% vs. 1.3% for Original NAFLD NASH, p = 0.0047)

Predicting Outcomes of NAFLD
Liver Related Mortality

Younossi Z et al Hepatology 2011
Association of Pathologic Features with LRM

Univariate survival analyses [HR (95% CI), p-value]

- Portal inflammation (grade ≥2) [6.68 (2.20-20.3), p<0.001]
- Ballooning degeneration (grade ≥2) [5.32 (1.89-14.9), p=0.001]
- Mallory-Denk bodies (grade ≥2) [4.21 (1.66-10.7), p=0.002]
- Portal fibrosis (grade >2) [14.1 (5.47-36.5), p<0.001]
- Pericellular fibrosis (grade >2) [4.86 (1.73-13.7), p=0.003]

On multivariate analysis, only significant fibrosis (grade > 2) was an independent predictor of LRM

In conclusion:
- Ballooning degeneration of hepatocytes is a surrogate marker for predicting hepatic fibrosis
- Advanced hepatic fibrosis is a surrogate of LRM

Younossi Z et al Hepatology 2011

ACG 2014 Annual Postgraduate Course • October 18-19, 2014

ACG 2014 Annual Postgraduate Course
Copyright 2014 American College of Gastroenterology
Predictors of Advanced Fibrosis or Liver-related Mortality

<table>
<thead>
<tr>
<th>Variables</th>
<th>Angulo</th>
<th>March</th>
<th>Ratziu</th>
<th>Dixon</th>
<th>Ong</th>
<th>Younossi</th>
<th>Ekstedt</th>
<th>Westin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST/ALT</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT or AST</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Highlights of the Pathogenesis of NAFLD and NASH

Since NAFLD is closely related to obesity (complex and heterogeneous diseases), its pathogenesis is also heterogeneous involving multiple pathways
Obesity

1st hit
Insulin Resistance (Adipose, liver and muscle)

Free Fatty Acids

Lipotoxicity

Oxidative Stress

Hepatic Steatosis

2nd hit
Hepatic Steatosis

2nd hit
Lipid Peroxidation

2nd hit
Oxidative Stress

2nd hit
Genes That Influence these Pathways and the Environment

NASH

FIBROSIS

Modified from Mishra P, Rafiq N, Younossi Z. Practical Management of Liver Disease 2008

ACG 2014

ACG Postgraduate Course • October 18-19, 2014
Diagnostic Tests for Non-alcoholic Fatty Liver Disease

What biomarkers or tests can identify the potentially progressive form of NAFLD or NASH?

- Liver Biopsy & Pathologic Protocols
- Clinical Presentation & Routine Laboratory Data
- Routine Radiologic Tests (Ultrasound, CT, MRI)
- New Biomarkers (NASH or Fibrosis)
- Predictive Panels Based on Clinical and Lab Data
- New Radiologic Modalities (Fibroscan MRS)
## Non-alcoholic Fatty Liver Disease

### Routine Radiologic Modalities in NAFLD

<table>
<thead>
<tr>
<th>Study</th>
<th>Yr</th>
<th>N</th>
<th>Mod</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver Stiffness: Ultrasound elastography or MR elastography (problems with reproducibility and inability to discriminate lower stages of fibrosis and no large validation study in longitudinal cohorts.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver Fat Content: MRI/MRS proton density fat fraction: ? Feasibility in population studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Current routine radiologic modalities are unable to consistently predict NASH or fibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not practical in large scale studies of NASH</td>
</tr>
</tbody>
</table>

### How about Non-invasive Biomarkers for NASH?

---

Copyright 2014 American College of Gastroenterology
Biomarkers for NASH and Fibrosis

**Predictive Panels (Clinical-Lab Data)**

**Blood-Based Biomarkers**

**Pathogenesis Based Biomarkers**

**NASH**

**Fibrosis**

- CK-18 fragments
- Circulating active caspase 3
- Fas/FasL
- Lipid Peroxidation products
- Antioxidant capacity
- Protein oxidation products

**APOPTOSIS**

**OXIDATIVE STRESS**

**INFLAMMATORY ADIPO-CYTOKINES**

- TNF-alpha, adiponectin, C40r, IL-6, CCJ2, R1-
- beta, resistin, visfatin, RBP-4

**FIBROGENESIS AND FIBROSIS**

- TGF-beta, HA, Laminin, Other ECM component, Tissue Elasticity

---

Pathogenic Pathways-based Biomarkers

Modified From Wieckowska A. et al. Seminars in Liver Disease/Volume 28, Number 4, 2008

Copyright 2014 American College of Gastroenterology
Biomarkers for NASH and Fibrosis

**Predictive Panels (Clinical-Lab Data)**

- **Pathogenesis Based Biomarkers**
- **Blood-Based Biomarkers**

**Other Technologies Used for Biomarker Development in NAFLD**

- **Gene Expression:**
- **Proteomics Technologies:**
  - Z Younossi 2009, N Chalasani 2009, Z Younossi 2010
- **miRNA:**
- **Lipidomics/Lipomics:**
  - A Sanyal 2009
- **NASH Metabolomics Tests** 2009, 2010
Despite the excitement for the application of these new technologies, a validated biomarker for NASH has not been developed.
**NAFLD Guideline Recommendations**

**Non-invasive Assessment**

- **NAFLD Fibrosis Score** is a clinically useful tool for identifying NAFLD patients with higher likelihood of having bridging fibrosis and/or cirrhosis (Strength – 1, Evidence - B)

- Although serum/plasma CK18 is a promising biomarker for identifying steatohepatitis, it is premature to recommend in routine clinical practice (Strength – 1, Evidence - B)

Given the limitation of clinical, laboratory, and radiologic modalities, liver biopsy continues to be used for diagnosis and staging NASH

---

**Liver Biopsy for Diagnosis and Staging of NASH**

- Pathologic criteria for NASH
- Limitations of liver biopsy
### Pathologic Criteria for NASH

<table>
<thead>
<tr>
<th>Ludwig, 1980 (Original)</th>
<th>• Steatosis, lobular inflammation, necrosis and Mallory bodies in zone 3, mild portal and periportal inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younossi, 1999 (NAFLD Subtypes)</td>
<td>• Steatosis, ballooning degeneration • Steatosis, ballooning with either Mallory’s hyaline or fibrosis</td>
</tr>
<tr>
<td>Brunt, 1999 (Brunt Criteria)</td>
<td>• Steatosis with mixed inflammation, occasional ballooned hepatocytes • Steatosis, ballooning and disarray in zone 3 • Panacinar steatosis + ballooning + disarray + portal inflammation</td>
</tr>
<tr>
<td>Kleiner, 2005 (NAS Criteria)</td>
<td>• Steatosis (0-3), lobular inflammation (0-3), ballooning (0-3) • 0-2 not NASH (\rightarrow) (\geq) 5 usually NASH</td>
</tr>
</tbody>
</table>

---

**Minimal Histologic Criteria for NASH**

- **Hepatocyte ballooning:** swollen liver cells with thin strands of cytoplasm
- **Masson trichrome stain:** deposition of collagen in the perisinusoidal spaces
- **Immunostain for ubiquitin:** small Mallory-Denk bodies in ballooned hepatocytes

---


ACG 2014 Postgraduate Course • October 18-19, 2014
Minimal Histologic Criteria for NASH

- Steatosis, lobular inflammation and ballooning degeneration with or without perisinusoidal fibrosis or Mallory hyalines

- Hepatocyte ballooning: swollen liver cells with thin strands of cytoplasm
- Masson trichrome stain: deposition of collagen in the perisinusoidal spaces
- Immunostain for ubiquitin: small Mallory-Denk bodies in ballooned hepatocytes

Potential Pitfalls of Liver Biopsy in NAFLD

- Costly and expensive
- Invasive (complications around 0.5%)
- Sampling problems
  - Inadequate length or fragmented specimen
  - In 35% of patients, one biopsy is not enough to rule out bridging fibrosis (??)
  - Not unique to NASH
- Morphometric assessment may overcome some of these issues
NAFLD Guideline Recommendations

Role of Biopsy

• Liver biopsy should be considered in patients with NAFLD who are at increased risk to have steatohepatitis and advanced fibrosis (Strength – 1, Evidence - B)

• 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 (Strength – 1, Evidence - B)

NAFLD and NASH: Treatment

Treatment Targets
Targets Used for Treatment of Non-alcoholic Fatty Liver Disease

NAFLD and NASH: Treatment

Does Treatment of Obesity Improve Liver Disease in NAFLD?

Diet +/-Exercise, Medication and Surgical Weight Loss
### Treatment of NAFLD: Weight Loss

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Intervention</th>
<th>Duration (months)</th>
<th>Design</th>
<th>ALT</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hickman</td>
<td>31</td>
<td>Diet</td>
<td>15</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Huang</td>
<td>16</td>
<td>Diet</td>
<td>12</td>
<td>Open label</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Palmer</td>
<td>39</td>
<td>Diet</td>
<td>2-111</td>
<td>Case series</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Andersen</td>
<td>41</td>
<td>Diet</td>
<td>4-23</td>
<td>Open label</td>
<td>+</td>
<td>+/-*</td>
</tr>
<tr>
<td>Kugelmas</td>
<td>8</td>
<td>Diet/Ex</td>
<td>3</td>
<td>Open- label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Ueno</td>
<td>15</td>
<td>Diet/Ex</td>
<td>3</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Zhu</td>
<td>34</td>
<td>Diet/Ex</td>
<td>12</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Harrison</td>
<td>10</td>
<td>Orlistat</td>
<td></td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sabuncu</td>
<td>13/12</td>
<td>Sibutramine/Orlistat</td>
<td>6</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Luyckx</td>
<td>69</td>
<td>Surgery</td>
<td>27</td>
<td>Case series</td>
<td>+</td>
<td>+/-*</td>
</tr>
<tr>
<td>Silverman</td>
<td>91</td>
<td>Surgery</td>
<td>2-61</td>
<td>Case series</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dixon</td>
<td>36</td>
<td>Surgery</td>
<td>26</td>
<td>Case series</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mummadi</td>
<td>766</td>
<td>Surgery</td>
<td>12</td>
<td>Meta-analysis</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### Bariatric Surgery and NAFLD

<table>
<thead>
<tr>
<th>Study First Author</th>
<th>Year of publication</th>
<th>Sample size</th>
<th>Surgery type</th>
<th>Mean follow-up time</th>
<th>Change in Steatosis</th>
<th>Change in Inflammation</th>
<th>Change in Fibrosis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranowitz</td>
<td>1990</td>
<td>15</td>
<td>RYGB or gastroplasty</td>
<td>12 months</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Improved liver enzymes</td>
</tr>
<tr>
<td>Silverman</td>
<td>1995</td>
<td>91</td>
<td>RYGB</td>
<td>18.4 months</td>
<td>↓</td>
<td>↓ / O</td>
<td>↓</td>
<td>Improved lobular but no change in portal fibrosis</td>
</tr>
<tr>
<td>Luyckx</td>
<td>1996</td>
<td>69</td>
<td>Gastroplication or LAGB</td>
<td>Not declared</td>
<td>↓</td>
<td>↓</td>
<td>O</td>
<td>NA</td>
</tr>
<tr>
<td>Dixon</td>
<td>2004</td>
<td>36</td>
<td>LAGB</td>
<td>25.5 ± 10 months</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>Significant improvement in all liver enzymes, increase in fibrosis overall was small</td>
</tr>
<tr>
<td>Kral</td>
<td>2004</td>
<td>104</td>
<td>Biliopancreatic diversion (BPD)</td>
<td>74 ± 27 months</td>
<td>↓</td>
<td>NR</td>
<td>↑</td>
<td>Improvement in lobular and portal fibrosis, ALT and AST</td>
</tr>
<tr>
<td>Clark</td>
<td>2005</td>
<td>16</td>
<td>RYGB</td>
<td>10 ± 4 months</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NAH grade improved. No significant reduction in LFTs</td>
</tr>
<tr>
<td>Keshishan</td>
<td>2005</td>
<td>78</td>
<td>BPD-duodenal switch (BPD-D5)</td>
<td>30 months</td>
<td>↓</td>
<td>↓</td>
<td>NR</td>
<td>↓</td>
</tr>
<tr>
<td>Mattar</td>
<td>2005</td>
<td>70</td>
<td>RYGB, LAGB or SG</td>
<td>15 ± 9 months</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Mummadi</td>
<td>2013</td>
<td>32</td>
<td>Roux-en-Y</td>
<td>24 months</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Stratopoulos</td>
<td>2005</td>
<td>216</td>
<td>VBG</td>
<td>18 ± 9.6 months</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>Improvement in ALT and AST</td>
</tr>
<tr>
<td>Menhardt</td>
<td>2006</td>
<td>30</td>
<td>End-to-side jejuno-jejunal bypass</td>
<td>70± 42.8 months</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>No change in histology scores, ALT and AST levels tended higher.</td>
</tr>
<tr>
<td>Barker</td>
<td>2006</td>
<td>19</td>
<td>RYGB</td>
<td>21.4 months</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>Improvement in portal and lobular fibrosis, Net enzymes.</td>
</tr>
</tbody>
</table>
Prospective cohort of 60 obese patients undergoing laparoscopic adj gastric banding Baseline liver biopsy during initial surgery, and second liver biopsy 29.5 ± 16.0 months Steatosis, inflammation, fibrosis and Mallory bodies all improved significantly with weight loss (P < 0.001 for all comparisons)

Dixon J et al Obesity Surgery 2006
Bariatric Surgery for very obese patients with NAFLD improves ALT and probably histology
Fibrosis may improve in some patients
No long term outcomes data are available to suggest a change in the natural history of NASH

**NAFLD Guideline Recommendations**

- **Weight loss generally reduces hepatic steatosis**, achieved either by hypocaloric diet alone or in conjunction with increased physical activity. (Strength –1, Evidence - A)
- **Loss of at least 3-5% of body weight appears necessary to improve steatosis**, but a greater weight loss (up to 10%) may be needed to improve necroinflammation (Strength – 1, Evidence - B)
- **Exercise alone in adults with NAFLD may reduce hepatic steatosis** but its ability to improve other aspects of liver histology remains unknown (Strength – 1, Evidence - B)
Pharmacologic Targets for Treating NAFLD

Pharmacologic Treatment of Insulin Resistance, Oxidative Stress, Dyslipidemia and Other Pathways

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Drug</th>
<th>Duration (months)</th>
<th>Design</th>
<th>ALT</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caldwell</td>
<td>10</td>
<td>Troglitazone</td>
<td>3-6</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acosta</td>
<td>8</td>
<td>Pioglitazone</td>
<td>2-12</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Shadid</td>
<td>5</td>
<td>Pioglitazone</td>
<td>4.5</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Sanyal</td>
<td>21</td>
<td>Pioglitazone + Vit E</td>
<td>6</td>
<td>RCT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Promrat</td>
<td>18</td>
<td>Pioglitazone</td>
<td>12</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tetri</td>
<td>30</td>
<td>Rosiglitazone</td>
<td>12</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Belfort</td>
<td>55</td>
<td>Pioglitazone ± Diet</td>
<td>6</td>
<td>RCT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Torres</td>
<td>49</td>
<td>R vs. R+M vs. R+Losartan</td>
<td>12</td>
<td>Open</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marchesini</td>
<td>14</td>
<td>Metformin</td>
<td>4</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Nair</td>
<td>15</td>
<td>Metformin</td>
<td>12</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Buglaries</td>
<td>55</td>
<td>Metformin</td>
<td>6</td>
<td>RCT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Uygun</td>
<td>17</td>
<td>Metformin</td>
<td>6</td>
<td>RCT</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Duseja</td>
<td>7</td>
<td>Metformin</td>
<td>6</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Schwimmer</td>
<td>10</td>
<td>Metformin</td>
<td>6</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Morita</td>
<td>5</td>
<td>Nateglinide</td>
<td>5</td>
<td>Open</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Metabolic Treatment Target: Insulin Resistance

- PIVENS: RCT NASH without DM (N=247)
  - Pioglitazone 30 mg/d (N=80) for 96 weeks
  - Vit E 800 IU/d (N=84) for 96 weeks
  - Placebo (N=83) for 96 weeks
  - Pre- and Post Liver Biopsy
  - Primary endpoint: NAS score decrease of 2 (no higher fib)
  - Secondary endpoints (Path features, enzymes, etc.)
- **Met primary endpoint:** Pi 34%, VE 43% & P 19%
- Histologic secondary endpoints were seen for both Pio and Vit E but no improvement in fibrosis scores

Conclusions:
- Vit E superior to placebo
- Pio did not meet pre-specified primary endpoint
- Other issues:
  - Weight gain
  - Improvement of liver enzymes not sustained

NAFLD Guideline Recommendations

- Metformin has no significant effect on liver histology and is not recommended as a specific treatment for liver disease in adults with NASH. (Strength – 1, Evidence - A)

- Pioglitazone can be (?) used to treat steatohepatitis in patients with biopsy-proven NASH. However, it should be noted that majority of the patients who participated in clinical trials that investigated pioglitazone for NASH were non-diabetic and that long term safety and efficacy of pioglitazone in patients with NASH is not established. (Strength – 1, Evidence- B)

Treatment: Vitamin E

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Author</th>
<th>N</th>
<th>Design</th>
<th>Duration (Months)</th>
<th>ALT</th>
<th>Histo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E</td>
<td>Lavine</td>
<td>11</td>
<td>Open</td>
<td>4-10</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Kawanaka</td>
<td>10</td>
<td>Open</td>
<td>6</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Sanyal</td>
<td>10</td>
<td>RCT</td>
<td>6</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Hasegawa</td>
<td>12</td>
<td>Open</td>
<td>12</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Vajro</td>
<td>14</td>
<td>RCT</td>
<td>5</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Bugianesi</td>
<td>25</td>
<td>RCT</td>
<td>6</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>Vitamin E/Ex</td>
<td>Kugelmas</td>
<td>8</td>
<td>Open</td>
<td>3</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>Vitamins E+C</td>
<td>Harrison</td>
<td>23</td>
<td>RCT</td>
<td>6</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Vitamins E+C</td>
<td>Ersoz</td>
<td>28</td>
<td>RCT</td>
<td>6</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>PIVENS</td>
<td>Sanyal</td>
<td>84</td>
<td>RCT</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
NAFLD Guideline Recommendations

- Vitamin E (a-tocopherol) administered at daily dose of 800 IU/day improves liver histology in non-diabetic adults with biopsy-proven NASH and therefore it should be considered as a first-line pharmacotherapy for this patient population. (Strength -1, Quality - B)

- Until further data supporting its effectiveness become available, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis (Strength - 1, Quality - C)

Treatment: Lipid Lowering Agents

<table>
<thead>
<tr>
<th>Study</th>
<th>Design (mts)</th>
<th>Meds</th>
<th>N</th>
<th>ALT</th>
<th>Hist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurin</td>
<td>Open label (12)</td>
<td>Clofibrate</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fernández-Miranda C Basaranoglu</td>
<td>RCT (1)</td>
<td>Gemfibrozil</td>
<td>46</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Horlander</td>
<td>Open label (12)</td>
<td>Atrovastatin</td>
<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kiyici</td>
<td>Open label (6)</td>
<td>Atrovastatin</td>
<td>27</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Hatzitolios</td>
<td>Open label (6)</td>
<td>Atrovastatin</td>
<td>+</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Gomez-Dominguez Rallidis</td>
<td>Open label (12)</td>
<td>Atrovastatin</td>
<td>25</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Ekstedt</td>
<td>Retrospect (10.3-16.3 yrs)</td>
<td>Statins</td>
<td>68</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Merat</td>
<td>RCT (6)</td>
<td>Probucol</td>
<td>30</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Nelson</td>
<td>RCT (12)</td>
<td>Simvastatin</td>
<td>14</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Park</td>
<td>Open label (24)</td>
<td>Ezetimibe</td>
<td>45</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>
NAFLD Guideline Recommendations

- Given the lack of evidence to show that patients with NAFLD and NASH are at increased risk for serious drug-induced liver injury from statins, statins can be used to treat dyslipidemia in patients with NAFLD and NASH (Strength – 1, Quality – B)
- Until RCTs with histological endpoints prove their efficacy, statins should not be used to specifically treat NASH (Strength – 1, Quality – B)

Non-alcoholic Fatty Liver Disease
Other Treatment Regimens

Antioxidants
- Betaine
- N-Acetyl-cysteine
- Lecithin
- Silymarin
- Beta-carotene

Anti-TNF agents (Pentoxifylline)
Probiotics (VSL#3)
ACE inhibitors/ARBs
Caspase inhibitors
Cytoprotective agents/Bile Acids
  - Ursodeoxycholic acid (UDCA)
  - Obeticholic Acid (OCA)-FLINT Study (NASH CRN)
**Treatment: Ursodeoxycholic acid**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Author</th>
<th>N</th>
<th>ALT</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDCA</td>
<td>Laurin</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>UDCA</td>
<td>Holoman</td>
<td>24</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>UDCA &amp; Diet</td>
<td>Ceriani</td>
<td>31</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>UDCA &amp; Diet</td>
<td>Guma</td>
<td>24</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>UDCA</td>
<td>Kiyici</td>
<td>17</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>UDCA</td>
<td>Lindor</td>
<td>160</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

**Randomized, Placebo-controlled Trial of UDCA**

![Graph showing Δ ALT (Pre and Post) and Δ Histology (Pre and Post)](image)

Lindor K et al., Hepatology 2004
**FLINT**
The Farnesoid X Receptor (FXR) Ligand Obeticholic Acid in NASH Treatment Trial

**Sponsor:** NIDDK

**Investigators:** US NASH Clinical Research Network

---

**FLINT Entry & Endpoint**

**Histological Entry Criteria:**
- Definite or borderline NASH
- NAFLD activity score (NAS) ≥ 4
- With > 1 in each NAS component
  - Steatosis: scored 0-3
  - Lobular inflammation: scored 0-3
  - Ballooning: scored 0-2
- No cirrhosis
- Fibrosis: scored 0-4

**Primary Endpoint:**
- A decrease in NAFLD Activity Score (NAS) ≥ 2
- With no worsening of fibrosis

**Preplanned interim analysis:**
- p=0.0024 on ITT

**Decisions:**
- Stop treatment phase of trial – as it met efficacy endpoint
- No further liver biopsies in remaining patients
- Lipid changes in OCA vs. placebo (HDL ↓, LDL ↑)
- Follow up patients at 6 months, as planned
- Study finishes ~ July 2014

---


Since the original description of NASH over 30 years ago, despite dozens of clinical trials of different agents, we have failed to identify effective treatment for most patients with NASH.

Research Flaws:
- Until recently, no standard histologic criteria for entry into the clinical trials.
- Outcomes which were followed in clinical trials not very robust.
- Open-label studies without the rigor of randomized clinical trials.
- Short duration of treatment, lasting from 6-12 months.
  - Since NAFLD is the hepatic manifestation of metabolic syndrome, it should be treated like its other complications such as DM, dyslipidemia (long term).
- Little attention to the complex pathogenic pathway.
  - Different pathogenic pathways may be involved in the NASH phenotypes.
  - A single pathway approach to treat all NASH phenotypes will likely fail.
- Future considerations: Given the heterogeneity of NASH phenotype, targeted treatment using a personalized medicine approach based on pathogenic pathways, clinical and prognostic biomarkers should be considered.

How Do We Manage our NAFLD Patients in 2014?

Elevated aminotransferases
Fatty Liver by imaging

- Exclude other causes of CLD
- Confirm lack of excessive ETOH
- Assess risk factors
- Consider Assessment for IR

- No evidence of other CLD
- Young age
- No evidence of adv LD

- Self directed life style modifications
  - Repeat lab in 6 months

- Goals achieved
  - Monitor q 6-12 m

- Unsuccessful
  - Risks (DM, IR)
  - Liver enzymes elevated
  - High NAFLD Fibrosis score

- Suspicion for other CLD
- Dx of NAFLD uncertain

Liver biopsy
How Do We Manage our NAFLD Patients in 2014?

Histologic NASH
- Continue life style and modifications
- If non-diabetic: VIT E
- If diabetic: Pioglitazone?

Simple Steatosis
- Refer to primary for management of MS and risk of CVD

Medical treatment unsuccessful
- Consider RCT of new agents
- Consider Bariatric surgery for those who meet criteria

Update in NASH

Summary
NAFLD is a complex disease tied closely to obesity
Prevalence of NAFLD parallels the epidemic of obesity
Only NASH patients can progress
NAFLD/NASH in the setting of DM/MS has adverse outcomes
Lack of effective treatment from dozens of clinical trials of NAFLD suggests the heterogeneity of the NAFLD phenotype
Personalized targeted treatment may be the best future option to treat NASH
Appropriate study population selection and treatment targets are key for future clinical trials
Current treatment considerations for current patients with NASH:
- Life style modifications for all
- Vitamin E for non-DM NASH
- ??Pio for DM with NASH but be aware of safety concerns
- Clinical trials (OCA and others)
- Consider bariatric surgery for morbidly obese+/-DM with NASH