The fat epidemic – NASH diagnosis and management

Chris Day
Newcastle University
United Kingdom

Nomenclature

NAFL
- Fat infiltration >5% with or without mild inflammation

NASH
- Fat infiltration + necro-inflammatory changes (ballooning degeneration, Mallory bodies, megamitochondria) and/or fibrosis

Cirrhosis
- Fibrosis with necrosis

Presentation

- Symptoms
  - Unusual – majority discovered by chance
  - Fatigue most common
- Most common
  - Incidental abnormal LFTs
  - Incidental hepatomegaly
  - Incidental “bright liver” on imaging
- Common scenarios
  - “Statin” monitoring
  - “Annual reviews” in T2DM/Lipid/HBP clinics
  - Medical insurance/occupational health checks
Symptoms & signs in 400 NAFLD
*Ramesh & Sanyal 2005*

<table>
<thead>
<tr>
<th>Symptom</th>
<th>NAFL</th>
<th>NASH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>Fatigue</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>RUQ pain</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>Hepat</td>
<td>22</td>
<td>28</td>
</tr>
<tr>
<td>Obesity</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>Diabetes</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>65</td>
<td>69</td>
</tr>
</tbody>
</table>

NAFLD vs age, gender and BMI matched controls
- Fatigue Impact Score (FIS): 51 ± 38 vs 8 ± 12; p<0.0001 - as bad as PBC patients
- FIS negatively correlated with activity and positively correlated with daytime somnolence (by Epworth Sleepiness Scale, ESS)
- Large negative impact on QoL (by CLDQ)
- No association with histological severity/IR
- As with other clinical features

“Incidental” abnormal LFTs
- Most studies have focussed on ALT & AST
- Prevalence depends on cut-off/exclusions
- If HBV, HCV, Alcohol excluded:
  - With “traditional” ALT/AST cut-off: 3-5%
  - With proposed “low” ALT cut-off: 12-14%
  
  *Prati 2002, Ruhl 2003, Clark 2003, Pendino 2005*

LFTs and NAFLD
- NAFLD is the commonest diagnosis in patients with “incidental” abnormal LFTs (ALT/ALP/GGT)
  
  *Daniel 1999, Skelly 2001, Pendino 2005*
- Majority of patients with NAFLD (~80%) have normal LFTs
  
  *Browning 2004*
- Severity of histology in NAFLD with normal LFTs no different from those with abnormal LFTs
  
  *Mofrad 2003, Sorrentino 2004, Franci et al 2008*

Suspect NAFLD in patients with risk factors: features of the metabolic syndrome

Screening in high risk groups is currently not advised due to uncertainties over diagnostic tests and treatment options

*Natural history*
Advanced (F3) fibrosis

NASH and/or F1-F2 fibrosis

Steatosis (5-10%)
Liver Death/OLTx (12-40%)

Liver related mortality and histology

Confirmed by Younossi et al in 257 NAFLD patients with >5 years follow up
*Hepatology 2011*

NAFLD and Cardiovascular Disease

Risk of Cardiovascular Disease in Patients with Nonalcoholic Fatty Liver Disease
    Giuseppe Targher, M.D., Christopher P. Day, M.D., Ph.D.,
    and Enzo Bonora, M.D., Ph.D.

Therapy of NAFLD

DEPENDS ON DISEASE STAGE:

- **Steatosis +/- lobular inflammation**
  - Treat the metabolic syndrome components by behavioural (lifestyle) approaches +/- drugs where necessary
  - Look for and treat extra-hepatic co-morbidities
  - ? No liver follow-up required

- **NASH +/- fibrosis**
  - "Tailored" treatment for metabolic syndrome
  - +/- Liver directed therapy
  - Liver follow-up for advanced disease (varices & HCC surveillance etc)
Can we grade/stage NAFLD with blood tests or imaging?

Blood tests predicting presence of steatosis
- Fatty liver index
  - BMI, waist, TGs and GGT
- Steatotest
  - alpha2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, fasting glucose, TGs, cholesterol, ALT adjusted for age, gender, weight and height

Blood tests predicting presence of NASH
- NashTest
- Shimada index
- Markers of apoptosis
  - CK-18 fragments
  - CK-18 + soluble Fas
  - CK-18 + FGF21
- None have been independently validated in NAFLD or are in routine clinical use

Blood tests assessing stage (fibrosis)

Cytokeratin-18 Fragments (CK-18)
- Marker of apoptosis
- For every 50 U/L increase in the plasma level of CK-18 fragments, the likelihood of having NASH increased by 30% ($P < 0.001$).
- CK-18 independent predictor of NASH after adjusting for fibrosis, ALT, AST, age, biopsy length.

Derivation of the NAFLD score
- 733 patients, 480 estimation group, 253 validation group
- Independent predictors of advanced (bridging or cirrhosis) fibrosis on MVA:
  - Age
  - BMI
  - AST/ALT ratio
  - Hyperglycaemia (FBG >6.1mmol/diabetes)
  - Platelet count
  - Albumin
Comparison of the Diagnostic Performance of the Simple Tests for F3/4 Fibrosis

<table>
<thead>
<tr>
<th>Tool</th>
<th>Cut-off</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<tr>
<td>ALT/ALT ratio</td>
<td>0.8</td>
<td>76</td>
<td>76</td>
<td>44</td>
<td>50</td>
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<tr>
<td>1</td>
<td>1</td>
<td>52</td>
<td>60</td>
<td>35</td>
<td>69</td>
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<tr>
<td>BMI</td>
<td>1.2</td>
<td>27</td>
<td>57</td>
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<td>64</td>
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<tr>
<td>BMI score</td>
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<td>89</td>
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<tr>
<td>SBP score</td>
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<td>76</td>
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<td>NAFLD fibrosis score</td>
<td>1.455</td>
<td>78</td>
<td>58</td>
<td>30</td>
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<tr>
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<td>0.87</td>
<td>73</td>
<td>78</td>
<td>79</td>
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</table>

ROC in model building group

AUC = 0.88 ± 0.02

Only 24% indeterminate

Risk score: \(-1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2\) + 1.13 \times \text{IFG/diabetes (yes=1, no=0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{NAFLD%fibrosis score} - 0.027 \times \text{PPV = 90%}

Performance of scoring system in validation group

Imaging: steatosis

- Magnetic Resonance Spectroscopy
- Magnetic Resonance Imaging
- CT
- Ultrasound (requires >33% steatosis)
- Currently, USS used to confirm suspected diagnosis of NAFLD, other techniques restricted to research studies
- Controlled Attenuation Parameter (CAP®) New technique based on elastography – uses the same equipment as “Fibroscan”

Imaging: NASH and Fibrosis

- No widely available imaging technique can reliably identify NASH or fibrosis in the absence of advanced cirrhosis
- Transient elastography (Fibroscan®)
  - BMI associated with technical failure
  - Improving data in adult NAFLD
  - Promising results in children
  - XL probe may be more reliable

Yoneda 2008, Wong 2010

Nobili 2008

Saadeh 2002

Myers et al. Liver Intern, 2012

McPherson, Gut 2010
### Fibroscan in NAFLD

- Wong 2010

### The Fibroscan XL Probe

- Myers Hepatology 2011

### Diagnostic accuracy of Fibroscan

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Cutoff (kPa)</th>
<th>Se (%)</th>
<th>Sp (%)</th>
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</thead>
<tbody>
<tr>
<td>Wong, 2010</td>
<td>247</td>
<td>10.3</td>
<td>92</td>
<td>87.8</td>
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<tr>
<td>Yoneda, 2008</td>
<td>97</td>
<td>17.5</td>
<td>100</td>
<td>96.6</td>
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<td>Nobili, 2008</td>
<td>50</td>
<td>10.2</td>
<td>100</td>
<td>100</td>
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</table>

A combination of AST/ALT ratio and fibroscan can reliably exclude advanced fibrosis in ~60-70% of patients.

For the rest liver biopsy is probably still indicated.

### Liver biopsy?

**AASLD/AGA/ACG Guidelines**

- Liver biopsy considered in patients with NAFLD who are at increased risk of NASH/advanced fibrosis.
- Metabolic syndrome and NAFLD fibrosis score may be used to identify the high risk patients.
- Biopsy should also be considered when other diagnoses cannot be excluded (GH, Autoimmune disease etc...)

### Summary: diagnosis

- Diagnosis currently relies on clinical features, blood tests, + USS
- For severity:
  - AST/ALT ratio < 0.8, Fibroscan < 10, NAFLD score < -1.455 excludes advanced fibrosis: NPV > 90%
  - Rest probably still require liver biopsy?
  - Validated NASH/fibrosis markers with PPV > 90% urgently needed
Therapies directed at features of the Metabolic Syndrome with potential “liver effects”

- Lifestyle changes directed at obesity and physical fitness
- Bariatric Surgery
- Insulin sensitizers
- Lipid-lowering agents
- Anti-hypertensives

Lifestyle changes: current status

- Weight reduction by lifestyle modification with diet and exercise should be recommended because it:
  - Improves cardiovascular risk profile
  - Improves steatosis
  - Probably ↓ inflammation (only with 7-9% weight loss)
- To date, no evidence of improved fibrosis
- Resistance = aerobic exercise
  - Hallsworth 2011
- Patients lack confidence to exercise
  - Frith 2010
- No independent effects of weight lowering drugs

Bariatric Surgery

- Histological effects:
  - Improves steatosis: 91% [82-98%]
  - Improves steatohepatitis: 81% [62-95%]
  - May improve fibrosis

⇒ NOT yet recommended as 1° treatment for NASH but NASH not a contraindication to Sx in an otherwise eligible patient
  - Chalasani 2012

Insulin sensitisers

- Metformin
  - Sound theoretical basis
  - Pilot data contradictory and recent RCT -ve
  - But: emerging evidence of anti-cancer effect including 62% ↓ in HCC in diabetics
  - Zhou 2001
  - ↓ Lipogenesis, ↑ fat oxidation
  - Lin 2000

- Glitazones
  - Sound theoretical basis & encouraging pilot data
  - Recent large RCT (in non diabetics) negative for fibrosis but beneficial effect on NASH
  - Sanyal NEJM 2010

Insulin sensitisers (1) metformin

- Sound theoretical basis
  - ↓ Lipogenesis, ↑ fat oxidation
  - Lin 2000
- Contradictory pilot data

- Improved hepatocyte ballooning in recent pediatric trial (“TONIC”)
  - 22/50 (44%) on metformin, vs 10/47 on placebo (21%), p=0.02
  - Lavine 2011
Insulin sensitisers (2) glitazones

- Sound theoretical basis - PPARγ agonists
  - Anti-steatotic: Maeda 2001, Mayerson 2002
  - Anti-fibrotic: Galli 2002
  - PPARγ mutations → NASH: Savage 2003
- Latest, largest RCT negative: Sanyal NEJM 2010

Lipid lowering agents

- Fibrates:
  - Good theory - PPARα agonists
  - No benefit in RCTs: Basaranoglu 1998, Laurin 1996
- Statins
  - Definitely safe in NAFLD patients
  - Do improve LFTs: Athyros Lancet 2010
  - May also ↓ HCC risk (OR: 0.63 [0.5-0.8]): El-Serag 2009

Anti-hypertensives

- RAS system
  - Anti-fibrotic:
      - 2011
  - Insulin sensitising (Telmisartan): Georgescu 2009
Anti-hypertensives: rationale for targeting RAS

- Drugs that induce HSC apoptosis will stimulate fibrosis reversion despite ongoing liver injury
- HSC have a RAS that prevents apoptosis

RelA pSer536 is associated with myofibroblasts in diseased human liver

Patient Histology Grade Average % Area of phospho-Ser536 RelA positive staining Responded
<table>
<thead>
<tr>
<th>Patient</th>
<th>HGC</th>
<th>F2</th>
<th>F3</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
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<tr>
<td>Patient 1</td>
<td>F1</td>
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<td>13.176</td>
<td>10.250</td>
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<tr>
<td>Patient 2</td>
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<td>6.196</td>
<td>5.216</td>
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<tr>
<td>Patient 3</td>
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<td>F2</td>
<td>18.800</td>
<td>7.550</td>
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<td></td>
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<tr>
<td>Patient 4</td>
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<td>F3</td>
<td>7.256</td>
<td>7.610</td>
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<tr>
<td>Patient 5</td>
<td>F2</td>
<td>F1</td>
<td>11.055</td>
<td>4.710</td>
<td>Yes</td>
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</tr>
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</table>

Tested “Liver-directed” therapies

- Antioxidants:
  - Vitamin E: benefit in 2 recent RCTs
    - Sanyal 2010, Lavine 2011
  - Pentoxifylline (anti-TNFα)
    - Zein 2011
  - Caspase inhibitors
    - GS-9450 (-1, -8 and 9) ↓ ALT
    - Ratziu 2012
  - Urso:
    - Neither 13-15 mg/kg nor 23-28 mg/kg/day any benefit in two large RCTs
    - Lindor 2004, Leuschner 2010
    - Urso + Vit E: encouraging pilot data
    - Dufour 2006
Vitamin E: panacea for NASH?

- PIVENS & TONIC indicate that Vitamin E may be effective in some patients

- BUT need for caution:
  - Increased all cause mortality risk at >400 IU/day
  - Increased haemorrhagic stroke risk (although reduced embolic stroke risk)
  - Increased prostate carcinoma risk

PTX in NASH RCT

- 26 patients on PTX 400mg for 1 year vs 29 on placebo

- Improved
  - NAS; p<0.001
  - Steatosis; p<0.001
  - Fibrosis; p=0.038

Recurrence of NASH post OLTx

- Contos 2001

Untested liver-directed therapies

- Probiotics
- ER stress (chaperones) – TUDCA, 4-phenylbutyrate
- IKK inhibitors (eg. Sulphasalazine)
- Anti-inflammatory/anti-fibrotic
- Improve NASH in experimental models
- Combined PPARs/PPARα agonists
- Peripheral CB, blockade
- Improves CHD and lipid metabolism
- FXR/TGR5 agonists

Newsome PN et al Guidelines for liver transplantation for patients with non-alcoholic steatohepatitis Gut 2012;61:484-500

Zein et al 2011

Miller 2005, Bjelakovic 2007

Schwartz, 2010

Lippman 2009, Klein 2011

Zein et al 2011

Ozkan Science 2006

Oakley 2005

Benza 2008

Carlou 2011

Jourdan 2012

Zhang 2009
Crosstalk Between BA Transport / Signaling & Hepatic Lipid Metabolism

A nuclear-receptor–dependent phosphatidylcholine pathway with antidiabetic effects

Summary: treatment
- Lifestyle advice for all patients with NAFLD
- For patients with NASH + DM
  - Metformin + Pioglitazone
- For patients with NASH + hypertension
  - A2RBs and Vit E
- Low threshold for statins (HCC*)
- For patients with NASH only
  - Best evidence for Vitamin E and should be considered as first line therapy

Acknowledgements

Farnesoid X receptor agonist WAY-364290 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis

NIH-Intercept funded trial of FXR agonist Obeticholic acid (OCA) ongoing

A ‘Virtuous Cycle’ of LRH-1 Activation…?

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