Histopathology of nonalcoholic fatty liver disease (NAFLD)

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Introduction

NAFLD (non-alcoholic fatty liver disease)

- A liver disease that resemble alcoholic liver disease in a patient who consume no alcohol
- NAFLD is the liver component of the metabolic syndrome defined as a clustering of metabolic complications of obesity:
  - **obesity** [body mass index (BMI) >30 kg/m²]
  - **visceral obesity** (waist/hip > 0.9 in males, 0.85 in females)
  - **glucose impairment** (fasting plasma glucose > 6.1 mmol/l; insulin resistance sd),
  - **dyslipidemia** (hypertriglyceridaemia and low high-density lipoprotein-cholesterol),
  - **systemic hypertension**
Introduction

A disease of emerging recognition - A public health problem

- Prevalence up to 25-30% in Western countries - the most common liver disease
- An often “silent” chronic liver disease,
  - may progress to “cryptogenic” cirrhosis
  - may result in hepatocellular carcinoma

NAFLD: a range of changes

<table>
<thead>
<tr>
<th>Condition</th>
<th>General Population</th>
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<tbody>
<tr>
<td>Steatosis</td>
<td>20%-30%</td>
</tr>
<tr>
<td>NASH</td>
<td>2%-3%</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>&lt; 1%</td>
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<tr>
<td>Cirrhosis</td>
<td></td>
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NASH: a 15% risk of cirrhosis within 5 yrs

HCC population 3.8%
- **Natural history of NAFL**
  - Uncomplicated steatosis
  - Steatohepatitis (NASH)
  - Cirrhosis & burnt-out NASH
  - Grading & staging
- Morphologic/evolutive variants
- Differential diagnosis
- Clinical correlation
- Physiopathology

<table>
<thead>
<tr>
<th>Natural history</th>
<th>Systematic histologic approach</th>
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<tbody>
<tr>
<td>Steatosis</td>
<td></td>
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<tr>
<td>Inflammation</td>
<td></td>
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<tr>
<td>Hepatocellular damage</td>
<td></td>
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<tr>
<td>Fibrosis</td>
<td></td>
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<td>Siderosis</td>
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</table>
Bland steatosis

Simple macrovesicular or mixed steatosis

Mild inflammation - *Inflammation does not constitute steatohepatitis*
Natural history

Steatosis

- Perivenular steatosis (most cases)
- Peri portal steatosis (children, parenteral nutrition, AIDS, malnutrition)
- Severe steatosis

Steatosis: mild (5-33%), moderate (33-66%), severe (>66%)
Hepatocellular damage; Fibrosis; Siderosis

Natural history

Steatohepatitis (NASH)

Steatosis - Slight inflammation - Hepatocellular ballooning in perivenular areas (inter observer variation) and/or perivenular/pericellular fibrosis (Fibrosis is not a required diagnostic feature)
Natural history

Steatohepatitis (NASH)

Hepatocellular ballooning and fibrosis in perivenular areas

Natural history

NAFLD

Additional features: Mallory bodies

Glycogenic nuclei
Natural history

Additional features: apoptotic bodies

Megamitochondria within hepatocyte cytoplasm

Natural history

Additional features: mild siderosis in periportal hepatocytes and/or acinar reticulo-endothelial cells
Fibrosis usually starts in acinar zone 3 (stage 1b).

Portal fibrosis more common in morbidly obese patients and pediatric NASH (stage 1c).

Portal/peripoportal plus zone 3 fibrosis (stage 2).

Bridging fibrosis (stage 3).
'Active' cirrhotic NASH (stage 4)
**Natural history**

**‘Burned out NASH’**

**Cryptogenic cirrhosis**

NAFLD is a significant cause of ‘cryptogenic’ cirrhosis (‘burned-out’ NASH)

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**Assessment of activity (grading) and fibrosis (staging)**

<table>
<thead>
<tr>
<th>NAFLD Activity Score (NAS)¹ (grading)</th>
<th>Assessment of activity (grading) and fibrosis (staging)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis grade (S)</td>
<td>Lobular inflammationᵃ (L)</td>
</tr>
<tr>
<td>0: &lt; 5%</td>
<td>0: None</td>
</tr>
<tr>
<td>1: 5%-33%</td>
<td>1: &lt; 2</td>
</tr>
<tr>
<td>2: 34%-66%</td>
<td>2: 2-4</td>
</tr>
<tr>
<td>3: &gt; 66%</td>
<td>3: &gt; 4</td>
</tr>
</tbody>
</table>

ᵃ. Counted in 20 x fields.
Total NAS score = S + L + B (range 0-8). A score of 5 or more is equivalent to NASH; a score of 3 or 4 is borderline NASH; a score of 2 or below equates to non-NASH NAFLD

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**Staging fibrosis¹**

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<table>
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</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No fibrosis</td>
</tr>
<tr>
<td>1</td>
<td>perisinusoidal or portal fibrosis</td>
</tr>
<tr>
<td>1a</td>
<td>Zone 3, perisinusoidal fibrosis, special staining (i.e. EvGᵃ) required for identification</td>
</tr>
<tr>
<td>1b</td>
<td>Zone 3, perisinusoidal fibrosis, detected with H &amp; E</td>
</tr>
<tr>
<td>2</td>
<td>Only periportal/portal fibrosis</td>
</tr>
<tr>
<td>3</td>
<td>Zone 3, plus portal/periportal fibrosis</td>
</tr>
<tr>
<td>4</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>

ᵃ. EvG Elastica-van-Gieson

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Natural history of NAFL

Morphological/ evolutive variants
- NAFLD concurrent with other liver diseases
- Pediatric NASH
- Liver transplantation
- Hepatocellular carcinoma

Differential diagnosis
Clinical correlation
Physiopathology

Morphologic variants
NASH + chronic hepatitis C

Importance of clinicopathologic correlation
- HCV infection coexistent with metabolic syndrome (5-10%)
- HCV infection with NAFLD features not related to metabolic sd:
  - Steatosis (genotype 3a) = «viral steatosis»
  - Insulin resistance (genotypes 1 and 4) = «metabolic steatosis»
Pediatric NASH

- 4–8% of children/adolescents are obese in western countries
- Lesions may or may not resemble those of adults
  - Type 1 (20%; most common in girls) resembles the adult pattern (zone 3 steatosis, ballooning and/or perisinusoidal fibrosis).
  - Type 2 (most common in boys and children with huge obesity) zone 1 steatosis or severe steatosis (with portal inflammation and/or fibrosis, without ballooning degeneration or perisinusoidal fibrosis)
- Prognosis?

Liver transplantation

- NASH: an increasing indication for liver transplantation
  - In Europe (34811 LT for cirrhosis, 01/1988 - 06/2006)\textsuperscript{ELTR 12/2004}: Cryptogenic (8%), active NASH (not precised)
  - In US (Pittsburgh; LT in 2012 adults, 06/1997 –06/2008)\textsuperscript{Malik, 2009}: Cryptogenic (NP); NASH (4.9%)
- Following liver transplantation\textsuperscript{Malik, 2009}
  - Recurrent NAFLD in 70%: NASH (24%), fibrosis (18%; at 18 months). No retransplantation (follow-up 3 years)
  - De novo NAFLD
Morphologic variants/ NAFLD evolution

**Steatosis**
General population
20%-30%

**NASH**
General population
2%-3%

**Fibrosis**
General population
< 1%

NASH: a 15% risk of cirrhosis within 5 yrs

**Cirrhosis**
HCC population
3.8%

HCC
HCC population
3.8%

A benign condition

HCC and metabolic syndrome

- Incidentally diagnosed at presentation (55%)
- ~0.5% of NAFLD cases develop HCC
  Relative risk in morbidly obese patients: from 2 to 4.5
  Diabetes: risk x 2
- NASH-related HCC ~3.8% of all HCC cases
- HCC occurs in the setting of:
  - Nonfibrotic liver (F0-F2) in 65% of cases
    some of them on a preexisting liver cell adenoma
  - As a late complication of NASH-related or cryptogenic cirrhosis
- HCC is more often well differentiated (65% versus 28%)
- Natural history of NAFL
- Morphologic/ evolutive variants
- **Differential diagnosis**
- Clinical correlations
- Physiopathology

### Alcoholic steatohepatitis (ASH)

#### Drug toxicity
- Amiodarone; Irinotecan; Tamoxifen; Glucocorticoids; highly active antiretroviral therapy in human immunodeficiency virus patients;

#### Surgical procedures
- Jejunoileal bypass; Bilio–pancreatic diversion; Extensive small bowel resection

#### Miscellaneous
- Total parenteral nutrition; Malnutrition; rapid weight loss
- Hepatitis C
- Аβ/hypoβ lipoproteinemia; Weber-Christian disease (nodular panniculitis); lipodystrophy

**Causes of fatty liver disease not primarily associated with insulin resistance**
Differential diagnosis

Alcoholic steatohepatitis (ASH)

Often undistinguishable, but
Abundant neutrophils
Mallory bodies
Perivenular lobular changes
- Natural history of NAFL
- Morphologic/ evolutive variants
- Differential diagnosis
- Clinical correlation
- Physiopathology
**Clinical correlation**

- In adults (fifth-sixth decades of life) and children

- **Risk factors: the metabolic syndrome** (at least 3 risk criteria) or its individual components
  - Obesity [body mass index (BMI) > 30], especially abdominal obesity (waist/hip > 0.9 in males/0.85 in females or waist circumference > 94 cm in males/80 cm in females)
  - Dyslipidemia (triglycerides ≥1.7 mmol/l or high-density lipoprotein cholesterol < 0.9 mmol/l in males/1 mmol/l in females)
  - Hypertension (blood pressure > 140/90 mm Hg)
  - Insulin resistance (baseline glycemia > 6.1 mmol/l)

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**Clinical correlation**

- Patients usually asymptomatic

- Incidental detection
  - Unexplained persistently mildly raised transaminase levels and γ-glutamyltranspeptidase
  - Imaging performed for other reason

- Noninvasive assessment of steatosis and fibrosis

- Liver biopsy, the gold standard, is controversial. Indications:
  - To rule out other causes of liver disease/comorbidities (viral hepatitis, drug reaction, autoimmune hepatitis, Wilson disease)
  - Before liver transplantation, during bariatric surgery: To confirm NAFL - distinguish steatosis from NASH - staging fibrosis
  - Hepatocellular carcinoma??
- Natural history of NAFL
- Morphologic/ evolutive variants
- Differential diagnosis
- Clinical correlation
- **Physiopathology**

- Uptake of free fatty acids
- De novo lipogenesis
- β-oxidation of free fatty acids
- Synthesis/ secretion of VLDL

**Two 'hits'?** Day and James, 1998

**First hit:** liver steatosis

Food
Visceral adipose tissue
uptake of free fatty acids

Lipogenesis

Insulin resistance
- peripheral lipolysis
- Glucose uptake by musculature

Two ‘hits’?

First hit: liver steatosis

Food
Visceral adipose tissue

uptake of free fatty acids

Lipogenesis

Insulin resistance
- peripheral lipolysis
- Glucose uptake by musculature

adipokines (adiponectin, leptin, resistin, TNF)

Second hit: oxidative stress
- abnormal cytokines production

Food
Visceral adipose tissue
Take-home messages

- **NAFLD is the manifestation of the metabolic sd**
  Not all patients with NASH are overweight

- **A public health problem in adults and children**, given
  the rising incidence of obesity and metabolic sd

- **Natural history ranges** from indolent to end-stage liver
  disease
  
  Non-NASH steatosis has a benign clinical course
  
  NASH can progress to cirrhosis and HCC

Take-home messages

- **Cryptogenic cirrhosis may have previously unrecognized NAFLD** (‘burned-out’NASH)

- **HCC can occur at any stage of NASH**
  
  NASH is often ignored until HCC is discovered

- **Regular screening for liver cirrhosis and HCC is important for patients with obesity or metabolic sd**
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