New Concept in Histopathology of NASH

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NAFLD: An Epidemic Disease

Global prevalence of NAFLD among T2DM patients 55.5% (95% confidence interval: 47.3-63.7)

Younossi ZM J Hepatol 2019
NAFLD
Hepatic Manifestation of Metabolic Syndrome

From Siegel Cancer 2009
Natural History of NAFLD
A dynamic disease

From Rinella ME J Hepatol 2019
Epidemiology of chronic liver diseases in the USA in the past three decades

- National Health and Nutrition Examination Surveys (NHANES)

Figure 2: Relative changes in the prevalence of chronic liver disease aetiologies (reference: 1988–1994 cycle). NAFLD, non-alcoholic fatty liver disease.
NAFLD for the Pathologist: Key points

1. Hepatic fat accumulation (>5% Steatosis) associated with insulin resistance

2. 2 pathologically distinct conditions
   - NAFL (steatosis) and NASH (steato-hepatitis)

3. NASH: a wide spectrum of disease severity
   - Fibrosis, Cirrhosis, and Hepatocellular carcinoma

4. Liver biopsy required for NASH diagnosis
   - Clinical, biochemical or imaging measures cannot distinguish NASH from steatosis

Recommendations

| NASH has to be diagnosed by a liver biopsy showing steatosis, hepatocyte ballooning and lobular inflammation | A | 1 |

EASL-EASD-EASO Clinical Practice Guidelines for the management of NAFLD
J Hepatol 2016
➢ Acute Alcoholic Hepatitis-like: Steatosis + Mallory hyaline + Polymorphonuclears


➢ Steatosis + ballooning + mixed acute and chronic lobular inflammation + zone 3 perisinusoidal fibrosis

*(Brunt E. Am J Gastroenterol 1999)*

➢ Steatosis + inflammation + hepatocellular ballooning

NASH « in a glance »

- Steatosis
- Ballooning
- Lobular inflammation
- Portal fibrosis
- Centrolobular fibrosis
Liver Biopsy: The « Reference » Standard

Issues
1. Assess a diagnosis of NASH
2. Evaluate the severity of the disease
   ▪ Activity (SH)
   ▪ Stage (Fibrosis)
3. Identify potential comorbidity risk factors

Recommendations
1. 15-20 mm long, > 10 portal tracts
2. Experienced liver pathologist
3. Zonal distribution / Sampling variability
New Concept in Histopathology of NASH

➢ Scoring Systems
➢ The Future
SCORING SYSTEMS
... to NASH

Table 1. Necroinflammatory Grading System for Steatohepatitis

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild, grade 1</td>
<td>Steatosis (predominantly macrovesicular) involving up to 66% of biopsy; may see occasional ballooned zone 3 hepatocytes; scattered peri- and intra-acinar lymphocytes; no or mild portal chronic inflammation.</td>
</tr>
<tr>
<td>Moderate, grade 2</td>
<td>Steatosis of any degree; ballooning of hepatocytes (predominantly zone 3) obvious; peri- and intra-acinar pml's noted, may be associated with zone 3 pericellular fibrosis; portal and intra-acinar chronic inflammation noted, mild to moderate.</td>
</tr>
<tr>
<td>Severe, grade 3</td>
<td>Panacinar steatosis; ballooning and disarray obvious, predominantly in zone 3; intra-acinar inflammation noted as scattered pml's, pml's associated with ballooned hepatocytes ± mild chronic inflammation; portal chronic inflammation mild or moderate, not marked.</td>
</tr>
</tbody>
</table>

Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease

- Steatosis (%) from 0 to 3
- Lobular inflammation from 0 to 3
- Ballooning from 0 to 2

NASH Clinical Resarch Network (NASH CRN)
Kleiner D Hepatology 2005

Brunt E Am J GastroEnterol 1999
A composite (Not diagnostic) score

NAFLD
A continuous morphological spectrum with many intermediates

NAS = 4
- Steatosis 3 + Infl 1: Not NASH
- Steatosis 1 + Infl 1 + ball 2: NASH

Kleiner D Hepatology 2005
Scoring system assessing semi quantitatively and separately
- Steatosis (0-3)
- Activity (0-4)
  - Ballooning (0-2)
  - Lobular inflammation (0-2)
- Fibrosis (0 – 4)

SAF \[S_0 \cdot A_0 \cdot F_0 \cdot 4\]

- Morbidly obese patients (n=679)
- Validation set of patients with MS (n=60)

Bedossa P et al Hepatology 2012
Activity score (SAF) strongly correlated with diagnosis of NASH

(92% of patients with A ≥2 had NASH)
Use of the SAF decreased interobserver variations among pathologists (with various levels of experience)
Some (non anecdotic) nuances

**NAS**
- All features combined
- Not diagnostic score
- Steatosis
  - From 1 to 3
- Ballooning
  - Few / Many
- Lobular inflammation
  - From 1 to 3

**SAF**
- Separately assessment
- Diagnostic score
- Steatosis
  - From 1 to 3
- Ballooning
  - Normal size / Large
- Lobular inflammation
  - From 1 to 2
Fibrosis in NASH

➢ Not a morphological diagnostic criteria but a strong prognostic factor of long-term outcome
  ▪ Complication (cirrhosis, HCC, ...)

➢ The most relevant histological endpoint

➢ « Burn-out NASH »
  ▪ Steato-Hepatitis ⇓ while fibrosis ⇆ (advanced stages)
NAFLD Staging (F)

Lobular Fibrosis

Portal Fibrosis

Brunt E Am J GastroEnterol 1999

Stage 1. Zone 3 perisinusoidal/pericellular fibrosis; focally or extensively present.
Stage 2. Zone 3 perisinusoidal/pericellular fibrosis with focal or extensive periportal fibrosis.
Stage 3. Zone 3 perisinusoidal/pericellular fibrosis and portal fibrosis with focal or extensive bridging fibrosis.
Stage 4. Cirrhosis.

Kleiner D Hepatology 2005

<table>
<thead>
<tr>
<th>Staging</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Perisinusoidal or periportal</td>
<td>1</td>
</tr>
<tr>
<td>Mild, zone 3, perisinusoidal</td>
<td>1A</td>
</tr>
<tr>
<td>Moderate, zone 3, perisinusoidal</td>
<td>1B</td>
</tr>
<tr>
<td>Portal/periportal</td>
<td>1C</td>
</tr>
<tr>
<td>Perisinusoidal and portal/periportal</td>
<td>2</td>
</tr>
<tr>
<td>Bridging fibrosis</td>
<td>3</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>4</td>
</tr>
</tbody>
</table>

Staging is robust (very low inter-observer variability, \( \kappa \) 0.83)

[Kleiner D Hepatology 2005]
Collagen Proportionate Area (quantitative analysis of collagen)

Staging: Lack of granularity

F2 (CPA 8.1%)
F3 (CPA 4.8%)
F3 (CPA 12%)
<table>
<thead>
<tr>
<th>NASH CRN</th>
<th>EPOS</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>1</td>
<td>Lumping together because:</td>
</tr>
<tr>
<td>1b</td>
<td></td>
<td>- Poor reproducibility, Sampling error</td>
</tr>
<tr>
<td>1c</td>
<td></td>
<td>- No clinical relevance</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Changing definition:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Central or Portal fibrosis extending to the midzone or portal + central fibrosis</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Increased granularity:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Few septa (no more than 2/10mm length of biopsy)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Many septa without nodule formation</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Increased granularity:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Many septa with occasional nodules</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>

**Glass vs e-slides**
- Intra observer agreement 0.83 (very good)
- Inter observer agreement 0.86 (very good)

Bedossa P et al EASL 2018
Changes in liver histology (paired biopsy, central review)

- Resolution of NASH
  - Complete resolution of hepatocyte ballooning, with inflammation score <1, in addition to no worsening of fibrosis
- Numerical reduction in scoring systems
  - ≥2 point improvement in total score be achieved with contribution from multiple parameters of the NAS, alongside no worsening of fibrosis
- Regression of fibrosis
  - At least 1 stage (+ CPA measurement), without worsening of SH
THE FUTURE
1. Improve accuracy of the histological analysis

➢ From a semi-quantitative (subjective) to a quantitative (objective) analysis using digital pathology

➢ More sensitive
  ▪ Assess more subtle changes
  ▪ Faster detection of treatment benefit

➢ Features easily quantified
  ▪ Steatosis
  ▪ Fibrosis (sirius red)
  ▪ Collagen Proportionate area (CPA)
DUAL PHOTON MICROSCOPY BASED QUANTITATION OF FIBROSIS-RELATED PARAMETERS (Q-FP) TO MODEL DISEASE PROGRESSION IN STEATOHEPATITIS

- Fibrosis-related parameters
  - collagen content, geometric and textural features of collagen fibers, number of cross-linked collagen fibers, ...

- 4 μm thick section without any specific staining

- 50 (test) and 42 (validation cohort) subjects with NAFLD

- 70 q-FPs measured in specific predefined regions of interest
  - Related to the NASH CRN fibrosis staging
  - 4-q-FPs independently associated with fibrosis stages

Wang Y Hepatology 2017
2. Define specific lipidomic *in situ* signatures

- Lipid species (toxic or protective)
  - **Steatosis**
  - **Steato-hepatitis**

- Combines a proteomic approach (MS) with morphological analysis
  - Spatial distribution of biomolecules in intact tissue samples
  - **Hundreds** of proteolytic ions are detected *simultaneously* on a single tissue section *without* the need for target-specific reagents

- **A**/ HE
- B/ m/z 772,55
- C/ m/z 820,54
- D/ m/z 760,61

(collaboration CEA C Junot)
3. Develop relevant experimental models

A multi-hit disease

A druggable disease

➢ No experimental models able to
  ▪ completely recapitulate the whole spectrum of the disease
  ▪ evaluate drug responses

Friedman S Nat Med 2018
Konerman J J Hepatol 2018
Human Multilineage 3D Spheroids as a Model of Liver Steatosis and Fibrosis

- *in vitro* model of human NAFLD with genetic predisposition
  - 3D spheroids [Hepatocytes (HepG2) and hepatic stellate cells (LX-2) homozygotes for the PNPLA3 I148M sequence variant]

- Compactness of the structure of HepG2 cells by co-culture with LX-2 cells (even at the lowest ratio of 24:1)

Pingitore P Int J Med Sci 2019
➢ Incubation with Fatty acids promotes lipid accumulation after 48h

➢ Incubation with Fatty acids results in fibrosis after 48h

Pingitore P Int J Med Sci 2019
Prevention of steatosis by drug treatment
NAFLD: A histological diagnosis, Liver biopsy “The referent standard”
- Diagnosis assessment, grading and staging
- Required for patient eligibility and drug evaluation (phase 3 CT)

Scoring systems: still useful
- NAS: a key-step, commonly used, not a diagnostic score
- SAF: more appropriate, need to be validated
- Fibrosis: current staging not enough discriminant

Liver biopsy: still unused
- Towards quantitative and *sans a priori* morphological approaches
Beaujon hospital
- Pathology (V Paradis)
- Radiology (V Vilgrain)
- Hepatology (F Durand)
- Liver surgery (O Soubrane)
- UNITY (D Valla)

Inserm U 1149, CRI
- « From inflammation to cancer in digestive diseases » (V Paradis)
  - A Couvelard, N Guedj, J Cros, V Rebours, A Beaufrère, N Poté
  - F Cauchy, L de Mestier (Doc)
    - S Paisley, S Frendi, O de Rycke (M2)
    - S Laouirem, J Le Faouder, M Albuquerque (IE)