Non-alcoholic fatty liver disease (NAFLD)
Atlas of histological images
Guidelines for diagnosis and scoring

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The NAFLD Atlas

• **Aim:** To provide a set of histological images useful to diagnose and score NAFLD in a consistent manner

• The selected images show either typical features of NAFLD or borderline lesions

• Multiple choice questions for self-testing follow the Atlas

• The Atlas is an ongoing LITMUS initiative that can be complemented by additional relevant images

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LITMUS aims to develop, robustly validate and advance towards regulatory qualification biomarkers that diagnose, risk stratify and/or monitor NAFLD/NASH progression and fibrosis stage.
The Liver Biopsy Report form used by the LITMUS Histopathology Group (LHG) members. It includes relevant items with sufficiently robust interobserver agreement.

Example of a reported liver biopsy with NAFLD in an Excel sheet used by LHG.

<table>
<thead>
<tr>
<th>Biopsy Reference</th>
<th>EX123659875</th>
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<tbody>
<tr>
<td>Biopsy_length (mm)</td>
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</tr>
<tr>
<td>Biopsy_nr_fragments</td>
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</tr>
<tr>
<td>Biopsy_Adequacy</td>
<td>Adequate</td>
</tr>
<tr>
<td>If inadequate, because</td>
<td></td>
</tr>
<tr>
<td>Steatosis</td>
<td>Grade 2</td>
</tr>
<tr>
<td>Microvesicular steatosis (patch)</td>
<td>Yes</td>
</tr>
<tr>
<td>Ballooning CRN</td>
<td>1</td>
</tr>
<tr>
<td>Ballooning SAF</td>
<td>1</td>
</tr>
<tr>
<td>Lob_Inflammation CRN</td>
<td>1</td>
</tr>
<tr>
<td>Lob_Inflammation SAF</td>
<td>2</td>
</tr>
<tr>
<td>Activity_NAS</td>
<td>4</td>
</tr>
<tr>
<td>Activity_A (SAF)</td>
<td>3</td>
</tr>
<tr>
<td>Portal_Inflammation</td>
<td>None or Minimal</td>
</tr>
<tr>
<td>Mallory-Denk bodies</td>
<td>None or Minimal</td>
</tr>
<tr>
<td>Mallory-Denk body</td>
<td>None or Minimal</td>
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<tr>
<td>Fibrosis (NASH CRN)</td>
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</tr>
<tr>
<td>Fibrosis (EPoS)</td>
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</tr>
<tr>
<td>Lobular inflammation</td>
<td>0</td>
</tr>
<tr>
<td>Activity</td>
<td>1</td>
</tr>
<tr>
<td>Other features</td>
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<tr>
<td>Other aetiology</td>
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</tr>
<tr>
<td>Diagnostic Category</td>
<td>Definite NASH</td>
</tr>
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</table>

The Liver Biopsy Report form used by the LITMUS Histopathology Group (LHG) members. It includes relevant items with sufficiently robust interobserver agreement.
Note that the number of portal tracts is not reported here. The pattern of injury in NAFLD is centrilobular (acinar zone 3), therefore the number of portal tracts is not as relevant as in other liver diseases. Furthermore, in advanced fibrosis/cirrhosis portal tracts may be difficult to identify.
LIVER BIOPSY REPORT FORM
1. Quality of biopsy

• **Number of fragments:**
  - Assessed on haematoxylin & eosin (H&E)
  - Ignore small splits if the core is in the same orientation
  - Ignore less than 1 mm fragment(s) (except if all are 1 mm fragments)
  - Ignore blood clots or not liver tissue fragments

• **Serial sections on the glass slides**
  - Use the section with most tissue present

• **Adequacy:**
  - Marginal quality means that some but not all features can be assessed or that the biopsy can be assessed but only with relative confidence because of technical issues
  - If marginal, form can be filled in at least partially. Items that cannot be assessed are left blank
  - If inadequate, the form cannot be filled at all
Small splits but 1 single fragment
fragments not counted
Number of fragments: 5
Number of fragments: 3
Biopsy Inadequate: fragmentation
(Attrition of material in paraffin block after multiple recuts)
Biopsy inadequate: technical issues
Biopsy marginally adequate: cannot stage fibrosis because of fragmentation but steatosis and other features can be assessed.
Biopsy marginally adequate: fragmentation but fibrosis may be assessed with relative confidence
**STEATOSIS**

Grade 0
Grade 1
Grade 2
Grade 3

**MICROVESICULAR STEATOSIS (PATCH) **

NO
YES

* Patch is defined as a group of at least 10 hepatocytes
Steatosis

• Grading according to NASH-CRN* (<5%, 5-33%, >33-66%, >66% of hepatocytes involved by steatosis)

• % of hepatocytes with either large (macrovesicular) or small (mediovesicular) fat droplets (not minute droplets/foamy cytoplasm)

• Assessed on H&E stained slide

• Assessed at low magnification (x4)

• When the extent of steatosis is variable along the biopsy core(s), take into account the whole section area

*NASH CRN: Nonalcoholic Steatohepatitis Clinical Research Network, National Institutes of Health, USA
GRADE 1 STEATOSIS
GRADE 2 STEATOSIS
GRADE 2 STEATOSIS
GRADE 3 STEATOSIS
GRADE 3 STEATOSIS

Masson trichrome
Microvesicular steatosis

(patch: a group of at least 10 hepatocytes with foamy cytoplasm)
Microvesicular steatosis (patch)
<table>
<thead>
<tr>
<th></th>
<th>NASH CRN</th>
<th>SAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>1</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>2</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>
• HEPATOCYTE BALLOONING

• Round shape **AND** clear, pale or reticulated cytoplasm

• Diagnosis on H&E and/or Masson trichrome (not with immunostaining)

• Scoring of ballooning:
  - According to NAS (0=no, 1=few, 2=many, prominent) (Kleiner et al. Hepatology 2005)
  - According to SAF (0,1,2)*
    0: normal hepatocytes with cuboidal shape, sharp angles and pink eosinophilic cytoplasm or rounded hepatocytes without cytoplasmic clearing
    1: presence of clusters of hepatocytes with round shape and pale cytoplasm, usually reticulated. Although the cell shape is different, the size is similar to that of normal hepatocytes
    2: as for score 1, but where there is also at least one enlarged ballooned hepatocyte (at least twice the size [2x] of a normal hepatocyte, within a cluster of hepatocytes with score 1 ballooning)

*Bedossa P, FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of non-alcoholic fatty liver disease. Hepatology 2014; 60:565-575.
Ballooning 0: few ballooned hepatocytes without cytoplasmic clearing
Ballooning 0: clear hepatocytes without round shape
Ballooning 1 (SAF & NASH CRN)

blue arrow: ballooned hepatocytes
white arrow: clear but not ballooned hepatocytes
Ballooning 1 (NASH CRN and SAF)
Ballooning 2 (NASH CRN and SAF)
Ballooning 2 (NASH CRN and SAF)
Ballooning 1 (NASH CRN, few ballooned hepatocytes)
Ballooning 2 (SAF, enlarged balloned hepatocytes)
Ballooning 2 (NASH CRN & SAF, many ballooned and enlarged hepatocytes)
Lobular inflammation

- Based on global assessment (not hot spots)
- Consider foci (clusters), not linear pattern of intrasinusoidal inflammatory cells
- Foci with more than 2 inflammatory cells
- x20 magnification

Scoring:
- According to NAS (1 to 3)
  - NASH CRN: 0 None; 1 < 2 foci; 2 2-4 foci; 3 > 4 foci

- According to SAF (1-2)
  - SAF: 0 none; 1 ≤2 foci; 2 >2 foci
Lobular inflammation 0
Lobular inflammation 1 (NASH CRN & SAF)
Lobular inflammation 1 (NASH CRN & SAF)
Lobular inflammation 2 (NASH CRN & SAF)
Lobular inflammation 3 (NASH CRN)
Lobular inflammation 2 (SAF)
Lobular inflammation 3 (NASH CRN)
Lobular inflammation 2 (SAF)
• **Portal inflammation**
  • Assess portal and/or septal inflammation
  • Lymphocytes, macrophages, plasma cells...
  • Assessed on hot-spots
Portal inflammation 1 (mild)
Portal inflammation 2 (more than mild)
Portal inflammation 2 (more than mild)
Portal inflammation 2 (more than mild)
• Mallory-Denk Bodies (MDB)
  • Few = at least 1, often not easy to recognise because they are rare or incomplete
  • Many = numerous and easy to recognise (might suggest associated ALD)
  • On H&E (not with immunostains)

<table>
<thead>
<tr>
<th>MALLORY-DENK</th>
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<tbody>
<tr>
<td>• No</td>
</tr>
<tr>
<td>• Few</td>
</tr>
<tr>
<td>• Many</td>
</tr>
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</table>
Incomplete/immature MDB
Fully developed MDB
MDB 2 (many)
MDB 2 (many)
Stage of fibrosis

• Stage according NASH-CRN (stage 0 to 4) and according to EPOS (stage 0 to 6)*
• Ignore subcapsular area (evaluate at a distance > 5mm from the capsule)
• Fibrous septa: from one vascular structure to another or a continuous transversal fibrous bridge that crosses the whole width of the biopsy
• Fibrous septa: thin or thick. Ignore bridging collapse with residual hepatocytes between individual collagen fibres

Stage of fibrosis  (NASH CRN)

- None = 0
- Perisinusoidal or periportal = 1
  - Mild, zone 3, perisinusoidal = 1A
  - Moderate, zone 3, perisinusoidal = 1B
  - Portal/periportal = 1C
- Perisinusoidal and portal/periportal = 2
- Bridging fibrosis = 3
- Cirrhosis = 4
Stage of fibrosis (NASH CRN)
EPOS staging of fibrosis (0-6)

<table>
<thead>
<tr>
<th>FIBROSIS (EPOS)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6a</td>
</tr>
<tr>
<td>6b</td>
</tr>
<tr>
<td>6c</td>
</tr>
<tr>
<td>NASH CRN</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1a</td>
</tr>
<tr>
<td>1b</td>
</tr>
<tr>
<td>1c</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>4</td>
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<td></td>
</tr>
</tbody>
</table>
EPOS staging of fibrosis (0-6)

- **Stage 0**: none or scant fibrosis (perivenular and/or portal)
  Mild portal fibrosis without periportal expansion OR scant perivenular fibrosis around up to 2 terminal hepatic venules with only delicate sinusoidal fibrosis but without pericellular fibrosis

- **Stage 1: Mild fibrosis.** Perivenular and pericellular fibrosis (up to 5 hepatocytes, fibrosis all around hepatocyte) and/or sinusoidal (linear) fibrosis involving more than 2 terminal hepatic venules OR Periportal fibrosis (collagen fibers extending to the periportal parenchyma in at least 1 portal tract)

- **Stage 2: Moderate fibrosis.** Sinusoidal and/or pericellular fibrosis extending from the portal tract and/or perivenular zone to the midzone without complete septa

- **Stage 3: Occasional bridging/septal fibrosis.** At least 1 complete fibrous septum and no more than 2 septa /10mm biopsy length (a septum is a fibrous band that transverses the biopsy or links 2 vascular structures. Avoid subcapsular area (evaluate at a distance > 0.5 cm from the capsule)

- **Stage 4: Advanced bridging/septal fibrosis.** More than 2 fibrous septa /10mm, no nodule

- **Stage 5 : Incomplete cirrhosis.** Septal fibrosis with isolated nodule/s (completely circumscribed by fibrosis) Avoid subcapsular area (evaluate at a distance > 0.5 cm from the capsule)

- **Stage 6 : cirrhosis** (6a – 6b – 6c). According to the Laennec staging system (see next slide)
Substaging of cirrhosis (4 a, b, c / Laennec staging system)*

Based on the thickness of fibrous septa and width of hepatocyte nodules

4a  Marked septation with rounded contours or visible nodules
    Most septa are thin (one broad septum allowed)

4b  At least two broad septa, but no very broad septa and
    less than half of biopsy length composed of minute nodules

4c  At least one very broad septum or more than half of biopsy length
    composed of minute nodules (micronodular cirrhosis)

  progressive hepatic fibrosis during long-term therapy with deferiprone in subjects
EPOS staging of fibrosis (0-6)

Masson trichrome
Stage 0 (NASH CRN and EPOS)
Stage 0 (tangential section of portal tract)
Stage 1a (NASH CRN), Stage 0 (EPOS)

CV: central venule

Masson trichrome
Stage 1b (NASH CRN), Stage 1 (EPOS)
Stage 1b (NASH CRN), Stage 1 (EPOS)

Masson trichrome
Stage 1c (NASH CRN), Stage 1 (EPOS)
Stage 2 (NASH CRN and EPOS)

Masson trichrome
Stage 2 (NASH CRN and EPOS)

Masson trichrome
Stage 2 (NASH CRN and EPOS)
Stage 3 (NASH CRN and EPOS, real fibrous septum crossing biopsy)
Stage 3 (NASH CRN and EPOS, fibrous septum)
Stage 2 (NASH CRN and EPOS)

- This is perisinusoidal fibrosis between hepatocyte trabeculae linking 2 vascular structures. Compared to previous slides, this is not a fully structured septum (septum in progress).
Stage 3 (NASH CRN), Stage 4 (EPOS)

- Many fibrous septa
- No nodular morphology

Masson trichrome
Stage 3 (NASH CRN), Stage 4 (EPOS)
Stage 3 (NASH CRN), Stage 4 (EPOS)
Stage 3 (NASH CRN), Stage 5 (EPOS)

- Many fibrous septa
- Focal but not complete nodular morphology
- Incomplete cirrhosis
Stage 3 (NASH CRN), Stage 5 (EPOS)
Stage 3 (NASH CRN), Stage 5 (EPOS)
Stage 4 (NASH CRN), Stage 6 (EPOS)
DIAGNOSTIC CATEGORY

• Normal

• NAFL (steatosis without definite NASH)

• Definite NASH

• Fibrosis / Cirrhosis without steatosis
  • Consistent with NAFLD
  • No NAFLD

• Other etiology

ADDITIONAL COMMENTS
CRITERIA FOR DEFINITION OF CATEGORIES

- **NORMAL**: fat in less than 5% of hepatocytes
- **NAFL** (steatosis without definite NASH features)
  - Fat only or
  - Fat and inflammation but no ballooning or ballooning but not inflammation
  - Fat and fibrosis or fat and cirrhosis
- **NASH**
  - Fat and
  - Ballooning and
  - Lobular Inflammation
- **NASH cirrhosis**:
  - Cirrhosis with steatosis+ballooning+inflammation
- **Fibrosis / Cirrhosis without steatosis**:
  - Consistent with NAFLD (presence of other NASH features/s i.e. ballooning, MDB, perisinusoidal fibrosis....)
  - No features of NAFLD («burned-out cirrhosis »)
- **Other**: possible other liver disease isolated or associated with NAFLD (granulomas, autoimmune hepatitis, .........)
Multiple Choice Questions

(correct answers follow at the end of the document)
How many fragments would you count in this biopsy?

- 1 = 3
- 2 = 6
- 3 = 2
- 4 = 1
Multiple Choice Question 2

• What is the grade of steatosis?
  1. Grade 0
  2. Grade 1
  3. Grade 2
  4. Grade 3
Multiple Choice Question 3

• Does this picture fulfill the criteria of:

1. NAFLD
2. NAFL
3. NASH
4. I don’t know
Multiple Choice Question 4

• Does this picture fulfill the criteria of:

1. NAFLD
2. NAFL
3. NASH
4. I don’t know
In this picture what are the features that are mandatory for the diagnosis of NASH?

1. Steatosis
2. Ballooning/clarification
3. Lobular Inflammation
4. Perisinusoidal fibrosis
5. Predominant in Zone 3
Multiple Choice Question 6

• How would you score this NASH case according to NAS?

1. <4
2. 5
3. 6
4. >6
Multiple Choice Question 7

- How would you grade Activity of this NASH case according to SAF?

1. A=1  
2. A=2  
3. A=3  
4. A=4
Multiple Choice Question 8

• How would you substage this cirrhosis case according to the Laennec staging system?
  • 1= 4a
  • 2= 4b
  • 3= 4c
  • 4= 4d
Multiple Choice Question 9

• How would you stage fibrosis in this biopsy according to NASH CRN?

• 1 = 2
• 2 = 3
• 3 = 4
• 4 = 5
Multiple Choice Question 10

• How would you stage fibrosis in this biopsy according to EPOS?

  • 1 = 3
  • 2 = 4
  • 3 = 5
  • 4 = 6
Answers to MCQ

- MCQ 1: 1
- MCQ 2: 3
- MCQ 3: 1, 2
- MCQ 4: 1, 3
- MCQ 5: 1, 2, 3
- MCQ 6: 4
- MCQ 7: 4
- MCQ 8: 1
- MCQ 9: 2
- MCQ 10: 3