Innovation in histological assessment of NASH
Relevance for biomarker discovery and validation

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Conflicts of Interest

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Histological assessment of NASH

Histology in NAFLD:
• NASH is defined by histology
• A gold standard for biomarker discovery and validation (imperfect)
• A primary endpoint in clinical trials (surrogate)

Caveats and limits of LB:
• Relative: sampling error, observer variation
• Absolute: invasive procedure

→ Histology still a central position in this field
Innovation in histological assessment of NASH
Relevance for biomarker discovery and validation

Agenda

1. Change in paradigm: from a dichotomous approach to a multidimensional continuous scaling system

2. Limit and Progress in evaluation of fibrosis

3. Advances in Histotechnologies and relevance for biomarker

Non Alcoholic Fatty Liver Diseases (NAFLD)

STEATOSIS (NAFL)

STEATOHEPATITIS (NASH)
THE NATURAL HISTORY OF NAFLD

STEATOSIS (NAFL)

STEATOHEPATITIS (NASH)

FIBROSIS

CIRRHOSIS

HCC
NASH: AN ENTITY BASED ON ASSOCIATION OF HISTOLOGICAL PATTERNS
UNDER THE LENS: THE 3 HISTOLOGICAL COMPONENTS OF NAFLD

FLIP consortium, Hepatology 2012, Hepatology 2014

- STEATOSIS: THE MARKER
- ACTIVITY: THE DRIVER
- FIBROSIS: THE KILLER
MANY VARIATIONS OF NAFLD
A MULTIDIMENSIONAL CONTINUOUS SCALING SYSTEM
Relevance for biomarkers

<table>
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<tr>
<th>STEATOSIS</th>
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Relevant with Liver Forum, Disease Definition WG
A MULTIDIMENSIONAL CONTINUOUS SCALING SYSTEM
Relevance for biomarkers and treatment

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- To release
- To be treated
- To follow
Agenda

• From a dichotomous to a multidimensional continuous scaling system

• Limit and Progress in scoring of fibrosis

• Advances in Histotechnologies and relevance for biomarker discovery
Histologic assessment of NASH
Limit and Progress in scoring of fibrosis

• Fibrosis: the most robust readout in NAFLD

• The NASH CRN fibrosis score*:
  – From 0 (normal liver) to 4 (cirrhosis)
  – Most widely used scoring system
  – Highly reproducible
  – Clinically meaningful**

* Kleiner D et al, Hepatology 2005
** Angulo P et al, Eksted M et al, Younossi Z et al
STAGE OF FIBROSIS - WHAT WE HAVE NOW AND WHAT WE NEED

WHAT WE HAVE

CIRRHOsis

F0 F1a F1b F2

F3

F4

NORMAL LIVER

WHAT WE NEED

CIRRHOsis

F1b

NORMAL LIVER


CPA
### NASH-CRN to EPOS Comments

<table>
<thead>
<tr>
<th>NASH-CRN</th>
<th>EPOS</th>
<th>Comments</th>
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<tbody>
<tr>
<td>1a</td>
<td>1</td>
<td><strong>Lumping together</strong> because:</td>
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<tr>
<td>1b</td>
<td></td>
<td>- Poor reproducibility, Sampling error</td>
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<tr>
<td>1c</td>
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<td>- No clinical relevance</td>
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</tbody>
</table>
| 2        | 2    | **Changing definition**:
|          |      | central or portal fibrosis + lobular fibrosis or portal + central fibrosis |
| 3        | 3    | **Increase granularity**:
|          |      | Few septa (no more than 2 /10mm length of biopsy) |
|          | 4    | Many septa (> 2....) without nodule |
| 4        | 5    | **Increase granularity**:
|          |      | Many septa with few nodules |
|          | 6    | Annular fibrosis with complete nodulation |

- More linearity: easier for biomarker discovery
- A highly reproducible scoring system between pathologists ($\kappa > 0.8$)
- Clinically relevant??
Innovation in histological assessment of NASH
Relevance for biomarker discovery and validation

Agenda

- From a dichotomous to a multidimensional continuous scaling system

- Limit and Progress in scoring of fibrosis

- Advances in Histotechnology and relevance for biomarker discovery
  - Virtual slides
  - Dual-photon microscopy
  - Lipidomic Imaging Mass Spectrometry (LIMS)
Advances in Histotechnology

e-slides

.sys files, 500 Mb, easy-to-use, top quality images
E- slides:
- Glass vs e-slide: high concordance in scoring lesions (fibrosis evaluation, $\kappa < 0.8$)
- Sharing images (double readings, second opinion...)
- Image analysis: CPA (fibrosis), SPA (steatosis)....
Advances in Histotechnology

Two-photon microscopy and second harmonic generation

- Ex vivo imaging and quantification of liver fibrosis using second-harmonic generation microscopy Sun TL et al. Journal of Biomedical Optics, May/June 2010
Specificity for fibrillar collagen +++
Assessment of multiple parameters (fibers shape, length, width, orientation, cross-linkage...)
Label free, FFPE, frozen, fresh
Quantitative, highly reproducible
Validation for fibrosis quantification in NAFLD *
Development of an expanded scoring system on a dynamic scale *
Clinical relevance to be demonstrated

* Dual-Photon Microscopy-Based Quantitation of Fibrosis-Related Parameters (q-FP) to Model Disease Progression in Steatohepatitis Wang Y et al, Hepatology 2017
Lipid In Situ Mass Spectrometry (LIMS)

• IMS: In situ analysis of tissue molecular composition (lipids, peptides...) using intact tissue section

• Coupling mass spectrometry with histology (imaging MS)

• Relevant for NAFLD because of differential distribution of lipids and lipid metabolism within the lobule (lipid zonation)*

*Lipid Zonation and Phospholipid Remodeling in Nonalcoholic Fatty Liver Disease, Hall Z et al Hepatology 2017
Lipid Imaging Mass Spectrometry (LIMS)
Matrix-Assisted Laser Desorption Ionisation (MALDI)

Frozen tissue section
Matrix spraying
Automated motorized slide

1 Lipid mass spectrum / spatial position
2-D ion intensity map (heat map) reconstructed for each individual peak above the histological image.
**Lipid In Situ Mass Spectrometry (LIMS)**

<table>
<thead>
<tr>
<th></th>
<th>NORMAL</th>
<th>NAFL</th>
<th>NASH</th>
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</thead>
<tbody>
<tr>
<td>Images</td>
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Scale: 0-2 cm
Lipid In Situ Mass Spectrometry (LIMS)

Unsupervised classification of tissue section using the whole LIMS dataset

NORMAL

NAFL

NASH
Ion density map of M1

Normal liver

Steatosis

NASH
ION DENSITY MAPS OF A NASH SAMPLE ACQUIRED WITH HIGH SPATIAL RESOLUTION.

A/ HE staining section (Z1: periportal, Z3: pericentral),
B/ Ion density map of m/z M1 co-localized with Z1 periportal area.
C/ Ion density map of m/z M2 co-localized with zone 3 (pericentral).

→ differential analysis of LIMS dataset of Z3-Steatosis vs Z3-NASH to increase sensitivity of NASH biomarker discovery
ION DENSITY MAP OF M1 IN HUMAN LIVER SECTIONS.
ION DENSITY MAP OF M2 IN HUMAN LIVER SECTIONS.
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lipidomic in biological fluid: a fishing expedition

→ Histology and LIMS as a guide for identification of circulating lipid biomarker
Thank you for your attention!

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