Imaging as an Outcome and Endpoint for Clinical Trials of NASH

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Objectives

• What are some of the main quantitative imaging biomarkers (QIBs) that are being used in drug development clinical trials?
  – MRI PDFF
  – MRE liver stiffness
  – cT1
  – VCTE CAP and FAST scores

• What is the Evidence?

• What's their use so far in NASH clinical Trials

• What’s the Future?
The NAFLD Spectrum

NAFLD Activity Score

<table>
<thead>
<tr>
<th></th>
<th>Steatosis (0 – 3)</th>
<th>Inflammation (0 – 3)</th>
<th>Ballooning (0 – 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 – 33%</td>
<td>1</td>
<td>&lt; 2 under 20x</td>
<td>Few</td>
</tr>
<tr>
<td>34 – 65%</td>
<td>2</td>
<td>2 – 4 under 20x</td>
<td>1</td>
</tr>
<tr>
<td>≥ 66%</td>
<td>3</td>
<td>&gt; 4 under 20x</td>
<td>Many</td>
</tr>
</tbody>
</table>
Baseline Fibrosis Stage but Not NASH Predicted Mortality and Time to Development of Severe Liver Disease

Overall Mortality

Severe Liver Disease

Log Rank $P < 0.001$

People with NASH

Liver Biopsy

Noureddin M.
Noninvasive Diagnosis of Fibrosis in NAFLD

Serologic Markers

- Simple
  - FIB-4
  - NFS

- Complex
  - Fibrospect
  - ELF
  - Pro C3

Imaging

- Elastography
  - VCTE
  - MRE
  - Multiparametric
  - ARFI

FIB-4 = \frac{\text{Age (years)} \times \text{AST Level (U/L)}}{\text{Platelet Count (10^9/L)} \times \sqrt{\text{ALT (U/L)}}}
Noninvasive Diagnosis of Steatosis in NAFLD

CAP

MRI-PDFF

?Steatohepatitis
Rationale for MRI-PDFF as Biomarker of Hepatic Steatosis

- Currently MRI-PDFF most accurate non-invasive biomarker to assess hepatic steatosis
- MRI accurate compared to MRS as reference-standard\textsuperscript{4-8}
- MRI accurate compared to histology as reference-standard\textsuperscript{9,10}
- MRI precise\textsuperscript{11-14} (repeatability, reproducibility)

Meta-analysis
- In analysis of 23 studies\textsuperscript{15}:
  
  “Excellent linearity, bias, and precision across different field strengths, imager manufacturers, and reconstruction methods”

Courtesy of M Middleton.
MRI-PDFF

- 3%
- 23%
- > 40%
NASH CRN FLINT Trial Results\textsuperscript{9}

- Adult cross-sectional and longitudinal relationships between PDFF and histologic steatosis grade (113 subjects, 8 sites)

Courtesy of M Middleton.
Higher Liver Fat May Be Prognostic

Higher liver fat on MRI-PDFF is associated with fibrosis progression* in NAFLD.

*Fibrosis progression defined as a transition from stage 0 fibrosis to stage 1 or greater on follow up liver biopsy.
Where Is the Evidence Coming From?

Where Is the Evidence Coming From?

Examples of MRI-PDFF in Phase2A Studies

![Optimal Dose Diagram]

MR Elastography

Anatomy
Normal Liver

Wave Image

Elastogram
Fibrotic Liver
Rationale for MRE as Biomarker of Liver Fibrosis

• Liver fibrosis increases shear stiffness and other parameters\textsuperscript{17-19}

• Accurate using histologic fibrosis stage as reference standard\textsuperscript{20}

• Repeatable and reproducible\textsuperscript{21-24}, predicts NASH\textsuperscript{25} and advanced fibrosis\textsuperscript{26}

• Precision in large meta-analysis study supports the claim\textsuperscript{27}:
  
  \begin{itemize}
    \item A measured change in hepatic stiffness of 19\% or greater, at the same site and with use of the same equipment and acquisition sequence, is inferred to indicate that a true change in stiffness has occurred with 95\% confidence
  \end{itemize}

Courtesy of M Middleton.

As Liver Becomes More Fibrotic, It Becomes Stiffer
Singh et al (2016) reported the following cutoffs for pooled data from nine carefully selected studies that used similar MRE technique:

- Stage ≥ 1 cutoff: 2.88 kPa
- Stage ≥ 2 cutoff: 3.54 kPa
- Stage ≥ cutoff: 3.77 kPa
- Stage 4 cutoff: 4.09 kPa

The best cutoff at each level will depend, amongst other things, on the COU to which it is intended to be used.
Diagnostic Performance of MRE-Stiffness for Bridging Fibrosis (F3–F4 vs F0–F2) and Cirrhosis (F4 vs F0–F3)

<table>
<thead>
<tr>
<th>Fibrosis Category</th>
<th>MRE Cutoff, kPa</th>
<th>AUROC (95% CI)</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Advanced fibrosis (F3–F4 vs F0–F2)</td>
<td>≥ 3.64*</td>
<td>0.85 (0.80, 0.90)</td>
<td>82 (76, 86)</td>
</tr>
<tr>
<td></td>
<td>≥ 3.92†</td>
<td></td>
<td>76 (70, 81)</td>
</tr>
<tr>
<td>Cirrhosis (F4 vs F0–F3)</td>
<td>≥ 4.67*</td>
<td>0.81 (0.76, 0.86)</td>
<td>79 (70, 86)</td>
</tr>
<tr>
<td></td>
<td>≥ 5.30‡</td>
<td></td>
<td>70 (61, 78)</td>
</tr>
</tbody>
</table>

- AUROC (95% CI) of MRE-stiffness to detect bridging fibrosis and cirrhosis were 0.85 (0.80, 0.90) and 0.81 (0.76, 0.86), respectively.
- In general, cutoffs from the literature and optimal cutoffs derived from this dataset had similar performance for classification of fibrosis by 2D MRE.

* Cutoff from Loomba, et al\(^3\); †Optimal cutoff based on maximal sum of sensitivity and specificity (Youden’s index) in this dataset.

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

The Controlled Attenuation Parameter (CAP) is a measurement of the ultrasound attenuation. It is correlated to the decrease in amplitude of ultrasound waves as they propagate through the liver. CAP has been designed on the assumption that fat affects ultrasound propagation. Therefore, the more steatosis, or liver fat there is, the higher the CAP result will be.

## Sensitivity Priority Based Steatosis Assessment

383 NAFLD Subjects With CAP & Paired Biopsy

<table>
<thead>
<tr>
<th>Steatosis Stage</th>
<th>Sensitivity Threshold (dB/m)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ S1</td>
<td>274</td>
<td>0.90</td>
<td>0.60</td>
<td>0.99</td>
<td>0.47</td>
</tr>
<tr>
<td>≥ S2</td>
<td>290</td>
<td>0.90</td>
<td>0.44</td>
<td>0.74</td>
<td>0.71</td>
</tr>
<tr>
<td>S3</td>
<td>302</td>
<td>0.90</td>
<td>0.38</td>
<td>0.45</td>
<td>0.87</td>
</tr>
</tbody>
</table>

The above threshold values are from the cited peer review publication. Clinical usage of threshold values are determined by the provider based on their preferred threshold value reference. 

# Youden’s Index Based Fibrosis Assessment

## 384 NAFLD Subjects With VCTE & Paired Biopsy

<table>
<thead>
<tr>
<th>Fibrosis Stage</th>
<th>Youden’s Threshold (kPa)</th>
<th>AUROC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0-F1 vs $\geq$ F2</td>
<td>8.2</td>
<td>0.77</td>
<td>0.71</td>
<td>0.70</td>
<td>0.78</td>
<td>0.61</td>
</tr>
<tr>
<td>F0-F2 vs $\geq$ F3</td>
<td>9.7</td>
<td><strong>0.80</strong></td>
<td>0.71</td>
<td>0.75</td>
<td>0.63</td>
<td>0.81</td>
</tr>
<tr>
<td>F0-F3 vs F4</td>
<td>13.6</td>
<td><strong>0.89</strong></td>
<td>0.85</td>
<td>0.79</td>
<td>0.29</td>
<td>0.98</td>
</tr>
</tbody>
</table>

The above threshold values are from the cited peer review publication. Clinical usage of threshold values are determined by the provider based on their preferred threshold value reference.

Baseline Characteristics of Patients Without Prior Biopsy According to Alternate NIT Combinations*

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Gastroesophageal Reflux Disease (GERD)</th>
<th>ENI</th>
<th>NIT</th>
<th>HCC</th>
<th>NASH</th>
<th>Cirrhosis</th>
<th>NAFLD</th>
<th>Other Liver Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 (43, 70)</td>
<td>51.7 (48, 70)</td>
<td>50.5 (43, 70)</td>
<td>50 (43, 70)</td>
<td>51.7 (48, 70)</td>
<td>50.5 (43, 70)</td>
<td>50.5 (43, 70)</td>
<td>50.5 (43, 70)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>55%</td>
<td>50%</td>
<td>55%</td>
<td>55%</td>
<td>50%</td>
<td>55%</td>
<td>55%</td>
<td>55%</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>29.3 (23.2, 41.5)</td>
<td>30.7 (24.0, 45.9)</td>
<td>29.3 (23.2, 41.5)</td>
<td>29.3 (23.2, 41.5)</td>
<td>30.7 (24.0, 45.9)</td>
<td>29.3 (23.2, 41.5)</td>
<td>29.3 (23.2, 41.5)</td>
<td>29.3 (23.2, 41.5)</td>
</tr>
<tr>
<td>Diabetes (n%)</td>
<td>33%</td>
<td>30%</td>
<td>33%</td>
<td>33%</td>
<td>30%</td>
<td>33%</td>
<td>33%</td>
<td>33%</td>
</tr>
<tr>
<td>Hypertension (n%)</td>
<td>48%</td>
<td>52%</td>
<td>48%</td>
<td>48%</td>
<td>52%</td>
<td>48%</td>
<td>48%</td>
<td>48%</td>
</tr>
<tr>
<td>History of smoking (n%)</td>
<td>55%</td>
<td>50%</td>
<td>55%</td>
<td>55%</td>
<td>50%</td>
<td>55%</td>
<td>55%</td>
<td>55%</td>
</tr>
<tr>
<td>History of alcohol abuse (n%)</td>
<td>33%</td>
<td>30%</td>
<td>33%</td>
<td>33%</td>
<td>30%</td>
<td>33%</td>
<td>33%</td>
<td>33%</td>
</tr>
</tbody>
</table>

*Data adapted from Loomba, et al. EASL. 2019.
## Obeticholic Acid (OCA) Improves Non-Invasive Markers of Fibrosis in Patients With NASH: A Secondary Analysis of the Phase 3 REGENERATE Study

<table>
<thead>
<tr>
<th>LS Mean (SE) Change from Baseline (P Value)</th>
<th>Placebo (n = 311)</th>
<th>OCA 10 mg (n = 312)</th>
<th>OCA 25 mg (n = 308)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Change (at month 6) in Serum-Based Biomarkers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIB-4</td>
<td>0.017 (0.04)</td>
<td>-0.099 (0.04) ( P = 0.0328 )</td>
<td>-0.120 (0.04) ( P = 0.0119 )</td>
</tr>
<tr>
<td>APRI</td>
<td>-0.018 (0.03)</td>
<td>-0.153 (0.03) ( P = 0.0011 )</td>
<td>-0.209 (0.03) ( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>CK-18 (M30), U/L</td>
<td>43.7 (32.56)</td>
<td>-127.1 (32.4) ( P &lt; 0.0001 )</td>
<td>-222.7 (32.45) ( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>Fibroscan</td>
<td>0.022 (0.0069)</td>
<td>-0.051 (0.0069) ( P &lt; 0.0001 )</td>
<td>-0.072 (0.0070) ( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>Change at Month 18 in Transient Elastography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Stiffness by TE (kPa)</td>
<td>1.11 (0.54)</td>
<td>-0.56 (0.55) ( P = 0.0187 )</td>
<td>-1.30 (0.56) ( P &lt; 0.0008 )</td>
</tr>
</tbody>
</table>
Figure 7. Liver Stiffness (VCTE) Over Time by Change in Fibrosis Stage (ITT Population*)
# FAST Score

<table>
<thead>
<tr>
<th>DERIVATION COHORT</th>
<th>EXTERNAL VALIDATION COHORTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td><strong>Malaysian NAFLD cohort</strong></td>
</tr>
<tr>
<td>Develop.</td>
<td>335</td>
</tr>
<tr>
<td>Bootstrap</td>
<td></td>
</tr>
<tr>
<td><strong>Age (year)</strong></td>
<td>54 (18)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>142 (42%)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>34 (9)</td>
</tr>
<tr>
<td><strong>T2DM</strong></td>
<td>167 (50%)</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>178 (53%)</td>
</tr>
<tr>
<td><strong>AST (UI/L)</strong></td>
<td>36 (24)</td>
</tr>
<tr>
<td><strong>ALT (UI/L)</strong></td>
<td>50 (37)</td>
</tr>
<tr>
<td><strong>E (kPa)</strong></td>
<td>8.9 (7.6)</td>
</tr>
<tr>
<td><strong>CAP (dB/m)</strong></td>
<td>343 (65)</td>
</tr>
<tr>
<td><strong>Steatosis grade</strong></td>
<td>S0=5% / S1=24%</td>
</tr>
<tr>
<td></td>
<td>S2=31% / S3=40%</td>
</tr>
<tr>
<td><strong>Fibrosis stage</strong></td>
<td>F0=18% / F1=23%</td>
</tr>
<tr>
<td></td>
<td>F2=22% / F3=29% / F4=7%</td>
</tr>
<tr>
<td><strong>NASH (FLIP)</strong></td>
<td>229 (68%)</td>
</tr>
<tr>
<td><strong>AUROC</strong></td>
<td>0.83 (0.78-0.87)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LiverMultiScan
Quantitative MRI Metrics That Correlate With Histology

In a single contrast-free MRI scan

3 metrics 15 minutes
for liver disease from start to finish#


#includes patient preparation time.
cT1 for Classification of NAFLD Disease Severity
MR Is Superior to TE for Distinguishing Simple Steatosis from NASH Based on Ballooning

LiverMultiScan had superior technical success rate versus TE (95% vs 59%).
cT1 able to assess both necroinflammatory and fibrotic components of NASH.

TE, transient elastography; MR, magnetic resonance; ROC, Receiver Operating Characteristic; AUROC, Area Under ROC.
cT1 Correlates With Clinical Outcomes
100% NPV for Ruling Out Future Clinical Events in CLD Patients from Varying Etiologies

All patients with mild/normal cT1 had no events and those with moderate/high cT1 had liver-related AEs.*

The higher the cT1, the sooner and more likely an AE may occur.

* Liver-related AE = Ascites (4), Encephalopathy (3), Liver-related death (2), HCC (1); AE, Adverse event; CLD, chronic liver disease; NPV, negative predictive value.

Identifying ‘High Risk’ NASH (NAS ≥ 4; F ≥ 2)
Composite Biomarker Based on cT1 Has High Diagnostic Accuracy

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>AUROC</th>
<th>Se.</th>
<th>Sp.</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDFF</td>
<td>0.66 (0.55 – 0.78)</td>
<td>0.74</td>
<td>0.58</td>
<td>0.58</td>
<td>0.73</td>
</tr>
<tr>
<td>cT1</td>
<td>0.77 (0.67 – 0.87)</td>
<td>0.86</td>
<td>0.58</td>
<td>0.62</td>
<td>0.85</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.78 (0.68 – 0.88)</td>
<td>0.61</td>
<td>0.85</td>
<td>0.77</td>
<td>0.73</td>
</tr>
<tr>
<td>cT1 + Glu.</td>
<td>0.89 (0.82 – 0.96)</td>
<td>0.92</td>
<td>0.73</td>
<td>0.73</td>
<td>0.92</td>
</tr>
<tr>
<td>cT1 + Glu + AST</td>
<td>0.93 (0.88 – 0.98)</td>
<td>0.89</td>
<td>0.81</td>
<td>0.79</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Cut-offs established from Youden’s index

CT1-based composite biomarker combining fasting glucose and AST identifies patients with high risk NASH in population of 110 patients with biopsy-confirmed NAFLD.

Confidential | 1.0
In NGM-282 Phase 2 study, cT1 has been shown to detect changes in 6 weeks that correlated with histological response.

Stephen A. Harrison,1,2; Stephen J. Rossi,3; Angelo H. Paredes,4; James F. Trotter,5; Mustafa R. Bashir,6; Cynthia D. Guy,7; Rajarshi Banerjee,9; Mark J. Jaros,9; Sandra Owers,2; Bryan A. Baxter,3; Lei Ling,3; and Alex M. DePaoli,3. Hepatology. 2019.
Detecting Efficacy at Week 12
Phase 2 NASH Study With MGL-3196

Significant decrease in fibroinflammatory disease (cT1), after 12 weeks (n = 17)

The Future

Treatment for Fibrotic NASH

Response

Continue Treatment

Partial: ?Add
None: ?Switch

NITs

No Response

NITs

18 months

NITs

18 months

NITs

18 months

NITs

18 months

Noureddin M.
Conclusions

- Imaging are useful NITs in clinical trials
- New data showed correlation with histological response (monitoring)
- Might be considered as diagnostic and monitoring tool in clinical practice