Disclosure

• None
Role of combined liver stiffness measurement (LSM) using vibration-controlled transient elastography and controlled attenuation parameter (CAP) testing in NAFLD/NASH clinical trials

- Single measurement
  - Diagnostic
  - Prognostic

- Multiple measurements over time ($\Delta$LSM/$\Delta$t or $\Delta$CAP/$\Delta$t)
  - Prognostic
  - Treatment response
Basics and Nuances of Fibroscan
Probe Size

<table>
<thead>
<tr>
<th>Probe size</th>
<th>Depth (mm)</th>
<th>US Freq (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+</td>
<td>25-65</td>
<td>3.5</td>
</tr>
<tr>
<td>XL+</td>
<td>35-75</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Liver Stiffness Measurement (VCTE)

Shear Wave Speed Measurement

Ultrasound Echo

Ultrasound Pulse

Pulse Echo Ultrasound

Measure
Shear Wave Speed $V_s$ (m/s)

Calculate
Equivalent Stiffness $E$ (kPa)

$E = 3\rho V_s^2$

Elasticity (Stiffness)
Liver Tissue Density
Velocity of Shear Wave

Range: 2.5 to 75 kPa
LSM (VCTE) and Fibrosis in NAFLD

### Optimal VCTE- LSM Cut-Off for ≥F2

**NAFLD with F0/F1 vs. F2 to F4 (Clinically Significant Fibrosis)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Probes</th>
<th>No. of Pts</th>
<th>% with ≥ F2</th>
<th>LSM Cut-off</th>
<th>AUROC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoneda (2008)</td>
<td>M+</td>
<td>97</td>
<td>52</td>
<td>6.6</td>
<td>0.86</td>
<td>88</td>
<td>74</td>
</tr>
<tr>
<td>Nobili (2008)</td>
<td>M+</td>
<td>50</td>
<td>48</td>
<td>7.4</td>
<td>0.99</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Wong (2010)</td>
<td>M+</td>
<td>246</td>
<td>17</td>
<td>7.0</td>
<td>0.84</td>
<td>79</td>
<td>76</td>
</tr>
<tr>
<td>Petta (2011)</td>
<td>M+</td>
<td>169</td>
<td>30</td>
<td>7.3</td>
<td>0.79</td>
<td>69</td>
<td>70</td>
</tr>
<tr>
<td>Gaia (2011)</td>
<td>M+</td>
<td>72</td>
<td>64</td>
<td>7.0</td>
<td>0.80</td>
<td>76</td>
<td>80</td>
</tr>
<tr>
<td>Myers (2012)</td>
<td>M+</td>
<td>75</td>
<td>78</td>
<td>7.8</td>
<td>0.86</td>
<td>79</td>
<td>64</td>
</tr>
<tr>
<td>Wong (2012)</td>
<td>M+</td>
<td>193</td>
<td>23</td>
<td>7.0</td>
<td>0.83</td>
<td>79</td>
<td>64</td>
</tr>
<tr>
<td>Naveau (2014)</td>
<td>M+ and XL+</td>
<td>100</td>
<td>22</td>
<td>7.6</td>
<td>0.81</td>
<td>73</td>
<td>78</td>
</tr>
<tr>
<td>Cassinotto (2015)</td>
<td>M+</td>
<td>291</td>
<td>71</td>
<td>6.2</td>
<td>0.82</td>
<td>90</td>
<td>45</td>
</tr>
<tr>
<td>Imajo (2016)</td>
<td>M+</td>
<td>142</td>
<td>54</td>
<td>11.0</td>
<td>0.82</td>
<td>65</td>
<td>89</td>
</tr>
</tbody>
</table>

Adapted from Yoshioka et al. Hepatol Res. 2015 Jan;45(2):142-51
## Optimal VCTE-LSM Cut-off for Cirrhosis

<table>
<thead>
<tr>
<th>Study</th>
<th>Probe</th>
<th>No. of Pts</th>
<th>% with F4</th>
<th>LSM Cut-off</th>
<th>AUROC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoneda (2008)</td>
<td>M+</td>
<td>97</td>
<td>9</td>
<td>17</td>
<td>0.99</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>Wong (2010)</td>
<td>M+</td>
<td>246</td>
<td>10</td>
<td>10.3</td>
<td>0.95</td>
<td>92</td>
<td>88</td>
</tr>
<tr>
<td>Gaia (2011)</td>
<td>M+</td>
<td>72</td>
<td>12.5</td>
<td>10.5</td>
<td>0.94</td>
<td>78</td>
<td>96</td>
</tr>
<tr>
<td>Myers (2012)</td>
<td>M+</td>
<td>75</td>
<td>22.3</td>
<td>10.3</td>
<td>0.89</td>
<td>81</td>
<td>83</td>
</tr>
<tr>
<td>Wong (2012)</td>
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<td>193</td>
<td>13</td>
<td>10.3</td>
<td>0.89</td>
<td>81</td>
<td>83</td>
</tr>
<tr>
<td>Cassinotto (2015)</td>
<td>M+</td>
<td>291</td>
<td>16.8</td>
<td>9.5</td>
<td>0.87</td>
<td>92</td>
<td>62</td>
</tr>
<tr>
<td>Imajo (2016)</td>
<td>M+</td>
<td>140</td>
<td>8</td>
<td>16.1</td>
<td>0.87</td>
<td>65</td>
<td>90</td>
</tr>
</tbody>
</table>

Adapted from Yoshioka et al. Hepatol Res. 2015 Jan;45(2):142-51
Controlled Attenuation Parameter

**Ultrasound Attenuation Rate**: dB/M (decibels per meter)

Range: 100 to 400 dB/M
**CAP and Hepatic Steatosis**

Normal liver

- Low Attenuation Rate

Fatty liver

- High Attenuation Rate

**Graph:**
- **x-axis:** Grade of steatosis on liver biopsy
- **y-axis:** CAP value (dB/m)
- **Legend:**
  - 0/1: Low attenuation
  - 2/3: High attenuation

**Statistical Significance:**
- $p<0.001$

*Reference*
CAP and $^1$H-MRS

- 1H-MR Spectroscopy and CAP in 50 patients with biopsy proven NAFLD and healthy volunteers
- Steatosis defined by % hepatocytes affected by steatosis
  - S1 – 5 to 33%
  - S2 – 34 to 66%
  - S3 – ≥ 67%
- CAP cut-off of 300 dB/m for detection of S3 steatosis
- CAP cut-off of 215 dB/m for healthy

CAP and MRI-PDFF

A

MRI-based proton density fat fraction (PDFF) and TE-based CAP for assessment of steatosis in 142 liver biopsy proven NAFLD

B

Imajo et al. Gastroenterology. 2016 Mar;150(3):626-637
## CAP and Hepatic Fat Fraction

<table>
<thead>
<tr>
<th></th>
<th>M probe</th>
<th>XL probe</th>
<th>P-Value</th>
</tr>
</thead>
</table>
| **S ≥ 2%** | **AUROC = 0.83 (0.71-0.95)**  
Cut-off 251 dB/m  
Se= 0.78 Sp= 0.78  
Acc = 0.80 | **AUROC = 0.84 (0.73-0.95)**  
Cut-off 254 dB/m  
Se= 0.83 Sp= 0.78  
Acc = 0.83 | 0.76 |
| **S ≥ 8%** | **AUROC = 0.87 (0.78-0.97)**  
Cut-off 267 dB/m  
Se= 0.80 Sp= 0.79  
Acc = 0.81 | **AUROC = 0.90 (0.82-0.99)**  
Cut-off 270 dB/m  
Se= 0.88 Sp= 0.79  
Acc = 0.85 | 0.50 |
| **S ≥ 16%** | **AUROC = 0.92 (0.85-0.99)**  
Cut-off 299 dB/m  
Se= 0.92 Sp= 0.88  
Acc = 0.90 | **AUROC = 0.91 (0.83-0.99)**  
Cut-off 301 dB/m  
Se= 0.92 Sp= 0.81  
Acc = 0.85 | 0.78 |

*S = steatosis; AUROC = area under the receiver operating characteristic curve; Se = sensitivity; Sp = specificity; Acc = accuracy*
NUANCES IN THE USE OF FIBROSCAN
Reliability: Effect of Confounders

Confounders for LSM

- Operator experience >100 exams
- Right heart failure
- Alcohol history
- Inexperience
- Inflammation
- Non-fasting
- Cholestasis
- Fast >3 hrs
- Check ALT
- Check ALP and T.Bili
- XL+ probe
- Alcohol
- Obesity

Effect of Food Intake on LSM

<table>
<thead>
<tr>
<th>Technology</th>
<th>Technician/Technique</th>
<th>Tissue</th>
<th>Tablets/Tonics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shear wave propagation</td>
<td>Operator experience</td>
<td>Probe to liver distance</td>
<td>Medication usage</td>
</tr>
<tr>
<td>TM mode</td>
<td>Variability</td>
<td>- Ascites</td>
<td>- Beta Blockers</td>
</tr>
<tr>
<td>A mode</td>
<td>- Intra-operator</td>
<td>- Adiposity</td>
<td>Etiology specific therapy</td>
</tr>
<tr>
<td>Algorithm</td>
<td>- Inter-operator</td>
<td>- Altered anatomy</td>
<td>Significant weight loss</td>
</tr>
<tr>
<td>Software</td>
<td></td>
<td>Acute hepatitis</td>
<td>- Bariatric surgery</td>
</tr>
<tr>
<td>Probe size</td>
<td></td>
<td>Cholestasis</td>
<td>Recent alcohol use</td>
</tr>
<tr>
<td>- Medium</td>
<td></td>
<td>Portal flow</td>
<td></td>
</tr>
<tr>
<td>- Extra-large</td>
<td></td>
<td>- Postprandial state</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TIPSS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cysts</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Infiltrative liver disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemangioma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Congestive hepatopathy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatic steatosis</td>
<td></td>
</tr>
</tbody>
</table>

ROLE OF FIBROSCAN IN NAFLD/NASH CLINICAL TRIALS
NAFLD Phenotype

- NAFLD
- NAFL
- NASH
  - Without Fibrosis
  - With Fibrosis
    - Early (F1)
    - Clinically significant (≥ F2)
    - Advanced (F3-F4)

Where does my patient fall on the spectrum?

<table>
<thead>
<tr>
<th></th>
<th>BMS</th>
<th>NuSirt</th>
<th>Intercept</th>
<th>Genfit</th>
<th>Galectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 2</td>
<td>≥10% hepatic fat</td>
<td>≥15% hepatic fat</td>
<td></td>
<td></td>
<td>NASH cirrhosis with HVPG ≥ 6 mm Hg</td>
</tr>
<tr>
<td>Phase 3</td>
<td></td>
<td></td>
<td>NASH with F2-F3</td>
<td>NASH with F1-3</td>
<td></td>
</tr>
</tbody>
</table>
Fibroscan – VCTE and CAP
Simultaneous VCTE & CAP Results

- **LSM**
  - 10.5 kPA
  - IQR/Med: 11%
  - ALT < 100 U/L
  - Reliable
  - ≥ F2

- **CAP**
  - 366 dB/m
  - Moderate/severe steatosis

- **NASH with clinically significant fibrosis and moderate to severe steatosis**
Fibroscan – Clinical Trial Setting

• Successful recruiting by enrichment
  • CAP > 300 dB/m for MRI-PDFF > 10%
  • LSM > 10.3 kPa for ≥ F2 stage of fibrosis
  • LSM ≥ 14 kPa for NASH cirrhosis
Feasibility in Multi-Center Trial

- A total of 511 NAFLD patients across eight clinical centers
- The cohort included 65% women with a mean (± SD) age of 56 (± 12) years, BMI 33.6 (± 6.8) kg/m² and waist circumference 107 (± 14) cm.
- The XL+ probe was required in 57% of patients based on automatic probe selection tool.
- Five patients (1.0%) refused the procedure after providing informed consent and five patients (1.0%) had a skin-to-capsule distance greater than 3.5 cm and the exam could not be completed.

Vuppalanchi et al. AASLD 2015
## Reliability of Fibroscan

### Reliability of VCTE and CAP

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver stiffness</strong></td>
<td></td>
</tr>
<tr>
<td>Number of dual exams</td>
<td>469</td>
</tr>
<tr>
<td>Mean ± SD- kPa</td>
<td>11.4 ± 10.9</td>
</tr>
<tr>
<td>Median (IQR)- kPa</td>
<td>7.8 (5.4 – 12.5)</td>
</tr>
<tr>
<td>Correlation (95% CI)</td>
<td>0.96 (0.95, 0.97)</td>
</tr>
<tr>
<td>Mean difference ± SD (1st-2nd exam)- kPa</td>
<td>0.16 ± 3.2</td>
</tr>
<tr>
<td>Median difference (IQR) (1st-2nd exam)- kPa</td>
<td>0.1 (-0.8 – 1.2)</td>
</tr>
<tr>
<td>Absolute mean difference ± SD - kPa</td>
<td>1.9 ± 2.6</td>
</tr>
<tr>
<td>Absolute median difference (IQR) - kPa</td>
<td>1 (0.4 – 2.2)</td>
</tr>
<tr>
<td>Bland-Altman 95% limits of agreement- kPa</td>
<td>-6.1, 6.4</td>
</tr>
</tbody>
</table>

| **CAP**                |         |
| Number of dual exams   | 310     |
| Mean ± SD- dB/m        | 301 ± 54 |
| Median (IQR)- dB/m     | 306 (266 – 341) |
| Correlation (95%CI)    | 0.81 (0.77, 0.85) |
| Mean difference ± SD (1st-2nd exam)- dB/m | -1.3 ± 34.7 |
| Median difference (IQR) (1st-2nd exam)- dB/m | 0 (-18 – 15) |
| Absolute mean difference ± SD- dB/m | 24 ± 25 |
| Absolute median difference (IQR)- dB/m | 16 (7 – 33) |
| Bland-Altman 95% limits of agreement- dB/m | -69.3, 66.8 |

Vuppalanchi et al. AASLD 2015
Reproducibility (intra-observer variability)
High Success Rate in Multi-Center

- A failure rate of 2% when FibroScan is used for estimation of LSM and CAP in patients with NAFLD
- Results unreliable in approximately 2%
- Female gender and waist circumference were associated with failed or unreliable exam
- Excellent intra-observer agreement for LSM and CAP

Vuppalanchi et al. AASLD 2015
Longitudinal Assessment

Change in LSM (VCTE) over time ($\Delta$LSM/$\Delta$t)

- Understand the natural history of NAFLD/NASH
- Understand the natural history of NASH cirrhosis
- Assess therapeutic response to treatment intervention

Hard outcomes
- Death or transplant

Composite endpoints
- Improvement in NAS
- Improvement in Fibrosis
- Resolution of NASH
Summary

- Fibroscan with VCTE and CAP at bedside is very useful to quickly define NAFLD phenotype
- Enhance successful recruitment
- One-time measurement of LSM and CAP – mostly prognostic
- Change over time (ΔLSM/Δt or ΔCAP/Δt) when linked to hard outcomes or surrogate endpoints may be a game changer
- Strategies to minimize confounders is very critical in a clinical trial setting