Imaging and Pathology

*Focus on NAFLD/NASH*

Michael S. Middleton, MD PhD

[msm@ucsd.edu](mailto:msm@ucsd.edu)

UCSD Department of Radiology
San Diego, CA
Liver Imaging Group (LIG)

31st European Congress of Pathology
Nice Acropolis Convention Center, France
September 8, 2019
Apollon: 12:15 - 13:00
Disclosures

- **Consultant:** Arrowhead, Kowa, Median, Novo Nordisk
- **Stockholder:** General Electric, Pfizer
- **Grants:** Guerbet
- **Lab services agreements through UCSD** (current and prior):
  - Alexion
  - AstraZeneca
  - Bristol-Myers Squibb
  - Celgene
  - Enanta
  - Galmed
  - Genzyme
  - Gilead
  - Guerbet
  - Intercept
  - Isis
  - Janssen
  - NuSirt
  - Organovo
  - Pfizer
  - Roche
  - Sanofi
  - Shire
  - Synageva
  - Takeda
Acknowledgements

- Patients who participated in the clinical trials discussed here
- Our collaborators within and outside UCSD
- Claude Sirlin MD (UCSD) for kindly allowing presentation of three of his slides
- Our team at UCSD who conducted many of these studies and analyzed the data
If you say there is an elephant in the room, you mean that there is an obvious problem or difficult situation that people do not want to talk about.
Blood tests / Anthropometric data / Demographics
Can imaging replace elements of pathology?

- Possibly

- Considerations:
  - Context of Use - multi-dimensional, critical
  - Cost
  - Safety
  - Benefit to risk ratio
  - Frequency
  - Required accuracy and precision
  - Supply vs. demand

Better perhaps to ask three questions:

1. How can imaging make pathology better?
2. How can pathology make imaging better?
3. What would be needed to choose one over the other?
UCSD experience

▪ **UCSD**: quantitative imaging biomarker (QIB) experience over the last 17 years

▪ **NASH-CRN**: imaging coordination and data analysis for FLINT and CyNCh Trials

▪ **Academic Research Organization (ARO)**:
  • UCSD ARO laboratory services agreements over last 12 years
  • 32 drug-development clinical trials to date as data analysis center
  • MRI-PDFF, MRS-PDFF, MRE liver stiffness
  • > 5,000 imaging exams evaluated to date at over 300 sites worldwide

▪ **Quantitative Imaging Biomarker Alliance (QIBA)**: PDFF and MRE committees

▪ **NIMBLE Trial**: rigorous independent multi-center prospective precision testing of selected promising QIBs under the auspices of the fNIH
Aims of this talk

- Focus on comparing MR imaging and pathology in NAFLD/NASH:
- Review of **biomarker validation, contexts of use**, and use in clinical trials of:
  - MRI estimation/validation of **Proton Density Fat Fraction (PDFF)** as biomarker of liver steatosis
  - MRE estimation/validation of **liver stiffness** as biomarker of liver fibrosis
- Discuss several unanswered questions and future directions
- Provide a framework/perspective on biomarker validation that might be generalizable to the development of quantitative biomarkers for ultrasound and other imaging methods, and perhaps also for pathology
Biomarker development

- Validation of a quantitative imaging biomarker (QIB) requires **feasibility**, **accuracy**, and **precision** that are all **fit for purpose** (i.e., aligned with a **context of use** [COU]).
  - Additional attributes should probably include: **acceptable percentages, and ratio of false positives and false negatives**

- FDA drug development qualification program defines 7 **categories**, and gives examples of 11 **contexts of use**\(^1\). 

- FDA and NIH refer to their **BEST** (**B**iomarkers, **E**ndpointS, and other **T**ools) resource to support this process\(^2\).

- RSNA currently sponsors QIB assessment programs as part of the **Quantitative Imaging Biomarker Alliance (QIBA)**\(^3\).

---


3 - RSNA QIBA website, accessed 09 May 2018; [https://www.rsna.org/QIBA/](https://www.rsna.org/QIBA/)
**Biomarker categories and COUs**

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples of contexts of use (COUs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic</td>
<td>Subject selection</td>
</tr>
<tr>
<td>Monitoring</td>
<td>Detect change in degree/extent of disease</td>
</tr>
<tr>
<td></td>
<td>Indicate toxicity or assess safety</td>
</tr>
<tr>
<td></td>
<td>Provide evidence of exposure</td>
</tr>
<tr>
<td>Predictive</td>
<td>Identify subjects on basis of effect of intervention or exposure</td>
</tr>
<tr>
<td>Prognostic</td>
<td>Stratify subjects</td>
</tr>
<tr>
<td></td>
<td><strong>Enrichment: inclusion/exclusion criteria</strong></td>
</tr>
<tr>
<td>Pharmacodynamic / Response</td>
<td><strong>Efficacy</strong></td>
</tr>
<tr>
<td></td>
<td>Demonstration of biological response</td>
</tr>
<tr>
<td>Safety</td>
<td>Presence/extent of toxicity</td>
</tr>
<tr>
<td>Susceptibility / Risk</td>
<td>Potential to develop disease or sensitivity</td>
</tr>
</tbody>
</table>

Need for objective quality control

- **Just as** biomarker validation (accuracy and precision) should be **appropriate to COU**, **So also** should QC be **appropriate to COU**

- Drug development clinical trials require more QC (and documentation) than pilot observational studies of new methodologies

- QIB evaluation with its often complicated analysis workflow **begs** an objective approach to minimize bias and ensure adequate precision

- **Pertains to:**
  - drug trials using MRI-PDFF and MRE liver stiffness as endpoints
  - indirectly to clinical trials using other QIBs
  - eventually, patient care
Proton Density Fat Fraction (PDFF)
PDFF Background

- Initial qualitative imaging estimates of hepatic fat reported in 1984[^4]
- Brief resurgence of interest in 1990's[^5]
- Correction for additional confounders in early 2000's (see next slide for representative refs)
- Currently MRI-PDFF most accurate and precise non-invasive imaging biomarker to assess hepatic steatosis - 189 papers now in PubMed ("PDFF" + "liver")
- Note that PDFF is **ratio of corrected fat signal, to sum of corrected fat and water signals**, whereas **histologic steatosis grade** is based on **percentage of hepatocytes with visible fat globules**

[^4]: Dixon et al, 1984; Radiology 153:189
[^5]: Thomsen et al, 1994; Mag Reson Imag 12:487
Rationale for MRI-PDFF as biomarker of hepatic steatosis

Accuracy
- MRI accurate compared to MRS as reference-standard\(^6-10\)
- MRI accurate compared to histology as reference-standard\(^11,12\)

Precision
- MRI precise\(^13-16\) (repeatability, reproducibility)

Meta-analysis
- In an analysis of 23 studies\(^17\):
  "Excellent linearity, bias, and precision across different field strengths, imager manufacturers, and reconstruction methods"

7 - Haufe et al, *JMRI* 2017; 1641
12 - Middleton et al, *Hepatology* 2018; 67:858
13 - Negrete et al, *JMRI* 2014; 39:1265
14 - Kang et al, *JMRI* 2011; 34:928
17 - Yokoo et al, *Radiology* 2018; 286:486
MRI-PDFF imaging method

6 echoes acquired at successive out-of-phase and in-phase TE values

1.15 msec
2.30 msec
3.45 msec
4.60 msec
5.75 msec
6.90 msec

3.0 Tesla
MRI-PDFF region-of-interest (ROI) analysis

High liver fat
(PDFF = 20.8%)

Low liver fat
(PDFF = 5.4%)
MRI PDFF accuracy - regression

506 adult subjects

MRI PDFF accuracy - Bland-Altman

506 adult subjects

NASH CRN FLINT trial results\textsuperscript{11}

- Adult cross-sectional and longitudinal relationships between PDFF and histologic steatosis grade (113 subjects, 8 sites)
NASH CRN CyNCh trial results

- Pediatric cross-sectional and longitudinal relationships between PDFF and histologic steatosis grade (169 subjects, 9 sites)

12 - Middleton et al, Hepatology 2018; 67:858-872
Cross-sectional trial of patients with NAFLD at UCSD\textsuperscript{18}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{Box plot showing MRI Proton Density Fat Fraction (%) across different steatosis grades.}
\end{figure}

\textsuperscript{18} Tang et al, \textit{Radiology} 2013; 267:422
Phase 2 trial of an ASK1 inhibitor: longitudinal PDFF change

- Phase 2 multi-center trial (GS-US-384-1497)\textsuperscript{19}, NASH and stage 2-3 fibrosis, MRI-PDFF and MRE liver stiffness evaluated compared to biopsy at baseline and at week 24 of treatment with selonsertib (selective inhibitor of apoptosis signal-regulating kinase 1).

- Steatosis grade was seen to correlate with MRI-PDFF (left), and histologic steatosis responders were seen to show decreases in MRI-PDFF (right):

\textsuperscript{19} - Jayakumar et al, \textit{Journal of Hepatology} 2019; 70:133
# PDFF cutoffs summary separating steatosis grades

<table>
<thead>
<tr>
<th>Study</th>
<th>(0) vs. (1,2,3)</th>
<th>(0,1) vs. (2,3)</th>
<th>(0,1,2 vs. 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLINT\textsuperscript{11}</td>
<td>-</td>
<td>16.3% PDFF at 90% specificity</td>
<td>21.7% PDFF at 90% specificity</td>
</tr>
<tr>
<td>CyNCh\textsuperscript{12}</td>
<td>-</td>
<td>17.5% PDFF at 90% specificity</td>
<td>23.3% PDFF at 90% specificity</td>
</tr>
<tr>
<td>Tang et al\textsuperscript{18}</td>
<td>6.4% PDFF at 100% specificity</td>
<td>17.4% PDFF at 91% specificity</td>
<td>22.1% PDFF at 90% specificity</td>
</tr>
</tbody>
</table>

\textsuperscript{11} - Middleton et al, *Gastroenterology* 2017; 153:753  
\textsuperscript{12} - Middleton et al, *Hepatology* 2018; 67:858  
\textsuperscript{18} - Tang et al, *Radiology* 2013; 67:858
How much change in PDFF is clinically meaningful?

- In a post-study secondary analysis of 35 patients in the MOZART study (ezetimibe)\textsuperscript{20}, the 10 who showed histologic response of $\geq 2$ point decrease in NAS had a relative MRI-PDFF decrease of $29.3\%$ ($-4.1\%$ PDFF compared to $-0.6\%$ PDFF).

- On the basis of that finding, it was suggested that, pending external independent validation by other groups, these results could be incorporated into designing future clinical trials.

- However, since NAS includes PDFF, however, a large drop in PDFF can drive a large drop in NAS; adding a requirement for NASH resolution may be helpful.

- Validation of this finding should be in a prospective study with a placebo group.

\textsuperscript{20} Patel et al, \textit{Ther Adv Gastroent} 2016; 9:692
Observations about PDFF and histology

- There is limited data comparing steatosis grades 0 and 1 to PDFF, probably because most studies have inclusion criteria excluding steatosis values.

- PDFF variability across the entire range of PDFF values is in the range of ± 2% PDFF.

- The Tang et al (2013) study included patients with low PDFF values, and in that study there was almost no overlap of PDFF values across grades 0 and 1.

- Thus, histology and MRI-PDFF may be nearly equivalent to separate grades 0 and 1.

- However, the two higher histologic categories are wide, and there is overlap across grades 1 and 2, and grades 2 and 3.

- Thus, PDFF appears to be more precise than histology for change in these ranges.
2D MRE Liver Stiffness
2D MRE Background

- First MRE estimates of hepatic liver stiffness reported in 1995\textsuperscript{21}
- Development continues to this day (see next slide for representative refs)
- Extensively reported - 295 papers now in PubMed ("PDFF" + "MRE" + "Elastography")
- **2D MRE** is FDA approved - used to estimate liver stiffness
- Available at over 1,000 sites, worldwide

\textsuperscript{21} - Muthupillai et al, 1995; Science 269:1789
Rationale for **MRE** as biomarker of liver fibrosis

- Liver fibrosis increases shear stiffness and other parameters\(^{22-24}\)
- Accurate using histologic fibrosis stage as reference standard\(^{25}\)
- Repeatable and reproducible\(^{26-29}\), predicts NASH\(^{30}\) and advanced fibrosis\(^{31}\)
- Precision in large meta-analysis study supports the claim\(^{32}\):
  
  > 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

\(^{22}\) Singh et al, *Clin Gastroenterol Hepatol* 2015; 13:440
\(^{23}\) Asbach et al, *Radiology* 2010; 257:80
\(^{24}\) Huwart et al, *Radiology* 2007; 245:456
\(^{25}\) Morisaka et al, *JMRI* 2017; 47:1268
\(^{26}\) Zhang et al, *JMRI* 2016; 43:704
\(^{27}\) Shi et al, *JMRI* 2014; 32:665
\(^{28}\) Serai et al, *Abdom Imaging* 2015; 40:789
\(^{29}\) Lee et al, *JMRI* 2014; 39:326
\(^{30}\) Chen et al, *Radiology* 2011; 259:749
\(^{31}\) Loomba et al, *Hepatology* 2014; 60:1920
\(^{32}\) Serai et al, *Radiology* 2017; 285:92
MRE source images

Magnitude images

Phase images
MRE post-processed images

Wave images

Elastogram Images
ROI placement

**white** - *manual* ROI placement
**green** - *automated* ROI placement

---

As liver becomes more fibrotic, it becomes stiffer
<table>
<thead>
<tr>
<th>Stage 0</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 kPa</td>
<td>2.5 kPa</td>
<td>3.2 kPa</td>
<td>4.9 kPa</td>
<td>9.7 kPa</td>
</tr>
</tbody>
</table>

As liver becomes more fibrotic, it becomes stiffer.

Courtesy Claude Sirlin MD, UCSD, 07 Sep 2019
As liver becomes more fibrotic, it becomes stiffer

Meta-analysis of MRE-stiffness in NAFLD
232 pts, 9 studies, 6 cohorts; Singh et al, 2016; Eur Rad 26:1431

“Advanced fibrosis”
3D MRE and cT1

3D MRE

- 3D MRE is currently investigational; used to estimate liver stiffness, and its real and imaginary component parts (G' and G''). G'' (loss modulus) and damping ratio (= G''/2G') correlate with liver inflammation in animals:\(^\text{34}\)

"Damping ratio and shear loss modulus can be used to distinguish inflammation from fibrosis at early stages of disease, even before the development of histologically detectable necroinflammation and fibrosis"

Ct1

- Corrected T1 has been reported to decrease in responders to NGM282, and to be a biomarker of inflammation.\(^\text{35}\)

The precision of both of these potential biomarkers of inflammation will be tested in upcoming clinical trials.

\(^{34}\) Yin et al, 2017; Radiology 284:694
\(^{35}\) Harrison et al, 2019; Hepatology (DOI 10.1002/hep.30590)
Technical MRE questions

- How low can we go on total MRE ROI total area and still be reliable?
- Should MRE reliability cutoffs be different for different COUs?
- How do these criteria perform in the presence of liver stiffness inhomogeneity?

Next steps

- Test MRE precision in the NIMBLE Trial
- Consider keeping MRE slice location constant and comparing precision
- Consider repeating MRE sequence several times to improve precision
- Assess whether these methods will better discern earlier fibrosis stages
How much change in MRE liver stiffness is clinically meaningful?

- In a 2017 poster\textsuperscript{36} reporting a study of selonsertib, a 15% reduction in MRE liver stiffness was suggested as being a clinically meaningful response:

  "Relative reductions of liver stiffness by MRE ≥ 15% at W24 were significantly associated with reductions in serum markers of fibrosis, high sensitivity C-reactive protein (hsCRP), and HbA1c."

- This poster is referenced in a review by Connolly et al (2018)\textsuperscript{37}

- This work would benefit from prospective validation in external independent studies that include a placebo arm.

\textsuperscript{36} - Loomba et al, \textit{J Hepatology} (2017); 66:S543 (poster, SAT489)
\textsuperscript{37} - Connolly et al, \textit{J Clin Translat Hepatol} (2018); 6:264
Criteria for improvement in NASH

- Response to therapy is often defined by histology either as:
  - ≥ 2-point improvement in NAS with ≥1-point reduction in either lobular inflammation or hepatocellular ballooning, and no worsening of fibrosis, or
  - ≥ 1-point improvement in fibrosis with no worsening of NASH.

- PDFF results are not part of those criteria

- MRE liver stiffness may be shown to be as good as or better than histology to distinguish improvement in advanced fibrosis, but probably not early fibrosis

- Informed by ongoing prospective multi-center imaging biomarker precision trials, there is hope that MRI methods may be able to assess inflammation adequately

- There is currently less evidence that hepatocellular ballooning can be assessed by imaging as well as it is now assessed by histology
Future directions

- MRI-PDFF and MRE results may:
  - augment pathology results at inter-study timepoints
  - provide supportive/ground truth results to develop automated assessment or deep learning algorithms in pathology

- Hybrid criteria (MRE for liver stiffness, pathology for the rest) may help assess drug response better than pathology criteria alone

- The language and formalism of imaging biomarker development and implementation into clinical trials may benefit parallel quantitative pathologic biomarker development and implementation into clinical trials
Summary of topics covered

- We discussed quantitative imaging biomarker validation, and the need to consider it in light of actual contexts of use.
- We reviewed MRI PDFF and MRE liver stiffness as biomarker for hepatic steatosis and fibrosis, respectively, and their use in clinical trials.
- We mentioned briefly ongoing technical multi-center clinical trials that are expected to more definitively assess the precision of current promising imaging biomarkers.
- We discussed several open questions related to imaging and pathology in NASH.
- Finally, we speculated on directions for future research and how advances in imaging and pathology may be mutually beneficial.
Thank you