Serum Biomarkers and Clinical Algorithms in NASH: Utility in Clinical Trials and Clinical Practice

NASH Leadership Forum, Washington DC, October 2019

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Disclosure Slide

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Abbvie, Allergan/Tobira, Astra Zenica, GlaxoSmithKline, Novartis Pharma AG, Pfizer Ltd., Vertex.

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Consultancy

Speaker
Abbott Laboratories, Allergan/Tobira, BMS, Clinical Care Options, Falk, Genfit SA, Gilead, Integritas.

This lecture contains discussion of off-label/investigative use of commercial products, medical devices, biologic or pharmaceutical agents. The lecture is for academic purposes only and does not constitute any form of medical advice regarding use of these compounds in routine clinical practice or any form of financial advice/recommendation regarding the companies or the products discussed.
NAFLD Natural History

Substantial inter-patient variation in disease natural history, rate of disease progression and outcome.

Steatohepatitis (NASH) is the biological driver of disease progression and fibrogenesis. The presence of advanced stage of fibrosis (F3-4) is the strongest predictor of long-term mortality.
An important paradox exists: a significant proportion of the population have NAFLD but only a minority progress to advanced liver disease or morbidity/mortality.
BEST Biomarker Category & Context of Use

Biomarker Categories
- Diagnostic
- Prognostic
- Monitoring
- Pharmacodynamic/Response
- Predictive
- Susceptibility/Risk
- Safety

FDA-NIH Biomarker Working Group. BEST (Biomarkers, Endpoints, and other Tools) Resource
https://www.ncbi.nlm.nih.gov/books/NBK338448/
BEST Biomarker Category & Context of Use

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- Susceptibility/Risk
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- Monitoring
- Pharmacodynamic/Response
- Predictive
- Safety

Likely/Generally Accepted Surrogates
- Histopathology (NAS + F)
- Cirrhosis
- Hepatic Decompensation
- Hepatocellular Carcinoma

Clinically Meaningful Outcomes
- Liver-Related Mortality
- Liver Transplantation
- All-Cause Mortality
Challenges of Histological Assessment for NAFLD

- Specific challenges related to how disease severity assessed:
  - Patient acceptance/safety,
  - Biopsy sampling error,
  - Not enough specialist hepatopathologists,
  - Inter- and Intra-observer variation in interpretation,
  - Resource intensive and logistical challenges shipping biopsies for central pathology reading in clinical trials.

- Inherent flaws in semi-quantitative scores:
  - Non-linear categorical descriptions of architectural changes, without reference to quantitative changes in liver collagen.
  - Miss-classification, especially at the boundary between categories.

- CPA only addresses fibrosis burden, not structural distribution or architectural distortion

- Clinical Benefit of surrogate endpoints related to changes in NASH activity or Fibrosis stage have not been formally established.

An imperfect histological reference standard complicates development of better non-invasive biomarkers and also hampers drug discovery pipelines
## Biomarker Needs

<table>
<thead>
<tr>
<th>Clinical Practice</th>
<th>Drug Development</th>
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<tbody>
<tr>
<td><strong>Is the diagnosis NAFLD?</strong></td>
<td><strong>Is the diagnosis NAFLD?</strong></td>
</tr>
<tr>
<td><strong>How active and/or advanced is the disease?</strong></td>
<td><strong>How active and/or advanced is the disease?</strong></td>
</tr>
<tr>
<td>– Is NASH present?</td>
<td>– Is NASH present?</td>
</tr>
<tr>
<td>– How much fibrosis?</td>
<td>– How much fibrosis?</td>
</tr>
<tr>
<td>– Should I refer the patient to specialist?</td>
<td>– Is patient suitable for clinical trial enrolment?</td>
</tr>
<tr>
<td><strong>Is it Stable / Progressing / Regressing?</strong></td>
<td><strong>Is it Stable / Progressing / Regressing?</strong></td>
</tr>
<tr>
<td><strong>Is treatment warranted?</strong></td>
<td><strong>Is treatment warranted?</strong></td>
</tr>
<tr>
<td><strong>What treatment to select?</strong></td>
<td><strong>What treatment to select?</strong></td>
</tr>
<tr>
<td><strong>Is the treatment/intervention working?</strong></td>
<td><strong>Is the treatment/intervention working?</strong></td>
</tr>
<tr>
<td><strong>If therapy does not work in all patients, are there a subset that do benefit?</strong></td>
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</tr>
</tbody>
</table>
BEST Biomarker Category & Context of Use

- A biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease.

Relevance to NAFLD:
- Degree of steatosis,
- Discriminate Steatosis (NAFL) vs. NASH,
- Stage of fibrosis
Non-Invasive Tests & Biomarkers Across the Spectrum of NAFLD

Normal Liver

Steatosis (NAFL)
- Fatty Liver Index (FLI)
- Ultrasound: FibroScan™ (CAP) MR-PDFF

Steatohepatitis (NASH)
- "Simple" Scores (FIB4, NFS)
- Direct Collagen Biomarkers (ELF Test™, PRO-C3™)
- CK-18
- NIS4

Fibrosis & Cirrhosis
- "Wet" Biomarkers
- "Dry" (Imaging) Biomarkers
- MR Liver MultiScan™
- FibroScan™ (VCTE)
- Ultrasound: SSI, ARFI
- MR Liver MultiScan™
- MR Elastography
Non-Invasive Tests & Biomarkers Across the Spectrum of NAFLD

- Normal Liver
- Steatosis (NAFL)
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  - MR Liver MultiScan™
  - MR Elastography
Routine Clinical Biochemistry (LFTs)

- NAFLD is the most common diagnosis in patients with ‘incidental’ abnormal LFTs Daniel, 1999; Skelly, 2001; Pendino, 2005
- Liver enzymes may be normal in up to 80% of NAFLD patients Browning, 2004
  - Transaminases are not a sensitive test for NAFLD/NASH.
  - Poor correlation between ALT and histology
  - ALT typically falls with advanced fibrosis
  - ALT > AST ➞ ALT < AST
- Severity of histology in NAFLD with normal LFTs no different from those with abnormal LFTs Mofrad, 2003; Sorrentino, 2004; Francaza, 2008

Grade/Stage of NAFLD with normal LFTs no different from those with abnormal LFTs

<table>
<thead>
<tr>
<th>Pattern</th>
<th>No. Normal ALT (n = 51)</th>
<th>No. Abnormal ALT (n = 60)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat alone</td>
<td>8</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Fat + scattered inflammation</td>
<td>8</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Fat + ballooning + inflammation</td>
<td>13</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Mallory hyaline + pericellular fibrosis</td>
<td>22</td>
<td>19</td>
<td>NS</td>
</tr>
</tbody>
</table>

Routine LFTs do not differentiate Steatosis/NASH or Stage of fibrosis

Mofrad et al, Hepatology 2003
Sources of Potentially Better Biomarkers...
Serum Tests to Detect NASH

- Markers of apoptosis
  - CK-18 fragments
  - CK-18 + soluble Fas
  - CK-18 + FGF21
- Ferritin
- PIIINP
- NASHTest®
  - α2-macroglobulin, apolipoprotein A1, haptoglobin, bilirubin, GGT, ALT, serum glucose, triglycerides and cholesterol, adjusted for age, gender and BMI.
- Palekar index
  - Sum of risk factors: Age >50 years, Female gender, AST >45 IU/l, BMI >30, AST/ALT Ratio >0.80, and HA >55 mcg/l
- Shimada index
  - Serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level.

All require further independent validation and none are in widespread clinical use
Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease

Kenneth Cusi,1,2,7,8,*, Zhi Chang1, Steve Harrison6, Romina Lomonaco1, Fernando Brill1, Beverly Orsak1, Carolina Ortiz-Lopez1, Joan Hecht1,7, Ariel E. Feldstein8,9, Amy Webb3,7, Christopher Louden4, Martin Goros4, Fermin Tio5,7


424 overweight/obese adults (MRS 275, Histology 318)

<table>
<thead>
<tr>
<th></th>
<th>NAFLD</th>
<th>NASH</th>
<th>Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>0.77</td>
<td>0.65</td>
<td>0.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>NASH1 (n = 318)</th>
<th>Fibrosis1 (n = 206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>58% (51-65%)</td>
<td>54% (44-63%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>68% (59-76%)</td>
<td>85% (75-92%)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>75% (68-82%)</td>
<td>83% (73-91%)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>49% (41-57%)</td>
<td>56% (47-65%)</td>
</tr>
<tr>
<td>Likelihood ratio (+)</td>
<td>1.81 (1.36-2.41)</td>
<td>3.51 (2.07-5.95)</td>
</tr>
<tr>
<td>Likelihood ratio (-)</td>
<td>0.62 (0.51-0.76)</td>
<td>0.55 (0.44-0.68)</td>
</tr>
</tbody>
</table>
Metabolomic Profiling to Distinguish NAFL from NASH

540 lipids and amino acids measured in serum samples from 467 + 192 histologically characterized individuals to distinguish Normal vs. NAFLD vs. NASH

A lipidomic signature performed best if patients with glucose levels <136 mg/dL, the area under the receiver operating characteristic curve for the discrimination between NASH and NAFL increased to 0.81 ± 0.04 with sensitivity and specificity of 0.73 and 0.80, respectively.

Two panels totalling 28 TGs identified:
• 11 TGs, that significantly differentiated NL vs. NAFLD but could not discriminate NASH from NAFL
• 20 TGs, that significantly distinguished NAFL vs. NASH but could not separate NL from NASH
Identifying “Higher Risk” NASH Patients

- Aim: to identify NASH (NAS ≥4*) and fibrosis (F≥2) patients ‘who should be considered for therapeutic intervention’

*NAS ≥ 4 with at least Steatosis ≥1, Ballooning ≥1, Inflammation ≥1
MicroRNAs as Potential Biomarkers for Hepatic Fibrosis

- Highly conserved.
- Small, 18-25 nucleotide, non-coding RNAs.
- Binds mRNA 3’-UTR repressing translation by destabilizing mRNA/silencing translation
- Regulate gene expression at post-transcription - translational level.

miRNAs circulating in:
- Protein complexes (Argo-2)
- Lipoproteins
- Microvesicles
- Exosomes

Szabo & Bala, Nat Rev GI Hep, 2013: 10, 542
Robustness of miR-34a-5p Across Multiple Cohorts

- Expression profiles obtained by HTG NGS show miR-34a-5p over-expressed in all 3 independent cohorts in patients with NAS ≥4 & F ≥2 vs. control patients defined as NAS <4 & F <2

<table>
<thead>
<tr>
<th>Cohort</th>
<th>CSD* vs NCSD**</th>
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<tbody>
<tr>
<td>GOLDEN-Diag (N=271)</td>
<td>110 vs 161</td>
</tr>
<tr>
<td>OBESE (N=249)</td>
<td>50 vs 199</td>
</tr>
<tr>
<td>RESOLVE-IT (N=249)</td>
<td>137 vs 112 (first screened patients)</td>
</tr>
</tbody>
</table>

*NAS ≥4, F ≥2, “Clinically Significant Disease” (CSD); **NAS <4; F <2; “Not- “Clinically Significant Disease” (NCSD)
Performance of the NIS4 Score for NASH (NAS≥4 + F≥2)

Train set: GOLDEN (220 pts)

Validation set: RESOLVE-IT-DIAG (467 pts)

Optimisation set: MERGED (687 pts)

Four components of NIS4: miR-34a, Alpha-2 Macroglobulin, HbA1c & YKL-40

Hanf et al, AASLD 2018
Non-Invasive Tests & Biomarkers Across the Spectrum of NAFLD

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- Steatohepatitis (NASH)
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- MR Elastography
Blood Tests for Liver Fibrosis

Indirect Serum Markers

- Routine clinical blood tests
  - ALT, AST
  - Albumin, PT/INR
  - Platelet count

- Markers of Inflammation
  - YKL-40, MCP-1
  - HsCRP
  - Haptoglobin
  - TNFα, IL-6, IL-8

- Markers of Apoptosis/Necrosis
  - CK18 (M30/M65)

- Metabolic/Liver Function
  - Apolipoprotein A1

Direct Serum Markers

- ECM components
  - Procollagen III N-peptide (PIIINP)
  - Type IV collagen (7S domain)
  - Laminin
  - Hyaluronic acid

- Factors regulating Fibrogenesis and/or Fibrolysis
  - Collagenases & inhibitors
  - α2-Macroglobulin
  - Metalloproteinases (MMPs)
  - TIMPs (TIMP-1, etc.)

Indirect and Direct Markers may be used Individually or in Combination

‘Simple Scores’ for Predicting Presence of Advanced (F3/4) Fibrosis

**NAFLD Fibrosis Score**

\[ = -1.675 + 0.037 \times \text{Age} + 0.094 \times \text{BMI} + 1.13 \times \text{IFG/diabetes} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{Platelets} - 0.66 \times \text{Albumin}. \]

- A score of less than -1.455 excludes fibrosis (NPV 88-93%).
- A score of greater than 0.676 predicts fibrosis (PPV 82-90%).

**FIB-4 Score**

\[ = \frac{(\text{Age} \times \text{AST})}{(\text{Platelets} \times \sqrt{\text{ALT}})} \]

- A score of less than 1.3 excludes fibrosis (NPV 95%)
- A score greater than 3.25 predicts fibrosis (PPV ~70%)

## Comparison of the Diagnostic Performance of Simple Tests for Advanced Fibrosis (F3/F4)

<table>
<thead>
<tr>
<th>Test</th>
<th>AUC</th>
<th>Cut-off</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST/ALT ratio</td>
<td>0.83</td>
<td>0.8</td>
<td>74</td>
<td>78</td>
<td>44</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>(0.74-0.91)</td>
<td>1</td>
<td>52</td>
<td>90</td>
<td>55</td>
<td>89</td>
</tr>
<tr>
<td>APRI</td>
<td>0.67</td>
<td>1</td>
<td>27</td>
<td>89</td>
<td>37</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>(0.54-0.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BARD score</td>
<td>0.77</td>
<td>2</td>
<td>89</td>
<td>44</td>
<td>27</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>(0.68-0.87)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIB-4 score</td>
<td>0.86</td>
<td>1.30</td>
<td>85</td>
<td>65</td>
<td>36</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>(0.78-0.94)</td>
<td>3.25</td>
<td>26</td>
<td>98</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>NAFLD fibrosis score</td>
<td>0.81</td>
<td>-1.455</td>
<td>78</td>
<td>58</td>
<td>30</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>(0.71-0.91)</td>
<td>0.676</td>
<td>33</td>
<td>98</td>
<td>79</td>
<td>86</td>
</tr>
</tbody>
</table>

McPherson et al. Gut 2010;59(9):1265
Indeterminate Results

- Avoid biopsy below lower threshold (TN + FN)
- Avoid biopsy above upper threshold (TP + FP)

- Low Cutoff (NPV) → Low Probability of F3/4
- High Cutoff (PPV) → High Probability of F3/4

False negatives: Normal → Diseased
False positives: Diseased → Normal

Indeterminate
Age as a Confounding Factor for the Accurate Non-Invasive Diagnosis of Advanced NAFLD Fibrosis

*Am J Gastroenterol* advance online publication, 11 October 2016; doi:10.1038/ajg.2016.453

Stuart McPherson, BSc, MBChB, MD, FRCP, Tim Hardy, BSc, MBBS, Jean-François Dufour, MD, PhD, Salvatore Petta, MD, PhD, Manuel Romero-Gomez, MD, PhD, Mike Allison, BSc(Hons), MD, PhD, Claudia P. Oliveira, MD, PhD, Sven Francque, MD, PhD, Luc Van Gaal, MD, PhD, Jörn M. Schattenberg, MD, PhD, Dina Tiniakos, MD, PhD, Alastair Burt, BSc (Hons), MBChB, MD (Hons), FRCPath, FRCP, FRCPA, FRSG, F AcadMed, FAHMS, Elisabetta Bugianesi, MD, PhD, Vlad Ratziu, MD, PhD, Christopher P. Day, MA, MB BChir, MD, PhD, FRCP, FRCP, FMedSci and Quentin M. Anstee, BSc, MB BS, PhD, FRCP.
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<table>
<thead>
<tr>
<th></th>
<th>Existing Threshold for Age &gt;65</th>
<th>New Threshold for Age &gt;65</th>
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<tbody>
<tr>
<td></td>
<td>Se/Sp</td>
<td>% H/L Risk</td>
</tr>
<tr>
<td><strong>NFS</strong></td>
<td>&lt; -1.455</td>
<td>93%/20%</td>
</tr>
<tr>
<td><strong>FIB4</strong></td>
<td>&lt;1.3</td>
<td>93%/35%</td>
</tr>
</tbody>
</table>
Serum Tests Assessing Fibrosis Stage

• ‘Expanded’ lab indices
  – FibroTest®
    • Age, gGT, Bilirubin, α2-Macroglobulin, Haptoglobin, Apolipoprotein A1
    • F3/4 fibrosis: AUC 0.88 (0.82-0.92)
  – FibroMeter®
    • Age, Weight, Glucose, ALT, AST, Ferritin, Platelets
  – HepaScore
    • Age, Gender, gGT, Bilirubin, Hyaluronic acid, α2-Macroglobulin

• ‘Direct’ fibrosis markers
  – Procollagen III N-Peptide (PIIINP)
  – The ELF-test®
    • HA, PIIINP, TIMP1
    • F3/4 fibrosis: AUC 0.90 (0.84-0.96)
  – Neo-Epitope “Protein Finger Print®” tests

Further independent validation in NAFLD cohorts needed before widespread clinical use
Equilibrium of ECM Turnover

Connective Tissue Equilibrium

↑ ECM Degradation

- Collagenases & inhibitors
- α2-Macroglobulin
- Metalloproteinases (MMPs)
- C3M

↑ ECM Formation

- PIIINP
- Hyaluronic Acid
- Type IV collagen (7S)
- TIMPs (TIMP-1, etc.)
- Pro-C3/C5

If validated, Direct Markers may better reflect the dynamics of matrix turnover and so could be useful not only as diagnostics but for monitoring response to treatment

Adapted from Karsdal et al, Am J Physiol 2015
Performance of Enhanced Liver Fibrosis (ELF®) Test in NAFLD

Combined 3 direct markers of fibrosis:
- Procollagen III N-terminal peptide (PIIINP)
- Hyaluronic acid (HA)
- Tissue inhibitor of metaloproteinase 1 (TIMP1)

**ELF Algorithm**

\[ DS = -7.412 + (\ln(\text{HA})*0.681) + (\ln(\text{P3NP})*0.775) + (\ln(\text{TIMP1})*0.494). \]

N = 192

<table>
<thead>
<tr>
<th></th>
<th>0 Versus 1/2/3/4 Any Fibrosis</th>
<th>0/1 Versus 2/3/4 Moderate Fibrosis</th>
<th>0/1/2 Versus 3/4 Severe Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFS</td>
<td>0.79 (0.69-0.88)</td>
<td>0.86 (0.78-0.94)</td>
<td>0.89 (0.81-0.97)</td>
</tr>
<tr>
<td>ELF</td>
<td>0.82 (0.73-0.90)</td>
<td>0.90 (0.84-0.96)</td>
<td>0.93 (0.88-0.98)</td>
</tr>
<tr>
<td>NFS + ELF</td>
<td>0.84 (0.76-0.92)</td>
<td>0.93 (0.88-0.99)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Guha et al, 2008: Hepatology 47, 455-460
Performance of the PRO-C3 collagen neo-epitope biomarker in non-alcoholic fatty liver disease

International cohort of Histologically characterised NAFLD with centrally read biopsies (N=449)

Performance of the PRO-C3 collagen neo-epitope biomarker in non-alcoholic fatty liver disease

International cohort of Histologically characterised NAFLD with centrally read biopsies (N=449)

<table>
<thead>
<tr>
<th>Non-invasive AUROC</th>
<th>Adj AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAR</td>
<td>0.67</td>
</tr>
<tr>
<td>APRI</td>
<td>0.75</td>
</tr>
<tr>
<td>BARD</td>
<td>0.71</td>
</tr>
<tr>
<td>FIB4</td>
<td>0.78</td>
</tr>
<tr>
<td>NFS</td>
<td>0.79</td>
</tr>
<tr>
<td>ADAPT</td>
<td>0.85</td>
</tr>
<tr>
<td>PRO-C3</td>
<td>0.76</td>
</tr>
<tr>
<td>FIB-C3</td>
<td>0.85</td>
</tr>
<tr>
<td>ABC3D</td>
<td>0.83</td>
</tr>
</tbody>
</table>

\[ ADAPT = \exp \left( \log_{10} \left( \frac{\text{Age} \times \text{PRO-C3}}{\sqrt{\text{Platelets}}} \right) \right) + \text{Diabetes} \]

\[ \text{FIBC3} = -5.939 + (0.053 \times \text{Age}) + (0.076 \times \text{BMI}) + (1.614 \times \text{T2DM}) - (0.009 \times \text{platelets}) + (0.071 \times \text{PRO-C3}) \]

Performance of the PRO-C3 collagen neo-epitope biomarker in non-alcoholic fatty liver disease

International cohort of Histologically characterised NAFLD with centrally read biopsies (N=449)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&gt; 50</td>
<td>1</td>
</tr>
<tr>
<td>BMI</td>
<td>&gt; 30</td>
<td>1</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>&lt; 200</td>
<td>1</td>
</tr>
<tr>
<td>Pro-C3</td>
<td>&gt; 15.5</td>
<td>1</td>
</tr>
<tr>
<td>T2DM</td>
<td>Yes / No</td>
<td>2</td>
</tr>
</tbody>
</table>

ABC3D > 3 = High Risk for F3/4 Fibrosis

Combinations & Algorithms
Combination of Serum Markers with Elastography

Suspected NAFLD
Features of the Metabolic Syndrome, radiological evidence of steatosis and/or abnormal liver biochemistry, raised FLI, alternative diagnoses excluded.

Calculate FIB-4 Fibrosis Score

- Age <65: NFS <1.3
- Age >65: NFS <2.0
  - Low risk (NPV 92-98%)
  - Indeterminate
  - High risk (PPV ~80%)

More than 2.67

Advanced [F3-4]
Fibrosis Excluded (NPV 89-97%)

Advanced [F3-4]
Fibrosis Likely (PPV 71-72%)

Indeterminate

Refer to Secondary Care

Transient Elastography (Fibroscan)

- M probe < 7.9k Pa
  - Advanced [F3-4]
    - Fibrosis Excluded (NPV 89-97%)
  - XL probe < 7.2k Pa
  - Indeterminate

- M probe 7.9 – 9.6 kPa
  - XL probe 7.2 – 9.3 kPa
  - Indeterminate

- M probe > 9.6 kPa
  - XL probe > 9.3 kPa
  - Advanced [F3-4]
    - Fibrosis Likely (PPV 71-72%)

Liver Biopsy

- Fibrosis F0-1
- Fibrosis F2-3
- Cirrhosis F4

Lifestyle Advice, Address CVD Risks, NAFLD Directed Therapy, HCC & Variceal Surveillance

Indeterminate

Lifestyle Advice, Address CVD Risks, NAFLD Directed Therapy

Suspected NAFLD

Features of the Metabolic Syndrome, radiological evidence of steatosis and/or abnormal liver biochemistry, raised FLI, alternative diagnoses excluded.

Recalculate FIB-4 Fibrosis Score in 3-5 years or if patient develops Type 2 diabetes.

Recalculate NFS 3-5 years.
Biomarker Performance in STELLAR-3 and 4 Phase 3 Studies

- The STELLAR studies enrolled patients with F3 or compensated cirrhosis (F4) due to NASH [NAS] ≥3.

**Two Sequential Tests**

**Detection of Advanced Fibrosis [F0-2 vs. F3-4]**

<table>
<thead>
<tr>
<th>Test Cutoffs</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Indeterminate</th>
<th>Misclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Novel</strong></td>
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<tr>
<td>FIB4 (1.23, 2.1) → ELF (9.35, 10.24)</td>
<td>78 (74, 82)</td>
<td>82 (76, 88)</td>
<td>91 (88, 94)</td>
<td>61 (55, 67)</td>
<td>13 (11, 16)</td>
<td>21 (18, 24)</td>
</tr>
<tr>
<td><strong>Literature</strong></td>
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<tr>
<td>FIB4 (1.3, 2.67) → ELF (9.8, 11.3)</td>
<td>69 (67, 71)</td>
<td>92 (90, 94)</td>
<td>96 (94, 97)</td>
<td>55 (53, 58)</td>
<td>24 (23, 26)</td>
<td>24 (23, 26)</td>
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<tr>
<td>FIB4 (1.23, 2.1) → FibroScan (9.6, 14.53)</td>
<td>78 (74, 82)</td>
<td>87 (82, 92)</td>
<td>94 (91, 96)</td>
<td>61 (55, 67)</td>
<td>20 (17, 23)</td>
<td>19 (16, 23)</td>
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<tr>
<td>FIB4 (1.3, 2.67) → FibroScan (9.9, 11.4)</td>
<td>77 (75, 78)</td>
<td>89 (87, 91)</td>
<td>95 (93, 96)</td>
<td>60 (58, 63)</td>
<td>20 (18, 21)</td>
<td>20 (18, 21)</td>
</tr>
</tbody>
</table>

**Three Sequential Tests**

**Detection of Advanced Fibrosis [F0-2 vs. F3-4]**

<table>
<thead>
<tr>
<th>Test Cutoffs</th>
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<th>NPV</th>
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<td>FIB4 (1.23, 2.1) → ELF (9.35, 10.24) → FibroScan (9.6, 14.53)</td>
<td>75 (71, 79)</td>
<td>82 (76, 87)</td>
<td>91 (88, 94)</td>
<td>58 (52, 64)</td>
<td>9 (7, 11)</td>
<td>23 (20, 26)</td>
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<tr>
<td>FIB4 (1.3, 2.67) → ELF (9.8, 11.3) → FibroScan (9.9, 11.4)</td>
<td>64 (60, 69)</td>
<td>93 (88, 96)</td>
<td>95 (92, 97)</td>
<td>52 (46, 57)</td>
<td>10 (7, 12)</td>
<td>28 (24, 31)</td>
</tr>
</tbody>
</table>

*Data are % (95% CI).

Combinations of tests in a sequential fashion maintains sensitivity and specificity and reduces the indeterminate zone, while maintaining misclassification at a rate that approximates the error of liver biopsy.

Anstee et al, Hepatology 2019
Management of Referrals to Secondary Care

Care Pathway

Before implementation of Care Pathway: 5% referrals F3-4, after implementation: 30% referrals F3-4.

Srivastava et al, J Hep 2019
Score VCTE+CAP+AST: Internal and External validation

Develop a composite FibroScan-based score to identify NASH+Fibrosis (NAS≥4 + F≥2)

<table>
<thead>
<tr>
<th>Derivation Cohort</th>
<th>External Validation Cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development population</td>
<td>Bootstrap validation</td>
</tr>
<tr>
<td>N</td>
<td>335</td>
</tr>
<tr>
<td>Prevalence of NASH+NAS≥4+F≥2</td>
<td>166 (50%)</td>
</tr>
<tr>
<td>VCTE+CAP+AST AUROC (95% CI)</td>
<td>0.83 (0.78-0.87)</td>
</tr>
</tbody>
</table>

* (95% CI) for prevalence

Newsome et al, AASLD 2018, Abstract # 140.
A biomarker used to identify **likelihood of a clinical event, disease recurrence or progression** in patients who have the disease or medical condition of interest.

**Relevance to NAFLD:**
- Stratify individuals by fibrosis progression risk,
- Identify likely “fast” or “slow” progressors,
- Predict long-term outcomes and hard endpoints

✗ Grade of NASH less informative to predict long-term outcome
NAFLD Fibrosis Score & FIB-4 Predict Long-term Outcome

Adjusted Hazard Ratio for Transplantation/Death

<table>
<thead>
<tr>
<th>NAFLD-FS</th>
<th>FIB-4</th>
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<tr>
<td>Intermediate vs. Low</td>
<td>High vs. Low</td>
</tr>
<tr>
<td>4.2 [95%CI 1.3-13.8]</td>
<td>9.8 [95%CI 2.7-35.3]</td>
</tr>
<tr>
<td>High vs. Low</td>
<td>6.9 [95%CI 2.3-20.4]</td>
</tr>
<tr>
<td>2.3 [95%CI 0.8-6.6]</td>
<td></td>
</tr>
</tbody>
</table>

n=320 NAFLD patients, 9-14 years follow-up.

BEST Biomarker Category & Context of Use

- **Sensitivity to Change** essential

  - Change must be Measurable and Objective.

![Diagram showing Biomarker Categories and their context of use]

- Susceptibility/Risk
- Diagnostic
- Prognostic
- Monitoring
- Pharmacodynamic/Response
- Predictive
- Safety
Change in Circulating Biomarkers Associated with Histological Improvement

- **Routine clinical chemistry values** and **direct fibrosis biomarkers** show response to improvement in histological fibrosis with therapy in early phase open-label trial of NGM282 (n=43).
  - ALT and AST
  - ELF Test (PIIINP, TIMP-1, not HA)
  - Pro-C3
- In a post-hoc analysis, **NIS4 score** fell with improvement in disease severity in GOLDEN-505 trial.

**Changes in Pro-C3 & ELF with NAS/Fibrosis improvement**

**Changes in NIS4 with NAS/Fibrosis improvement**

Conclusions

• NAFLD is highly prevalent, largely asymptomatic disease characterised by substantial inter-patient variability in disease severity and outcome.

• Biomarkers may be considered as:
  – Indirect & Direct Serum biomarkers.
  – Direct Serum biomarkers.

• At present, the staged application of available ‘simple panel’ biomarkers (NFS, FIB4) followed by a second non-invasive test (e.g. Fibroscan, MRE) helps to rule out cases that are unlikely to have significant disease.

• **The biomarker field is developing rapidly** and so the objective assessment of biomarker performance for specific predefined contexts of use is important to understanding their utility.

• **There is an urgent need for more sensitive and specific, independently validated and qualified biomarkers for use in NAFLD.** Promising experimental biomarkers include novel direct biomarkers related to ECM turnover, Metabolomic profiling and miRNAs however all require further validation.
Acknowledgements

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Ms Laura Haigh,
Prof Fiona Oakley,
Dr Jenny Gallacher.