# Optimizing I/E Criteria for Early NASH Clinical Studies with FibroScan®

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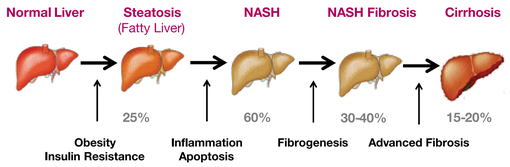
## Introduction

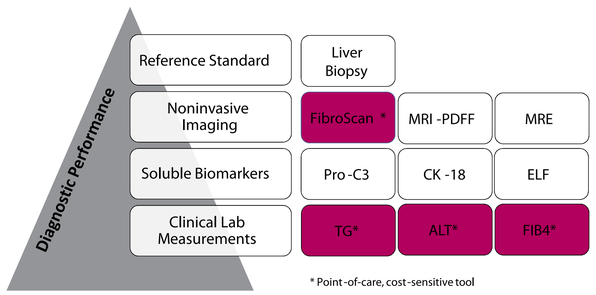
- Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are the hepatic manifestation of metabolic syndrome. NASH is a chronic, progressive disorder that can lead to cirrhosis, end-stage liver disease, and even hepatocellular carcinoma.
- Currently there is no FDA-approved treatment for NASH; however, there are over 90 investigational drugs in development to treat this disease.

**Figure 1. The natural history and prevalence of NAFLD/NASH.**




- While liver biopsy remains the reference standard for disease diagnosis, the biopsy may not be practical or appropriate for early clinical phase studies. Therefore, noninvasive biomarkers can be applied as inclusion criteria for these studies and can expedite study enrollment. Noninvasive assessments include imaging, soluble biomarkers and clinical laboratory (lab) measurements.
- For early phase clinical studies, steatosis and liver stiffness inclusion criteria are determined with magnetic resonance imaging proton density fat fraction (MRI-PDFF) and magnetic resonance elastography (MRE) assessments respectively. However, these tests are expensive and not feasible for pre-screening.
- The FibroScan® (Echosens, Paris, France) is an ultrasound-like device that simultaneously captures steatosis via controlled attenuation parameter (CAP) and liver stiffness (fibrosis) via vibration controlled transient elastography (VCTE). The FibroScan holds several advantages over other imaging modalities; it is a point-of-care system, with a quick examination and is cost-sensitivity compared to MRI-PDFF and MRE, making it an excellent pre-screening tool.
- Recent studies show the FibroScan has good performance for identifying steatosis and fibrosis compared to liver biopsy, MRI-PDFF and MRE [1-3].
- Clinical lab measurements include triglyceride (TG), alanine aminotransferase (ALT) and FIB4 index. FIB4 is a noninvasive biomarker used to identify NASH patients at risk of advanced fibrosis. The index was developed to stage liver disease in subjects with HIV-hepatitis C virus co-infection [4], and more recently has been applied as a marker of fibrosis in NASH [5].

**Figure 2. Noninvasive biomarkers of NASH.**



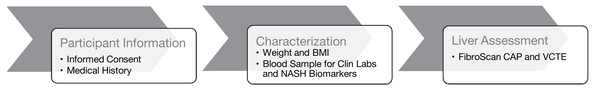

## Aim

- Celerion clinics are actively recruiting participants to take part in a 'pre-screening' initiative. A robust database of 'pre-screened' subjects facilitates swift study enrollment and reduces screen failures.
- In building this database, we sought to examine the concordance between traditional clinical labs and hepatic steatosis and liver stiffness (fibrosis) measured with FibroScan. Specifically, the concordance between FibroScan CAP and TG as well as VCTE and ALT and FIB4 were examined.

## Methods

- More than 200 subjects have participated in the pre-screening at Celerion clinics in Tempe AZ and Lincoln, NE.
- The screening event consists of simple anthropometric measurements, fasting blood sample for clinical labs and FibroScan assessment. Transient elastography and CAP measurements were performed with the FibroScan Touch 502 device using M or XL probes.
- Eligible participants are ≥18 (or ≥19 in Lincoln) years of age with a BMI ≥30 kg/m$^2$. For this study, 159 participants were included in the analysis.

**Figure 3. Flow of events for Celerion's NAFLD pre-screening database.**




**Database Snapshot:**
Over 200 subjects screened
80%, 67%,55% with a CAP value ≥260, ≥280, ≥300 dB/m respectively
28% with a VCTE value ≥ 7kPa
15% with type 2 diabetes

- Liver stiffness VCTE ≥7 kPa and liver fat CAP ≥260 dB/m cutoff values were used based on liver biopsy grading and staging of NASH [1-3].
- Elevated fasting serum values were defined as TG ≥150 mg/dL, ALT ≥43 IU/L (males) or ≥28 IU/L (females). A FIB4 cutoff value of 1.3 was applied to identify potential NASH subjects [5].

**FIB4 = (Age [years] x AST [U/L]) / (Platelet count [10$^9$/L] x √ALT [U/L])**

- Results were analyzed in a 2x2 contingency table with positive predictive value (PPV) and likelihood ratio reported. Wilson-Brown test was used to compute the 95% confidence intervals (CI). A two-tailed p-value was computed by Fisher's exact t-test with statistical significance set at $p<0.05$. Statistical analysis was performed with using Prism 7 (GraphPad Software, La Jolla, CA).

**PPV = fraction of subjects with elevated clinical labs who have liver fat/stiffness**
**Likelihood ratio = chances of liver fat/stiffness if a subject has an elevated clinical lab**

- Likelihood ratio is independent of prevalence and a robust measure of concordance, a likelihood ratio close to 1.0 suggests little to no chance.

## Results

**Table 1. Participant characteristics.**

| Parameter | NAFLD Database |
|---|---|
| Male/Female | 62/97 |
| Age (years) | 45.4±11.0 |
| BMI (kg/m$^2$) | 36.1±5.4 |
| TG (mg/dL) | 144.5±97.2 |
| ALT (U/L) | 27.5±17.8 |
| AST (U/L) | 22.9±12.6 |
| Platelet (10$^9$/L) | 265.2±58.1 |
| FIB4 | 0.82±0.48 |
| CAP score (dB/m) | 305.8±56.1 |
| VCTE score (kPa) | 8.15±8.9 |
| IQR%* | 16.2±10.9 |
| Probe size (% Medium) | 56% |

Results presented as mean±SD. *IQR% is a metric of FibroScan quality, values <30% indicate good quality, reproducible scan.

- Based on the specified cutoffs for TG, ALT and FIB4; 31%, 20% and 10% of the study population displayed elevated values respectively. Fatty liver and liver stiffness were observed in 78% and 28% of the study group respectively.

**Figure 4. Contingency tables.**

| CAP-TG | CAP≥260 | CAP<260 |
|---|---|---|
| TG≥150 | 44 | 6 |
| TG<150 | 80 | 29 |

| VCTE-ALT | VCTE≥7 | VCTE<7 |
|---|---|---|
| High ALT | 14 | 19 |
| Low ALT | 31 | 95 |

| VCTE-FIB4 | VCTE≥7 | VCTE<7 |
|---|---|---|
| FIB4≥1.3 | 7 | 9 |
| FIB4<1.3 | 38 | 105 |

**Table 2. Contingency analysis.**

| Analysis | CAP-TG | VCTE-ALT | VCTE-FIB4 |
|---|---|---|---|
| Specificity (%) | 82.6 [67.3, 91.9] | 83.3 [75.4, 89.1] | 92.1 [85.4, 95.8] |
| Sensitivity (%) | 35.5 [27.6, 44.2] | 31.1 [19.5, 45.7] | 15.6 [7.7, 28.8] |
| Negative Predictive Value (%) | 26.6 [19.2, 35.6] | 75.4 [67.2, 82.1] | 73.4 [65.6, 80.0] |
| Positive Predictive Value (%) | 88.0 [76.2, 94.4] | 42.4 [27.2, 59.2] | 43.8 [23.1, 66.8] |
| Likelihood Ratio | 2.1 | 1.7 | 2.0 |
| P value | 0.04 | 0.05 | 0.15 |

Results presented as % [95% CI].

## Discussion & Conclusion

- Fatty liver determined by CAP was identified in nearly 80% of the study group, which is consistent with other population-basedstudies [6]. Elevated TG levels were observed in a third of the cohort, resulting in a PPV of 88%.
- ALT and FIB4 demonstrated lower PPV at 42% and 44% respectively, with low concordance determined by the likelihood ratio.
- Taken altogether, elevated clinical labs may adequately reflect a NAFLD/NASH population for early clinical studies, however imaging modalities such as the FibroScan can optimize study pre-screening. FibroScan is a reproducible, quick and painless procedure that can be easily integrated into screening events.
- Moreover, recent FDA draft guidance indicates that early phase inclusion criteria for NASH studies may include biochemical (clinical labs) characterization with imagining assessments[7].
- Therefore, FibroScan assessment of liver fat and stiffness may be a more applicable I/E criteria for early clinical NAFLD/NASH studies than traditional labs alone.

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