Characterization and validation of regional variation in fibrosis within traditional fibrosis stages

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Introduction

- Histological assessment has anchored the field of metabolic dysfunction associated steatotic liver disease (MASLD) as a measure of the "ground truth".
- However, variability in human staging of fibrosis has driven the development of digital pathology approaches to improve the fidelity of the histological assessments.
- Second harmonic generation/two-photon excitation fluorescence (SHG/TPEF) imaging is such a method which generates a quantitative fibrosis (qF) score and has provided deep insights into regional differences in fibrosis evolution and during resolution in MASH.

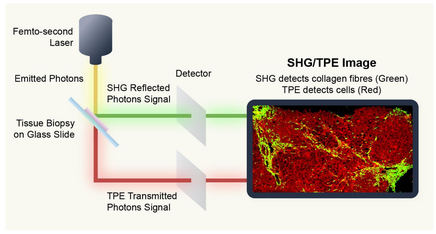
Aim

- The current study represents an independent validation of this method by the NIDDK NASH Clinical Research Network (CRN).
- The analysis will delineate the changes in collagen components observed in baseline biopsies across different stages of the disease and aims to identify specific patterns or alterations in collagen that correlate with the baseline disease stage.

Method

- A curated set of 100 paired liver biopsies, separated by a median of 4 years, was selected from the NASH CRN histological database. Of these, 45% demonstrated fibrosis progression, 10% showed no change, and 45% exhibited regression.
- SHG/TPEF imaging was performed. Overall qF scores and zonal qF scores (zones 1, 2, 3, central vein, and portal areas) were also independently reported.
- Image-based tissue qualification criteria are applied before proceeding to image analysis which include:
  - i. No. of Central Veins (CV) > 2
  - ii. No. of Portal Tracts (PT) > 5
  - iii. Biopsy Length > 10 mm
  - iv. Average width > 0.5 mm
  - v. Percentage of fragmentation < 50%
- A prespecified statistical plan and strict data chain of custody ensured study integrity and independence.
- Concordance with conventional fibrosis staging and zonal changes are assessed as part of a cross-sectional analysis by stage in the baseline biopsies.

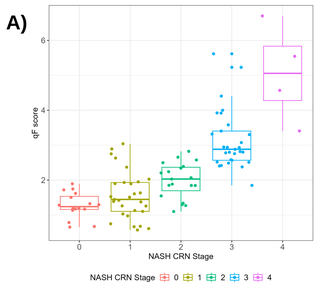
Figure 1. *SHG/TPEF stain-free imaging platform.*



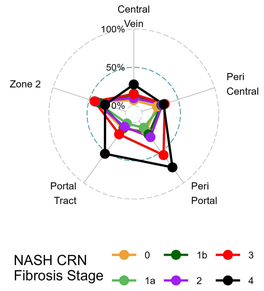
Results

Figure 2. *A) Spearman's correlation between qFibrosis continuous scores with NASH CRN fibrosis staging. B) Zonal qF readouts of n=97 baseline-only samples presented as a radar map to provide a graphical view of fibrosis in 5 areas of liver lobule, according to the C) NASH CRN staging system.*

A)



B)

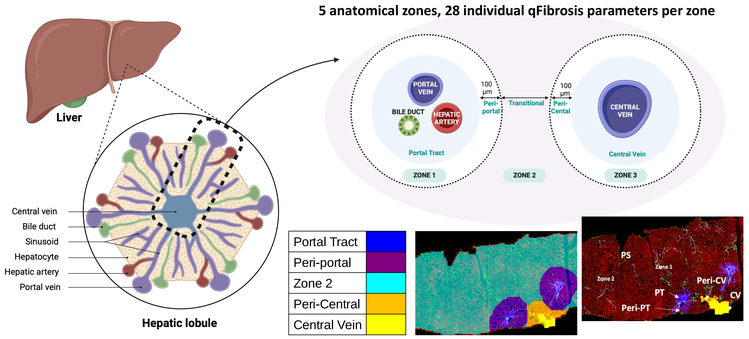


C)

NASH CRN fibrosis stage	Description
0	No fibrosis
1A	Mild perisinusoidal
1B	Moderate perisinusoidal
1C	Portal/periportal fibrosis
2	Perisinusoidal and portal/ periportal
3	Bridging fibrosis
4	Cirrhosis

- **Fig. 2A:** The overall mean qF score increased progressively with fibrosis stage and Spearman's correlation between overall qF scores and CRN staging was strong (rs=0.75).
- **Fig. 2B:** Portal, zone 1, and zone 3 qF scores increased with advancing fibrosis, while interestingly zone 2 fibrosis scores were lower in cirrhosis compared to stage 3 (p=0.023).

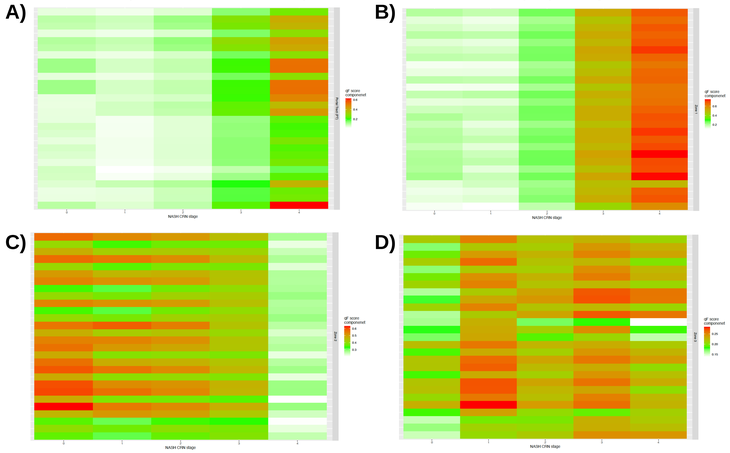
Figure 3. *Schematic representation of the liver zones as analysed by SHG/TPEF imaging.*



Correlation analysis of the individual qFibrosis parameters with fibrosis staging revealed:

- **Zone 1** – All parameters including peri-portal showed strong positive correlation
- **Portal tract** – 11 out of 28 parameters showed strong positive correlation
- **Zone 2** – 23 out of 28 parameters showed weak, but negative correlation

Figure 4. *Heatmap generated for visualization qFibrosis parameters in A) Portal tract, B) Zone 1, C) Zone 2 and D) Zone 3, stratified by NASH CRN fibrosis stage, across n=97 baseline samples. Detailed parameter-level descriptions are not shown due to proprietary reasons.*



- Heatmaps of the portal, zones 1 and 2 show the trend of how fibrosis components evolve with fibrosis stage
- The big change in zone 3 (pericentral region) occur from F0 to F1, and not much changes after, reflecting reality of early perisinusoidal fibrosis development in zone 3.

Conclusions

- Digital pathology with SHG microscopy provides enhanced granularity in assessing MASH fibrosis, allowing the detection of detailed fibrillar properties beyond whole-stage changes identified through ordinal scoring, supporting its capability in providing deeper insights into the complexities of fibrosis progression and regression.
- Subsequent analyses on the follow-up biopsies will evaluate the prognostic significance of these specific qFibrosis parameters in relation to different clinical outcomes.

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