Evaluation of a novel two-photon microscopy-based fibrosis phenotypic composite score and its correlation with serum neo-epitope collagen biomarkers in patients with NASH

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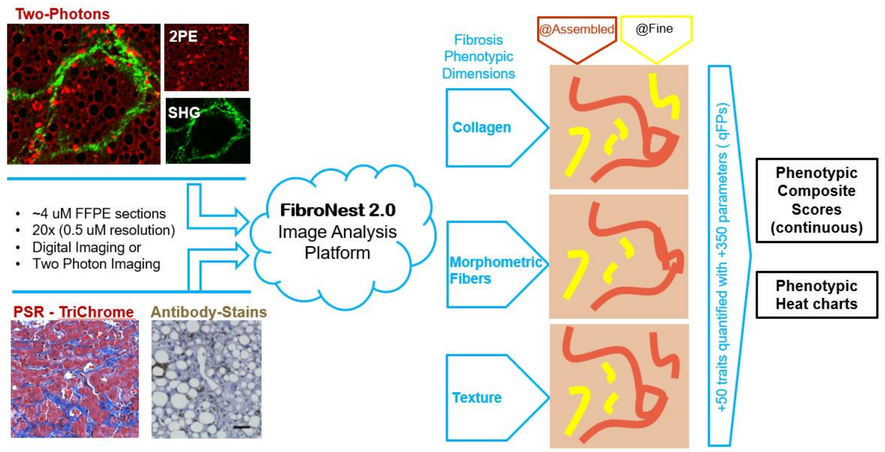
BACKGROUND

The current standard to diagnose nonalcoholic steatohepatitis (NASH) is based on categorical histologic staging. Two-photon microscopy-based image analysis provides quantitative and continuous measurements of fibrosis. We have previously reported that collagen formation biomarker PRO-C3 is significantly related to fibrosis stages in NASH patients. In this study, we evaluated the performance of a new phenotypic fibrosis composite score and its relationship with serum neo-epitope collagen biomarkers.

METHOD

TISSUE PREPARATION, INSTRUMENTATION, AND WORKFLOW



STUDY DESIGN

This retrospective study comprised a cohort of patients (n=98) with NASH diagnosed by histologic assessment of liver biopsy according to NASH CRN criteria by pathologists. Fibrosis stages 0-4

Fibrosis 0	Fibrosis 1	Fibrosis 2	Fibrosis 3	Fibrosis 4
N= 24	N=24	N=25	N=20	N=5

1. **Unstained histological slides from biopsies were imaged with Genesis200® Two-Photon microscopy system.**
2. **FibroNest, a novel cloud-based image analysis platform, quantifies fibrosis at (1) at the tissue level (2) at each collagen fiber level, individually and statistically, and (3) for texture (relative arrangement of the fibers). Each trait is described by several quantitative fibrosis parameters (qFPs) to account for mean, variance, outliers and progression (cut-off values are optimized for each trait). Selected from 300+ quantifiable fibrosis parameters (qFPs) are combined to form the Phenotypic- Fibrosis Composite Scores, a continuous phenotypic quantifier of fibrosis.**
3. **Serum neo-epitope biomarkers were measured by Nordic Bioscience. Kruscal Walis analysis was used to evaluate the correspondence of the Ph-FCS with fibrosis stages. Spearman Analysis was performed to evaluate the correlation of serum biomarkers and Ph-FCS.**

Conclusion

- The Ph-CFS and all qFPs significantly correlated with fibrosis stages ($p<0.00001$) and differentiates between F2-3-4 stages.
- Serum collagen neo-epitope biomarkers (PRO-C3, PRO-C6, PRO-C5, P4NP, C3M, and C4M) were measured, but only collagen III fibrogenesis biomarker PRO-C3 demonstrated significant correlation with Ph-CFS ($p<0.00001$).
- In addition, PRO-C3 also correlated with individual qFPs including morphometric measurement for collagen fiber length, perimeter, area, and structure index (collagen network complexity) ($p<0.00001$).

- **FibroNest** is a valuable image analysis tool to quantify fibrosis. It generates Composite Fibrosis Scores from quantitative fibrosis morphometric and texture parameters to provide an objective and reliable evaluation of fibrosis severity and progression in NASH. This can potentially be used to assess fibrosis regression with pharmacological agents.

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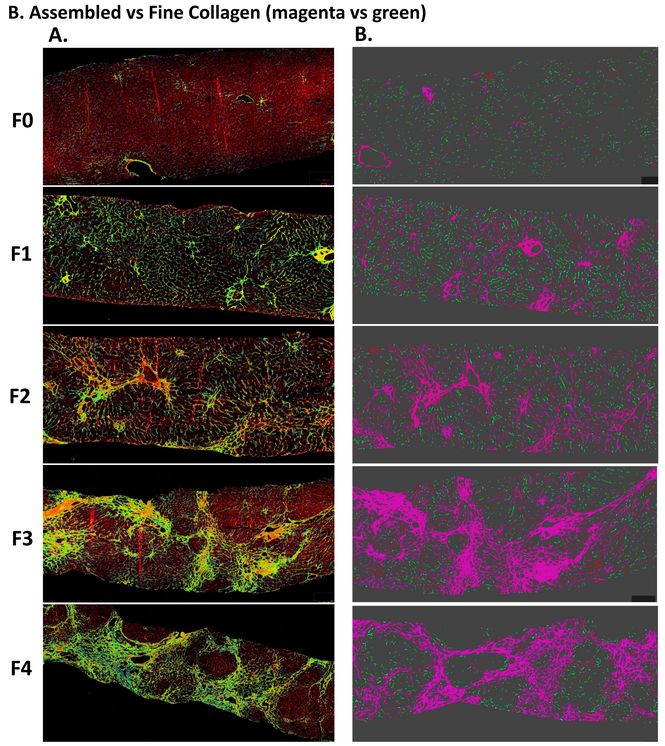
RESULTS

Figure 1. Patient liver biopsies are grouped into fibrosis stages F0 to F4.
A. SHG/ 2PE histological livers. Collagen (fluorescent green), Tissue (red)
B. Assembled vs Fine Collagen (magenta vs green)



C. Collagen Area Ratio (CAR)%

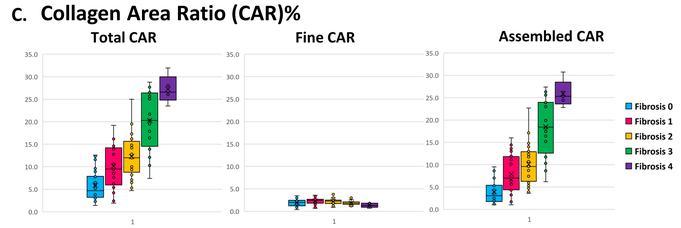
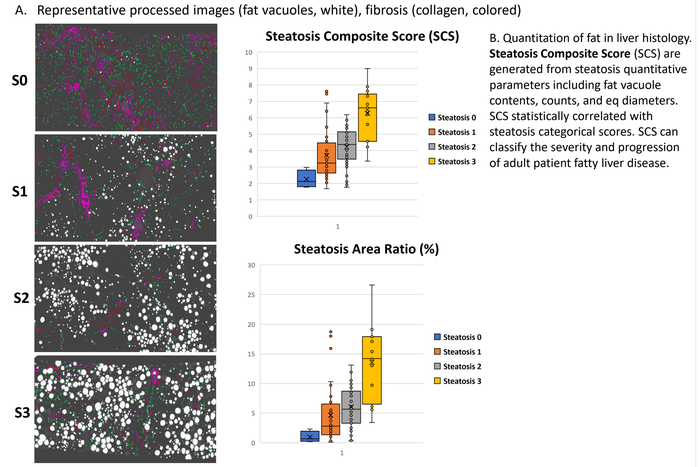


Figure 2. Steatosis Analysis

Patient liver biopsies are grouped into pathology grades (Steatosis S0-3).

A. Representative processed images (fat vacuoles, white), fibrosis (collagen, colored)

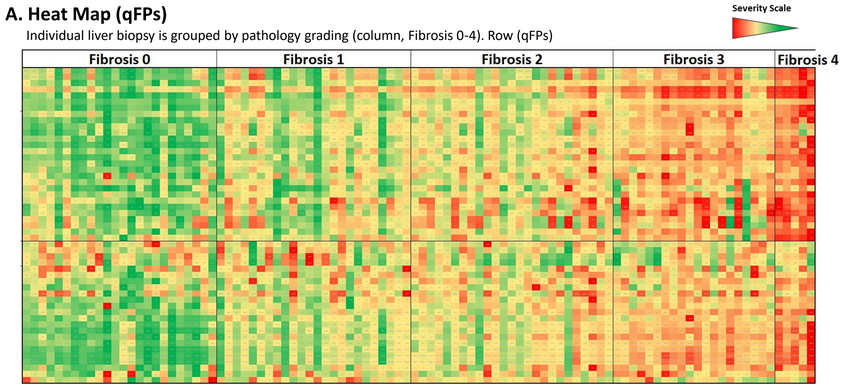


B. Quantitation of fat in liver histology. **Steatosis Composite Score** (SCS) are generated from steatosis quantitative parameters including fat vacuole contents, counts, and eq diameters. SCS statistically correlated with steatosis categorical scores. SCS can classify the severity and progression of adult patient fatty liver disease.

Figure 3. Fibrosis Collagen Phenotypic Analysis

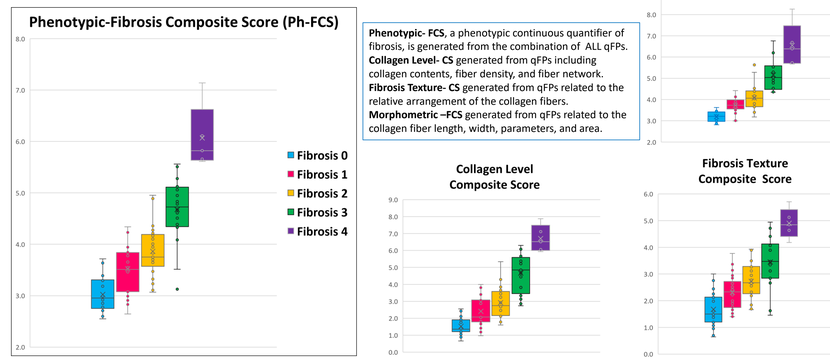
A. Heat Map (qFPs)

Individual liver biopsy is grouped by pathology grading (column, Fibrosis 0-4). Row (qFPs)



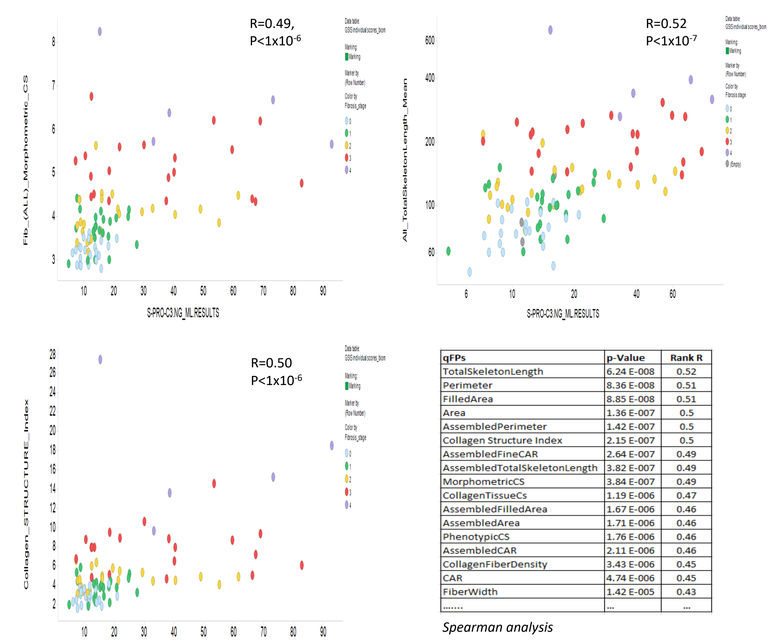
B. Quantification of fibrosis parameters (qFPs)

The Fibrosis Composite Scores demonstrated statistically significant trends with the fibrosis stages.



Phenotypic- FCS, a phenotypic continuous quantifier of fibrosis, is generated from the combination of ALL qFPs.
Collagen Level- CS generated from qFPs including collagen contents, fiber density, and fiber network.
Fibrosis Texture- CS generated from qFPs related to the relative arrangement of the collagen fibers.
Morphometric –FCS generated from qFPs related to the collagen fiber length, width, parameters, and area.

Figure 4. Moderate correlation of serum PRO-C3 levels with many fibrosis parameters (qFPs) (spearman analysis)



qFPs	p-Value	Rank R
TotalSkeletonLength	6.24 E-008	0.52
Perimeter	8.36 E-008	0.51
FilledArea	8.85 E-008	0.51
Area	1.36 E-007	0.5
AssembledPerimeter	1.42 E-007	0.5
Collagen Structure Index	2.15 E-007	0.5
AssembledFineCAR	2.64 E-007	0.49
AssembledTotalSkeletonLength	3.82 E-007	0.49
MorphometricCS	3.84 E-007	0.49
CollagenTissueCs	1.19 E-006	0.47
AssembledFilledArea	1.67 E-006	0.46
AssembledArea	1.71 E-006	0.46
PhenotypicCS	1.76 E-006	0.46
AssembledCAR	2.11 E-006	0.46
CollagenFiberDensity	3.43 E-006	0.45
CAR	4.74 E-006	0.45
FiberWidth	1.42 E-005	0.43
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Spearman analysis

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