Development of an Optimal Continuous Pediatric Fibrosis Score to Assess Severity and Progression of Fibrosis in NAFLD

Elena Reynoso1, Li Chen2, Mathieu Petitjean2, Cynthia Behling3, Joel Lavine1

1 Columbia Vagelos College of Physicians & Surgeons, NY, NY 2 PharmaNest, Princeton, NJ 3 Univ of California, San Diego, CA

ABSTRACT

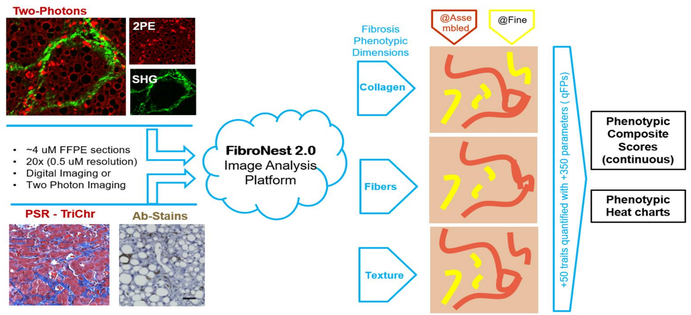
- Histological staging of NAFLD is essential to determine severity and progression to NASH.
- Fibrosis a key manifestation of progression, assessed by current standardized categorical staging as F0-F4.
- Pediatric histology demonstrates a different pattern and quality of fibrosis in a significant subset of children, not found in adults.
- Pathologists are thus limited by categorical limitations to quantify and qualitate the variations and complexities between the distinct types of steatohepatitis and progression of disease.
- This study presents the utility of an automated morphometric image analysis method of label-free two-photon images to score fibrosis in liver biopsies as a continuous measure.

OBJECTIVES

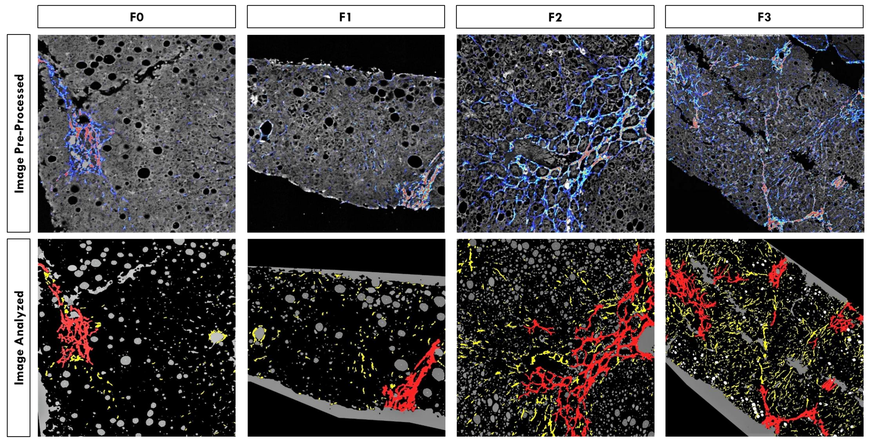
To develop a continuous pediatric Fibrosis Score for the assessment of fibrosis development in pediatric NASH.

METHODS

- Pediatric fatty liver biopsy slides (N=90) were read by pathologists for standard fibrosis staging.
- Unstained slides from the same scored biopsies were imaged with Genesis200® 2PE and Second Harmonic Generation (SHG) images .
- Image analysis using cloud-based computational methods (FibroNest©, PharmaNest, USA) exploited the SHG images to quantify multiple traits of the collagen phenotype.
- Statistical analysis with ANOVA was conducted to compare pathology staging F0(29), F1(43), F2(10) and F3(7), with calculated composite scores.

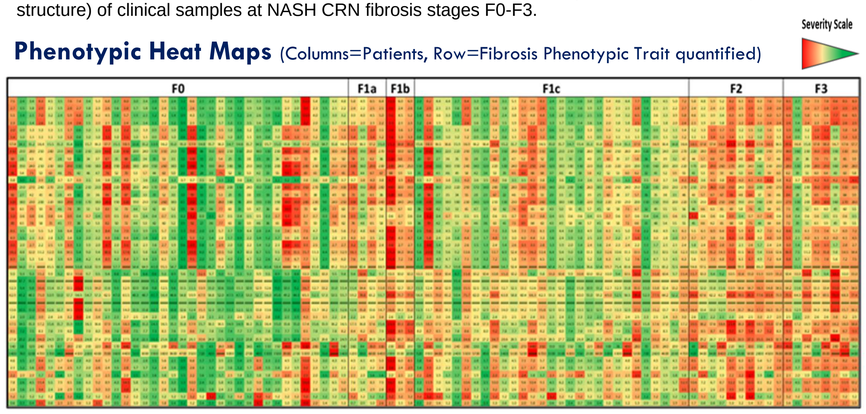


Results – Imaging



SHG/2PE Images are preprocessed (Tissue: white, Collagen: blue|low to red|high density) and then analyzed to quantify steatosis (grey with centroid) and collagen (yellow|fine structure and red|assembled structure) of clinical samples at NASH CRN fibrosis stages F0-F3.

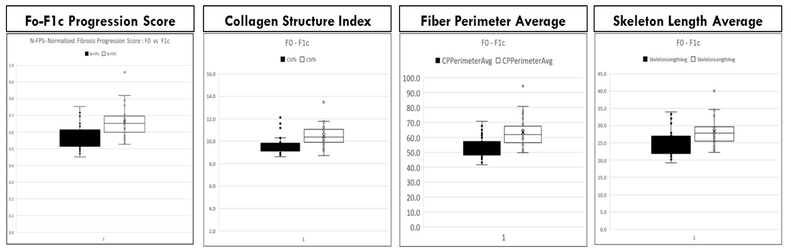
Phenotypic Heat Maps (Columns=Patients, Row=Fibrosis Phenotypic Trait quantified)



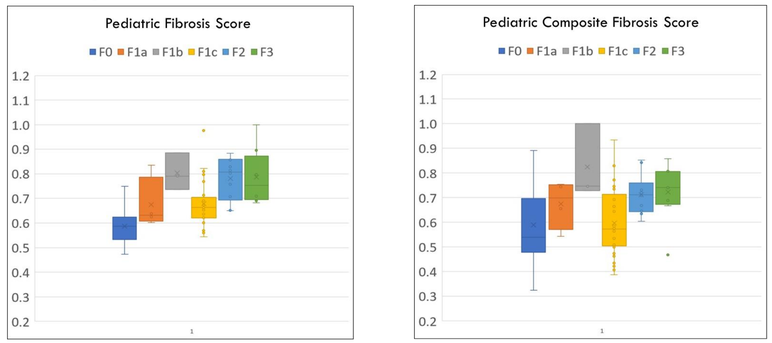
- The pediatric Composite Fibrosis Score (pCFS) was calculated from a selection of the 44 phenotypic parameters.
- The pCFS was significant (p=.001) when compared to fibrosis stage. The progression of the thickness and form factor of the skeleton of the fibrosis fibers are found to be specific to the F0/F1c transition and are used to generate a pediatric Fibrosis Progression Score (pFPS).
- The quadratic mean of the pCFS and pFPS yields a pediatric Fibrosis Score (pFS) that performs better when compared against pathologist staging (p<.000) and significantly demonstrates progression (p=.008).

RESULTS -Analysis

F0-F1c Progression Scoring (3 phenotypic traits help differentiate F0 from F1c)



Phenotypic Composite Scores correlate with NASH-CRN fibrosis Scoring



The inclusion of the F0-F1c Progression score in the Pediatric Composite Score (from the 44 phenotypic traits) to create the final Pediatric Fibrosis Score with better correlation with the NASH-CRN scores

CONCLUSIONS

- A continuous pFS calculated by phenotypic image analysis of unstained pediatric liver biopsies correlates with histological staging.
- Development of this automation/technology should permit quantitation of linear changes in fibrosis that may assist in limiting time commitments or sample sizes in clinical trials
- Demonstrate meaningful continuums in progression/regression.
- This methodology also has the potential to distinguish the two NASH subtypes
- Classify F0 from F1c, mitigating current limitations of pathology.