Automated Morphometric Fibrosis Phenotyping of NAFLD Biopsies Digital Images Helps Classify NASH-type 1 Vs NASH-type 2 in Early Fibrosis Pediatric Patients.

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) in the pediatric population encompasses liver histology ranging from isolated steatosis to fat with hepatocyte injury, inflammation and varying severity of fibrosis. Standard histological criteria for pediatric NAFLD and NASH are underdeveloped and two distinct forms of steatohepatitis have been reported: Type 1 is characterized by steatosis, ballooning degeneration, and centrilobular perisinusoidal fibrosis while Type 2 (most prevalent) is characterized by steatosis, portal inflammation, and portal fibrosis. Because the traits from both phenotypes can coexist, it can be difficult, in some cases, to adjudicate a definitive type. This study develops an automated image analysis of two-photon images from unstained biopsies to classify NASH-1 vs NASH-2 in a pediatric patient at early fibrosis stages (F1-F2).

Methods: Samples (N=51) are pediatric fatty liver biopsy slides obtained with IRB approval from local NAFLD repository. Stained slides were read by local pathologists for standard NAS, NASH-1 and NASH-2 classification. Unstained slides from the same biopsies were imaged with a Genesis200® (Histoindex, Singapore) Two-Photon microscope to generate label-free, Two-Photon Excitation fluorescence (2PE) and Second Harmonic Generation (SHG) images at 20X and 0.4 micron resolution. Sophisticated cloud-based image analysis computational methods (FibroNest©, PharmaNest, USA) are used to exploit the SHG images and quantify the fibrosis Phenotype by quantifying multiple fibrosis traits.

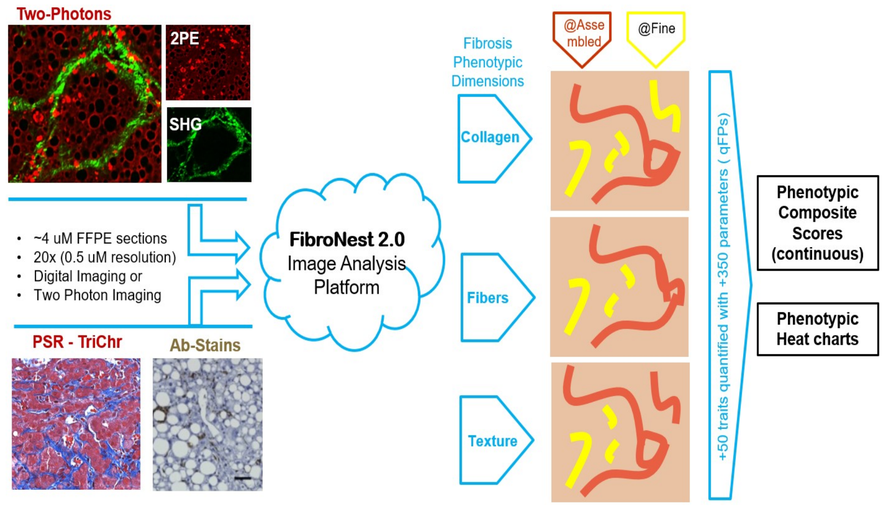
Results: Among these traits, the normalized density of thick and reticulated fibers and the average length of the skeleton of the collagen fibers are significantly different between the NASH-1 (N=32) and NASH-2 (N=19) groups. Used in combination with optimized cut-off values, these two parameters are used to classify NASH-1 vs NASH-2 biopsies with a specificity of 85% and sensitivity of 84%.

Conclusion: Automated Morphometric analysis with FibroNest © of digital images obtained from pediatric biopsies can help classify NASH-1 from NASH-2 patient in the F1-F2 region. The performance of the test can be improved by taking into account a “borderline” category when the traits of both NASH-1 and NASH-2 are observed by pathologists in the same biopsy, but the occurrence of such events is low at low fibrosis stages.

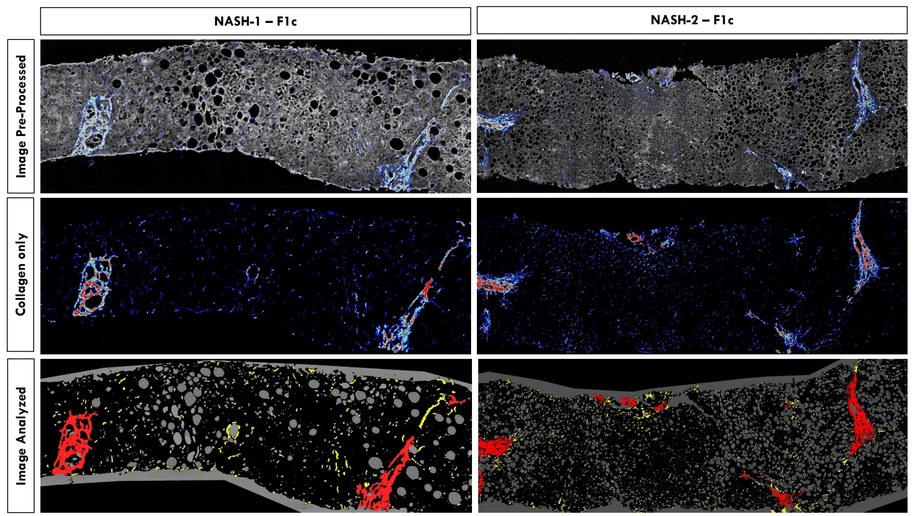
OBJECTIVE

To develop a liver biopsy-based method to classify NASH-1 pediatric phenotypes from NASH-2 phenotypes

METHODS - Tissue Preparation, Instrumentation and Workflow



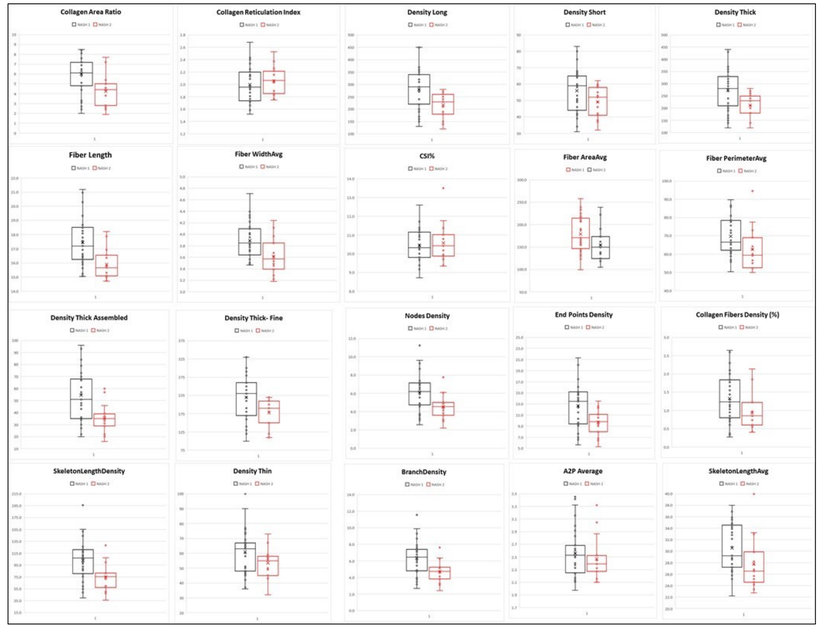
RESULTS – Imaging and Analysis



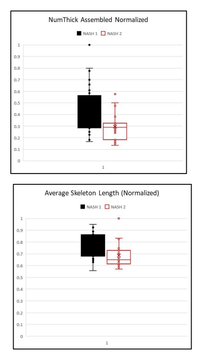
SHG/2PE Images are preprocessed (Tissue: white, Collagen: blue|low to red|high density) and then analyzed to quantify steatosis (grey with centroid) and collagen (yellow|fine structure and red|assembled structure) of clinical samples at NASH CRN fibrosis stages F1c (both) classified as NASH-1 and NASH-2

RESULTS - Quantification

Fibrosis Phenotypic traits compared between NASH-1 and NASH-2 (Selection)



Selected Traits and Selected Cut-off values help design a liver biopsy-based test to classify NASH-1 from NASH2 patients.



N=51
NASH-1= 32 (F1-2)
NASH-2= 19 (F1-2)
Specificity 85%
Sensitivity 84%