Automated Morphometric Fibrosis Phenotyping of NAFLD Biopsies Digital Images Helps Classify NASH-type 1 Vs NASH-type 2 in Early Fibrosis Pediatric Patients. Elena Reynoso1, Mathieu Petitjean1, Li Chen1, Elena Reynoso2, Cynthia Behling3, Joel Lavine2

1 PharmaNest, Princeton, NJ 2 Columbia Vagelos College of Physicians and Surgeons, New York, NY 3 University of California, San Diego, CA

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%.

RESULTS - Quantification

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

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 reassembled structure) of clinical samples at NAS CRN fibrosis stages F1c (both) as classified by NASH-1 and NASH-2.

OBJECTIVE

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

RESULTS - Imaging and Analysis

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