Automated Steatosis Morphometric Scores Benchmark the Pathology-Based Quantification of Steatosis in Pediatric NASH/NAFLD Populations

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) in the pediatric population encompasses a spectrum of liver pathology. Currently, steatosis of a pediatric liver biopsy is classified into a quantitative grade (0-3) estimating percentage of hepatocytes with macro vesicular steatosis, limiting accuracy and reproducibility.

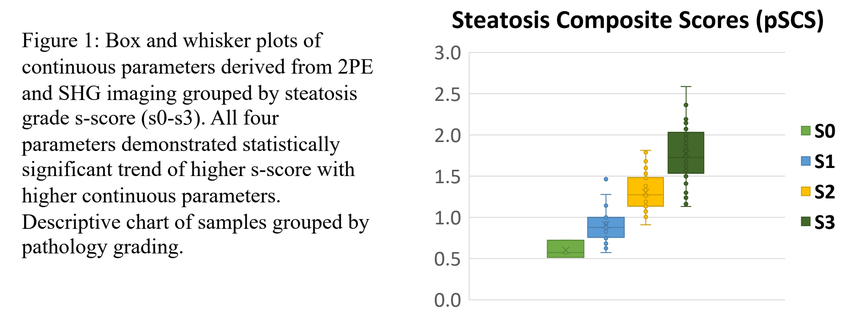
OBJECTIVE and METHOD

Develop an automated image analysis of label-free Two Photon Excitation Fluorescence imaging (2PE) to score steatosis in liver biopsies on a continuous scale, incorporating morphometric parameters, to obtain Steatosis Composite Scores (pSCS).

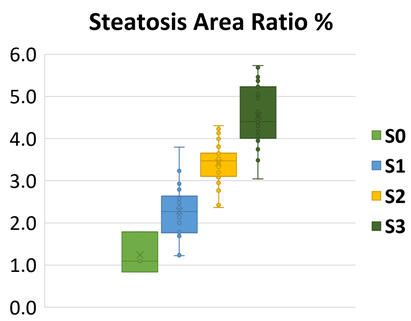
Samples (N=90) are pediatric fatty liver biopsy slides (IRB approved). Stained slides were read by pathologists for standard fat scoring s0(N=3), s1(18), s2(33), s3(37). Unstained slides from the same scored biopsies were imaged with Genesis200® 2PE and Second Harmonic Generation (SHG) images at 20X and 0.4micron resolution. Cloud-based image analysis (FibroNest©, PharmaNest, USA) is used to identify and quantify multiple liver tissue phenotypic features. A Pediatric Steatosis Composite Score (pSCS) was generated that included the steatosis content and the statistical distribution of the morphometric dimensions of the fat vacuoles. Statistical analyses were conducted to compare pathology s-scores with pSCS.

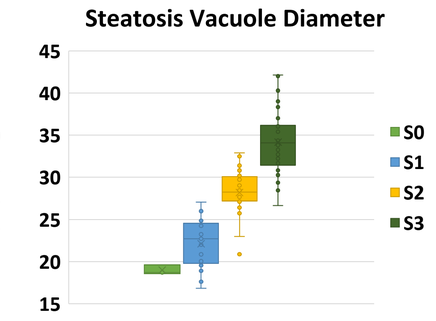
Figure 2: FibroNest© generated analysis highlighting steatosis and fibrosis. Steatosis is represented as white vacuoles. Other colors demarcate fibrosis (collagen) also analyzed and quantified
From L to R: NASH-CRN Steatosis Grades (s0, s1, s2, s3).

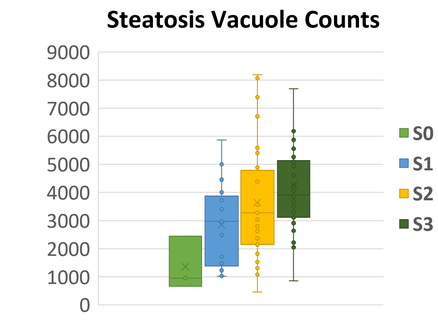
Figure 1: Box and whisker plots of continuous parameters derived from 2PE and SHG imaging grouped by steatosis grade s-score (s0-s3). All four parameters demonstrated statistically significant trend of higher s-score with higher continuous parameters. Descriptive chart of samples grouped by pathology grading.

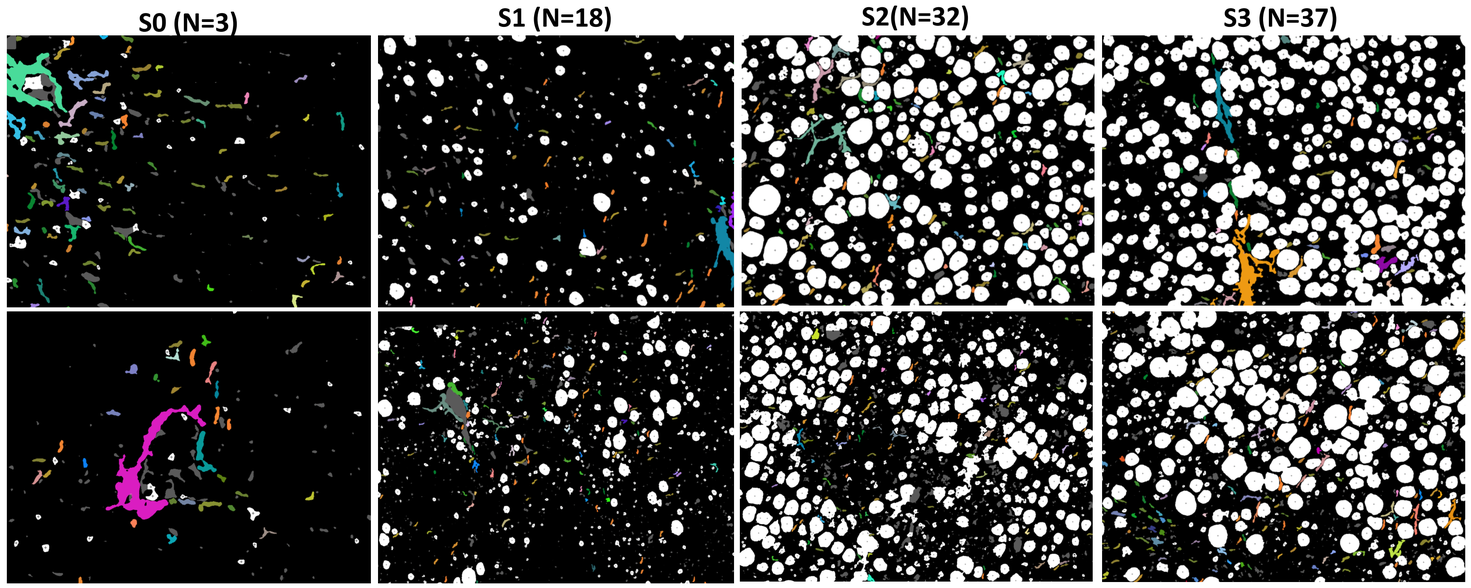


Steatosis Score	# of Samples
S0	3
S1	18
S2	32
S3	37









RESULTS

The FibroNest© image analysis of each biopsy included fat area ratio in biopsies and several morphometric parameters to describe the statistical distribution of fat vacuoles including normalized count and diameter. The pSCS is calculated as the weighted average of these parameters. This was compared to pathology scores, that classified biopsies based on level of steatosis in hepatocytes as s0(<5%), s1(5%-33%), s2(34%-66%), and s3(>67%). ANOVA of pSCS and morphometric fat vacuole diameter by s-groupings was highly statistically significant ($p<2e-16$, $p<2e-16$). ANOVA of steatosis area ratio by s-grouping was also statistically significant ($p<2.2e-16$). Kruskal-Wallis test of fat vacuole count by s-grouping was statistically significant ($p=0.003946$). A sSCS 4-level scale can be proposed (pSCS0 (<1.30), pSCS1(1.30-1.80), pSCS2(1.80-2.27), pSCS3 (>2.27)) to match the s scale, each with a sensitivity (specificity, N) of 72%(84%,18), 66%(86%,33), 84%(93%,37) when benchmarked to s1, s2, s3 values.

CONCLUSIONS

A pSCS calculated by morphometric image analysis of unstained pediatric biopsies classifies the severity and progression of pediatric liver fat in a continuous and linear manner. The pSCS highly correlates with the pathology categorical s-scores, and provides a linear, more accurate and reproducible means to quantitate changes in severity in this histologic feature.