Quantitative Histopathological Analysis of a Diet-Induced NASH Mouse Model Using Artificial Intelligence

REVEAL BIOSCIENCES
Data Powered Pathology

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease. It results in an accumulation of fat in the liver, and can progress to a more pathologically significant form of NAFLD known as non-alcoholic steatohepatitis (NASH). Patients with NAFLD and NASH present on a spectrum of the disease, characterized by hepatitis (inflammation) and hepatocellular ballooning (cellular injury), which can lead to excessive fibrosis and scarring. Currently, diagnosis is confirmed by liver biopsies that are qualitatively analyzed by experienced pathologists who assign scores for each feature. However, documented inter-pathologist variability in scoring and the semi-quantitative nature of the scoring system itself highlight the need for new methods to differentiate NAFLD and NASH, and ensure the unbiased, consistent assessment of disease to better diagnose and stratify patients for treatment options. To support this effort we developed imageDx™: NASH, a collection of artificial intelligence (AI)-based pathology models, to histopathologically validate and characterize NASH in a novel Diet-Induced NASH BL6 Model. Livers from mice on a NASH-inducing diet and a control diet were processed, sectioned, stained and digitally scanned to generate whole slide images. Quantification of each of the pathological features in mice was performed using imageDx™: NASH, including steatosis (macro- and micro-vesicular), inflammation, hepatocellular ballooning/degeneration, fibrosis and the presence of Mallory bodies. These validated machine learning algorithms were developed with input from experienced pathologists with the goal of providing a more informative analysis of the pathology. The AI-generated results were compared with semi-quantitative scores provided by a board-certified doctor of veterinary medicine. Using this diet-induced NASH rodent model both with and without therapeutic interventions, these algorithms effectively identified small differences in tissue morphology that may not be easily discernible by visual examination. By developing highly reproducible and sensitive computational models, this study provides a foundation for a better understanding of disease mechanism and the development of effective therapeutics for NASH.

INTRODUCTION

- As the incidence of NASH/NAFLD rises, so does the need for new methods to detect NASH earlier, differentiate NAFLD and NASH, and ensure reliable, consistent measurements of the disease process.
- Analysis and designation of each stage of steatosis, inflammation, ballooning, and fibrosis can be very subjective.
- Reveal Biosciences has developed a novel digital assay providing quantitative analysis for each of the pathologic features of NAFLD/NASH.
- This study uses machine learning and deep learning to provide a more quantitative and objective approach to measuring and staging the liver pathology.

EXPERIMENTAL DESIGN

6 week old male C57BL/6NTac mice (n=30) (Taconic Biosciences, Inc., Rensselaer, NY, USA) were conditioned for 30 weeks on high-fat, modified Amylin liver NASH rodent diet D09100310i (Research Diets, Inc., New Brunswick, NJ USA), containing 40 kcal% Fat (Palm Oil), 20 kcal% Fructose and 2% Cholesterol. Age-matched control mice were fed standard diet NIH-31M chow (n=5). *In vivo* work was performed by Explora BioLabs (San Diego, CA, USA).

METHODS

TISSUE PREPARATION AND STAINING

Mouse liver punch biopsies were collected (Explora BioLabs) fixed, processed, embedded, sectioned, mounted on slides and stained with Hematoxylin and Eosin (H&E), Masson's trichrome (MTC), and/or Picrosirius red (PSR) (Reveal Biosciences, San Diego, CA, USA).

AI-POWERED IMAGE ANALYSIS

Highly quantitative data were generated using imageDx™ NASH (Reveal Biosciences), an automated, integrated workflow that analyzes and manages whole slide images.

Figure 1. Digital Assay Workflow

Summary of the process that Reveal Biosciences follows to obtain analysis data. The analysis process includes automated identification of tissue, followed by segmentation of regions of interest and then classification of stained positive and negative cells and structures for measurement of expression. The identified regions were then quantified for precise positivity.

imageDx™ NASH

Steatosis Analysis	The amount of total lipid accumulation, subcategorized into macro or micro vesicular, amount of lipid/hepatocyte, and the mean vesicle size within the entire section area analyzed of the H&E stained section.
Ballooning Hepatocyte Density	The density of ballooning hepatocytes within the entire section area analyzed of the H&E stained section.
Immune Cell Density	The total number of inflammatory cells and inflammatory cell density within the entire section area analyzed of the H&E stained section.
Fibrosis Percentage (%)	The amount of total fibrosis within the entire section area analyzed of the MTC or PSR stained section.
Mallory Bodies	The presence or absence of Mallory Bodies within the entire section area analyzed of the H&E section.
Tissue Area Analyzed (mm^2)	The total tissue area used for analysis in the sample. This metric excludes any region classified as artifact, out of focus or otherwise purposely excluded from the analysis and is used for each feature analysis.

Figure 2. NASH Image Analysis Measurements

A summary of numerical quantitative data generated by imageDx™ NASH.

RESULTS

STEATOSIS ANALYSIS

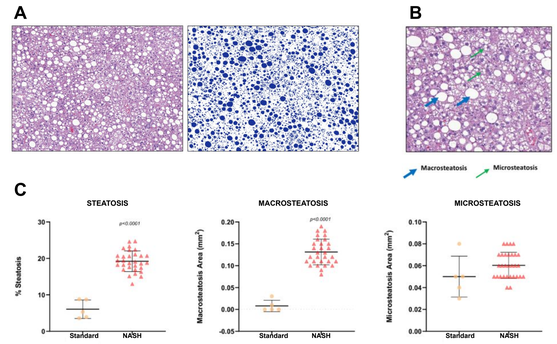

Figure 3: Cell-by-Cell Segmentation for Steatosis

(**A**) NASH mouse liver stained with H&E (left) and corresponding mask (right) identifying cells positive for lipid accumulation (blue) used for quantification. (**B**) Hepatocytes with fatty change (steatosis); examples of macrovesicular vesicles (blue arrows) and microvesicular vesicles (green arrows) within hepatocytes. (**C**) Quantification showed steatosis was significantly elevated in NASH animals, with separation of macro- and micro-vesicular steatosis. **Pathology scores:** 3 (>67%) in all NASH diet sections; hepatocytes had both microvesicular and macrovesicular fatty vacuoles in the cytoplasm. 0 (<5%) in all control NIH31M diet sections.

HEPATOCYTE BALLOONING ANALYSIS

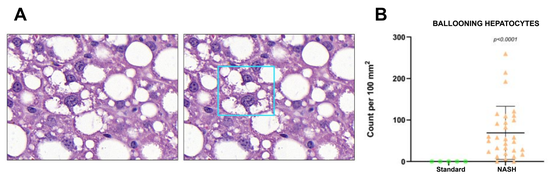

Figure 4: Cell-by-Cell Segmentation for Hepatocyte Ballooning

(**A**) NASH mouse liver stained with H&E (left) and corresponding mask used over the original image (right, blue box) to identify ballooning cells across the entire whole slide image used for quantification. (**B**) In graphical format, the data indicate the presence of hepatocellular ballooning in the NASH diet animals and lack thereof in animals fed the standard, control diet. **Pathology scores:** Ballooning in NASH diet sections ranged from few (1) to many (2), and involved all NASH diet sections. No ballooning (0) was present in control group standard diet.

MALLORY BODIES ANALYSIS

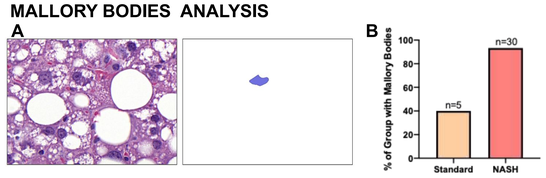

Figure 5: Cell-by-Cell Segmentation for Mallory Bodies

(**A**) NASH mouse liver stained with H&E (left) and corresponding purple mask used (right, purple) to identify Mallory bodies across the entire whole slide image used for quantification. (**B**) Graphical representation of the percentage of each group in which Mallory bodies were present. **Pathology scores:** Most of the NASH diet groups have one or more hepatocytes containing Mallory-Denk bodies. The control diet group did not have ballooning hepatocytes or Mallory bodies.

RESULTS

INFLAMMATORY CELL ANALYSIS

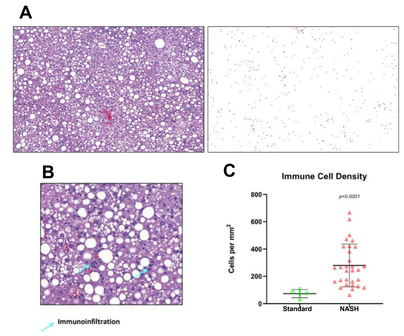

Figure 6: Cell-by-Cell Segmentation for Inflammatory Cells

(**A**) NASH mouse liver stained with H&E (left) and corresponding red mask used over the original image (right, red) to identify inflammatory cells across the entire whole slide image used for quantification. (**B**) H&E staining shows immune infiltration (blue arrows). (**C**) Graphical representation of increased immune cell density in NASH diet animals. **Pathology scores:** Inflammation in the NASH diet sections was 1-4 foci/20x field (1 or 2). Control diet animals had no measurable increase in immune cell foci.

FIBROSIS ANALYSIS

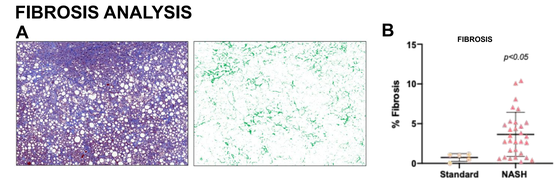

Figure 7: Cell-by-Cell Segmentation for Fibrosis

(**A**) Segmentation for fibrosis analysis shows NASH liver stained with Masson's Trichrome (left) and corresponding green mask (right, green) to identify fibrosis across the whole slide image for quantification. (**B**) Graphical representation of the percentage of fibrosis. **Pathology scores:** Fibrosis in the NASH diet and treatment groups consisted of perisinusoidal and periportal fibrosis (2) and bridging fibrosis (3).

CONCLUSIONS

- The quantitative output of the imageDx™ NASH digital assay provides highly reproducible, non-subjective, scaled measures of multiple pathological features in NASH livers.
- Detailed, quantitative measures of steatosis, inflammatory cells, ballooning hepatocytes and fibrosis can provide insight into the disease mechanism, differences between models, and response to potential therapies.
- Development of highly reproducible and sensitive computational models provides an effective approach to identification of small differences in tissue morphology that may not be easily discernible by visual examination.

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