Nonalcoholic steatohepatitis (NASH) is a common, often "silent" chronic liver disease. While resembling alcoholic liver disease, it occurs in people who drink little or no alcohol. The lesions most commonly accepted for NASH include steatosis, hepatocyte ballooning degeneration, mild diffuse lobular mixed activity and chronic inflammation, and perivenular, perisinusoidal collagen deposition. Progression of fibrosis may result in bridging septa and cirrhosis, ultimately leading to liver failure. There are no specific therapies for NASH. Current treatment focuses on controlling associated medical conditions, such as diabetes and obesity, and on monitoring for progression. Emerging antifibrotic therapies are aimed at inhibiting the accumulation of fibrogenic cells and/or preventing the deposition of extracellular matrix proteins. Development of a reproducible murine model recapitulating the progressive nature of NASH with accurate and reproducible detection and analysis of fibrosis progression would be a useful tool for studying the natural history, molecular mechanisms and biology of NASH.

Animal models mimicking NASH are in development. A major obstacle in their utilization is the identification and quantification of induced fibrosis and its amelioration due to therapy. Previous attempts to quantify such models have reproduced the metabolic and inflammatory aspects of NASH without the development of progressive liver fibrosis. A diet-induced murine model has resulted in a liver with many clinical features of NASH including hepatic fibrosis. However, fibrosis generally occurs at a low area percentage in tissue, and manual subjective evaluation cannot accurately discern discrete changes at low levels. Quantitative tissue image analysis (TIA) solutions have been developed to accurately and consistently determine percentages of induced fibrosis in various experimental animal models. This data in combination with morphologic and molecular data allows a greater utilization of these models in antifibrotic drug development.

METHODS: Mice from male fed a NASH-like inducing diet were collected and analyzed by IUA using StainMap®, a proprietary Flagship algorithm.Liver sections were stained with H&E, Picrosirius Red (PSR) and Masson's Trichrome (MTR) to visually determine increased collagen levels associated with induced fibrosis. Histology was assessed according to the Brunt staging system. Quantitative tissue image analysis for percent collagen was performed on liver sections stained with Masson's Trichrome stain (MTR) or PSR. While both stains have correlated measurements using StainMap®, PSR was chosen as the stain of choice for IUA, based on ease of visualization of PSR-positive collagen deposition and correlation with image analysis markup data. Figure 1: PSR staining. (a) Mouse fed fibrosis-inducing diet developed increased body weight, elevated fasting meal and impaired glucose tolerance. Animals also developed hyperglycemia and insulin resistance and some mice developed obesity, AST and ALT levels. The liver area grade progressively worsened, with microvascular dilatation evident at Day 90 and microvascular ischemia, with balloon degeneration present by Day 180. These findings were corroborated by a 4-fold increase in serum levels of MIA, a marker of hepatic injury. (b) Liver biopsy for steatohepatitis. (c) Serum biomarkers for steatohepatitis (ALT, AST, LDL and HDL) increased, with a 4-fold increase at Day 180. (d) The hepatic collagen expression was quantified by StainMap’s deconvolution is applied isolating the positive staining of interest (c), and the area of positive staining is determined as percent PSR Positive. (e) Liver sections stained with H&E, Masson’s Trichrome (MTR) and Picrosirius Red (PSR). Collagen is identified by blue and red coloration in the MTR and PSR stained tissues, respectively. While IUA was successful in detecting collagen crosslinking, increased perisinusoidally (not shown). Serum biomarkers for fibrosis (ALT, AST, TP, and FIB-4) progressively increased, with a 4-fold increase at Day 180.

RESULTS: Liver collagen content was determined in a stepwise process to determine area of PSR positivity and total tissue area. From these area measurements, percent PSR positive area was determined. In order to accurately compare control to late stage inducing models and in different species, the algorithm was optimized to measure collagen (based on PSR positivity) and total cross-section tissue area. The results were used to evaluate therapeutic intervention in the development of fibrosis. Progression of disease presents varying histomorphologic appearances to the liver with corresponding challenges to image analysis solutions. Optimized IUA algorithms were applied against liver sections to determine total tissue area and area of collagen deposition. Results allow discrimination of induction and therapy-related reductions in collagen amount at less than 5% increments.

CONCLUSIONS: Quantitative tissue image analysis is effective in determining percent fibrosis using collagen as the marker of fibrosis. Over 500 tissue sections from multiple animal models have been evaluated with IUA methodology. Results allow discrimination of induction and therapy-related reductions in collagen amounts at less than 5% increments. Studies have demonstrated that even at low incremental changes, therapeutic intervention may be effective. Such small incremental changes in collagen deposition may be subjectively observed by trained pathologists, but cannot be accurately measured without computer-assisted aided assistance. This tool of modeling fibrosis is being applied to different liver fibrosis-inducing models and different species. This same approach has also been applied to measure fibrosis in cardiac and renal tissues and is being investigated for use in pulmonary fibrosis animal models.

Image Analysis Quantification of Liver Fibrosis in Animal Models of Nonalcoholic Steatohepatitis (NASH)

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Figure 1: Image analysis approach to identify PSR-positive staining in fibrotic liver tissue sections. The PSR stained tissue images were first converted and extending from hepatic veins (1), the other vascular structures are identified (2), color deconvolution is applied isolating the positive staining of interest (3), and the area of positive staining is determined as both the total liver area (4).

Figure 2: Image analysis approach to identify PSR-positive staining in fibrotic liver tissue sections. The PSR stained tissue images were first converted and extending from hepatic veins (1), the other vascular structures are identified (2), color deconvolution is applied isolating the positive staining of interest (3), and the area of positive staining is determined as both the total liver area (4).

Figure 3: Example of quantitative area determination of PSR positivity. StainMap’s tuned approach exclusively detects and measures PSR positivity, and total area determination is unaffected by noncollagenous vascular structures.