Unique fibrosis progression and regression features in NAFLD, validation of concept in animal and human studies using artificial intelligence analyses (DP-AI)

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DIGITAL EXPERIENCE

INTRODUCTION

Natural history of fibrosis regression in non-alcoholic fatty liver disease (NAFLD) is not well understood and **is assumed to be a simple reversal process of the fibrosis progression**. Current fibrosis assessment relies on an ordinal staging system which reflects **static assessment** of collagen burden and its distribution. A relatively large reduction in collagen burden and distribution is needed to be reflected in a full stage change.

Hypothesis: Machine learned quantification of collagen fibrillar properties, mean septal width and cellularity of septae can:

1. Capture changes in fibrosis stage with fidelity
2. Detect both fibrosis progression and regression even when the ordinal fibrosis stage does not change

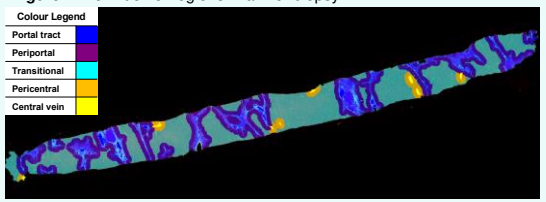
METHODS AND AIM

Aim: Correlation of conventional versus SHG-based fibrosis assessment by using SHG imaging of unstained tissue sections to provide visual mapping of collagen burden and distribution, as well as measurement of quantifiable collagen fibrillar properties.

In the animal study, mice were fed chow diet (normal) or a high fat diet with adlib sugar water administration as described before[1] for varying periods up to 48 weeks. Regression studies were done at an early time point (8 weeks, chow diet-normal water (NW) and after 48 weeks) with mice euthanized 4 weeks after diet reversal.

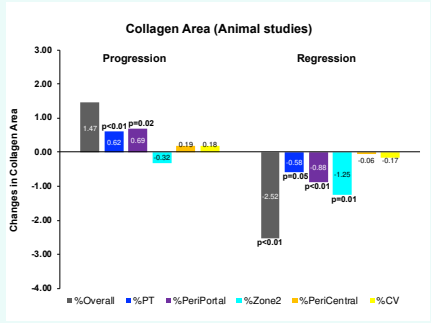
RESULTS

Figure 1. Definition of regions in a liver biopsy



- Illustration of the different regions in a liver biopsy
- "Peri" region is defined as 100 µm from either the portal tract (PT) or central vein (CV)

Figure 2. Collagen changes in different regions in animal studies.



- Data in **Figure 2** shows a **concordance** of collagen area with conventional fibrosis staging system.
- But the zonal analysis of these changes in collagen area suggest that **fibrosis regression is not a simple reversal of fibrosis progression**
- A decrease in the Zone 2 collagen area was observed in fibrosis regression. Conversely however, there was no increase in Zone 2 fibrosis during progression.

To better understand the pathobiology of progression and regression, deeper examination into the individual collagen parameters is required to show how SHG-related fibrosis parameters correlate with conventional fibrosis stages for both ≥ 1 stage fibrosis progression/regression by CRN system.

Table 1. Definition of positively/negatively correlated parameters.

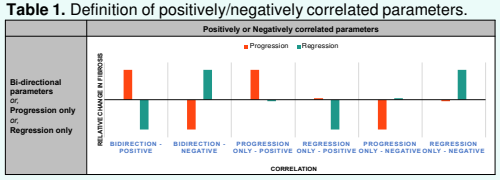


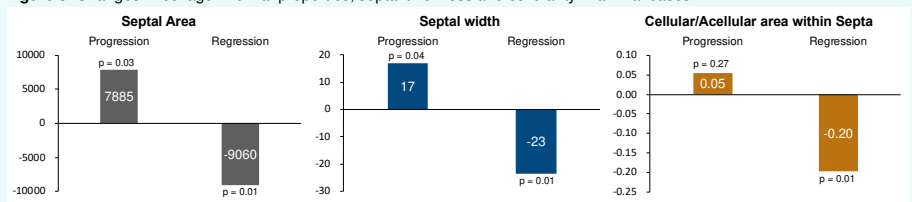
Table 1 demonstrates the systematic approach in which we **uncover collagen parameters that track either fibrosis progression or regression only; or in a bi-directional manner.** These parameters can have a positive or negative correlation. **Table 2** provides an example of some of these collagen parameters.

It is possible that at the parameter level, fibrosis evaluation is **more sensitive to change**, and we demonstrate this in **Figure 3** below.

Table 2. Example of some of these parameters. Green denotes positive correlation and red denotes negative correlation.

Bi-directional parameters	Progression only	Regression only
Percentage of total collagen at portal tract region	Number of short strings at portal tract region	Length of all strings at portal tract region
Number of strings at portal tract region	Number of distributed strings at portal tract region	Width of strings at portal tract region
Number of long strings at portal tract region	Number of short and distributed strings at portal tract region	Number of long and distributed strings at zone 2 region
Number of thick strings at portal tract region	Width of distributed strings at portal tract region	Percentage of total collagen at central vein region
Number of strings at peri-central region	Number of strings at zone 2 region	Number of thin and aggregated strings at central vein region
Number of short strings at peri-central region	Number of short strings at zone 2 region	Length of aggregated strings at central vein region
Number of thick strings at peri-central region	Number of short and aggregated strings at zone 2 region	Number of long and distributed strings at central vein region
Number of thin strings at central vein region	Number of thick and aggregated strings at zone 2 region	Width of distributed strings at central vein region
Length of all strings at central vein region	...	...
...		

Figure 3. Changes in collagen fibrillar properties, septal thickness and cellularity in animal cases.



- Further validation of the collagen parameters were conducted using DIAMOND animal models.
- We observed **concordant changes with septal area, septal width and cellularity** which track with progression on a high fat diet, and regression induced by reversal to chow diet.
- Future studies will include evaluation of these SHG-derived parameters versus biomarkers of fibrogenesis and progression to cirrhosis.

CONCLUSIONS

Quantitative image analysis of SHG/TPE generated images of unstained liver sections allow measurements of **collagen fibrillar properties, septal thickness** and **cellularity**. Similar observations have been seen in human studies as well. By applying DP-AI in a systematic evaluation, parameters associated with fibrosis progression and regression were able to reveal **greater granularity in capturing fibrosis changes**.

We believe that **SHG-derived fibrosis parameters** are **more reflective of the dynamic nature of fibrosis** in NASH than conventional static staging systems.

DISCLOSURES

Arun J. Sanyal discloses the following financial relationship(s) with a commercial interest:
Ownership interests: Sanyal Bio, Durect, Tiziana, Genfit, Exhalenz
Consultant: Gilead, Intercept, Novartis, Novo Nordisk, Inventiva, Merck, Pfizer, Boehringer Ingelhiem, Bristol Myers Squibb, Eli Lilly, Genentech, Amgen, Alnylam, Regeneron, Thera Technologies, Madrigal, Salix, Malinckrodt, Gatehouse, Rivus, Siemens, Lipocine
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CONTACT INFORMATION

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