

Quantitative Assessment For Fibrosis In NAFLD Using Collagen String Features In Specific Regions For Liver

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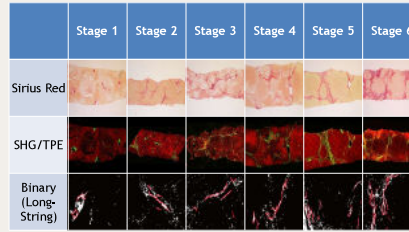
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BACKGROUND

- Digital image analysis on stained liver biopsies reveals significant correlation of collagen proportionate area (CPA) with traditional semi-quantitative staging system
- Histological staining may introduce operator-dependent variations and collagen progression dynamics has not been considered
- New SHG/TPEF microscopy can be used to observe collagen and hepatocyte morphology without staining.
- Morphological features of collagen can be extracted by sophisticated image analysis algorithms

Figure 1: Image comparison

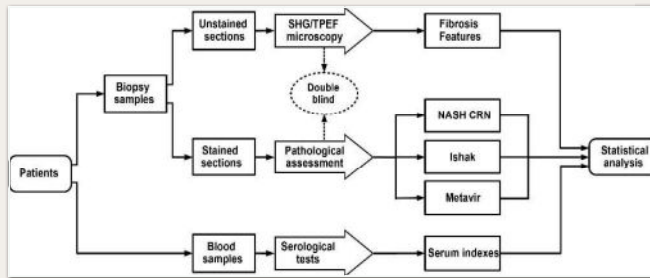


Legend: Comparison of stained images with SHG/TPEF images. The progression of hepatic fibrosis can be quantified by the SHG parameters, such as a significant feature, called long string, has been found out and the outlines of all long strings have been drawn in red.

DESIGN

- Biopsies from 101 patients with NAFLD and NASH were staged according to NASH CRN and with the seven category Ishak staging system modified to be appropriate for NASH
- Quantitative analysis was done on the unstained biopsy samples using SHG/TPEF microscopy to generate 100 quantitative collagen features including overall, portal, septal and fibrillar collagen in liver tissue
- For comparison, digitized images of Sirius red stained sections were also acquired to calculate the collagen proportionate area (CPA)
- The SHG collagen features were compared with CPA on the correlation with both staging systems and clinical data of the patients

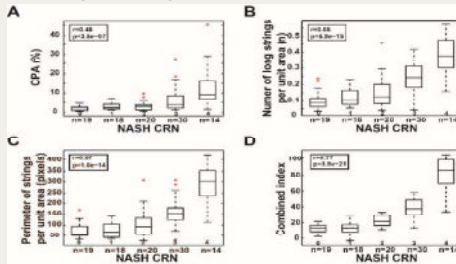
Figure 2: Study methodology



RESULTS

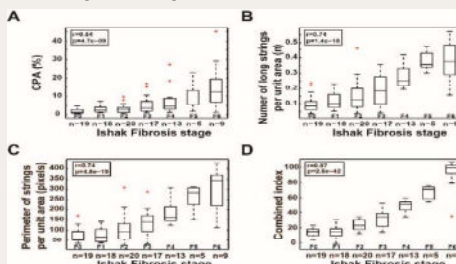
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Figure 3: Changes of Parameters with NASH CRN



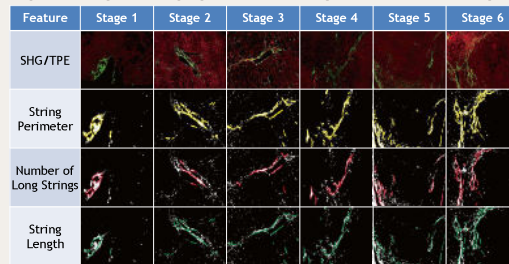
Legend: SHG features faithfully match NASH CRN. (A) Changes of CPA with NASH CRN. (B-C) Changes of two most significant SHG parameters with NASH CRN. (D) A combined index based on 20 SHG parameters has an excellent performance ($r = 0.77, p < 0.0001$). On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

Figure 4: Changes of Parameters with Ishak Score



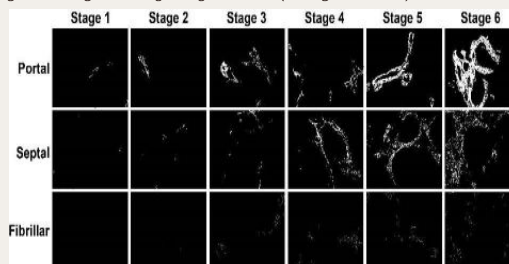
Legend: SHG features faithfully match Ishak Score. (A) Changes of CPA with Ishak Score. (B-C) Changes of two most significant SHG parameters with Ishak Score. (D) A combined index based on 20 SHG parameters has an excellent performance ($r = 0.87, p < 0.0001$). On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

Figure 5: Images showing highest r-valued string features at different stages



Legend: The features at different fibrosis stages are quite different. String Perimeter is the average perimeter of collagen strings per unit area. A collagen string is a long string if its length is longer than a threshold. In the figure, the outlines of all strings are drawn in yellow, the outlines of all long strings are drawn in red. The long axis of each string is labeled in green and the length is the length of string.

Figure 6: Images showing String Perimeter (the highest r-value) at different zones



Legend: The strings at portal, septal and fibrillar grow more large with fibrosis progression. Obviously, String Perimeter at each zone is increasing.

CONCLUSIONS

Compared with traditional stained image analysis, quantitative assessment using SHG collagen features recorded by SHG/TPEF microscopy is more robust for evaluating fibrosis in NAFLD