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Development and Validation of an Automated Assessment of Liver Fibrosis using Second Harmonic Generation Microscopy in Patients with Non-Alcoholic Fatty Liver Disease

P.E. CHANG[1,4], G.B.B. GOH[1,4], W.Q. LEOW[2,4], Y.Y. REN[3], W.K. WAN[2,4], K.H. LIM[2,4], C.K. TAN[1,4]

[1]Department of Gastroenterology & Hepatology, Singapore General Hospital, [2]Department of Anatomical Pathology, Singapore General Hospital, [3]HistoIndex Pte Ltd, [4]Duke-NUS Medical School

Singapore General Hospital
SingHealth

INTRODUCTION

- Assessment of liver fibrosis is essential in the management of patients with non-alcoholic fatty liver disease (NAFLD).
- Second Harmonic Generation (SHG) microscopy is a novel optical tissue imaging system that provides reliable automated quantification of fibrosis based on unique architectural features of collagen.
- Although SHG has been validated against METAVIR fibrosis staging in chronic hepatitis B, the utility of SHG in assessment of NAFLD fibrosis has not been established.

AIM

The aim of this study is to develop and validate unique SHG-based characteristics for the automated assessment of fibrosis stage in patients with NAFLD.

METHOD

- SHG microscopy using Genesis™ (HistoIndex, Singapore) was performed on archived liver biopsy specimens from 43 patients with NAFLD (training group).
- Unique algorithms were developed to identify specific SHG collagen characteristics that corresponded with the severity of fibrosis. A composite SHG-based index (the "***B-index***") was developed to predict fibrosis stage.
- This was compared against the histological grading by expert liver histopathologists using Brunt fibrosis staging.
- The accuracy of the algorithm to stage NAFLD fibrosis was then assessed in a separate group of 40 NAFLD patients (validation group) using AUROC analysis.
- The diagnostic performance of B-index to identify NAFLD specimens with advanced fibrosis (Brunt 3 and above) was evaluated.

RESULTS

- Mean age of the study cohort was 51.8 ± 11.7 years, with 41% males and 77% of Chinese ethnicity. Demographic, anthropometric and biochemistry data are summarized in Table 1. There were no significant differences in age, gender, ethnicity, liver function and platelet count between the training and validation groups. Subjects in the validation group had a lower BMI.
- Mean biopsy length, calculated by addition of all available fragments, was 2.4 ± 0.9 cm. Distribution of fibrosis according to Brunt classification was 23% B0, 30% B1, 8% B2, 17% B3 and 22% B4.
- A fibrosis index (***B-index***) was developed comprising 6 unique SHG-based collagen parameters related to NAFLD fibrosis (Figure 1 and Figure 2).
- The SHG-derived B-index correlated well with Brunt fibrosis staging by the histopathologist (Spearman's correlation 0.820, p<0.001) with AUROCs of 0.895, 0.982, 0.989, 0.964 for prediction of B1, B2, B3 and B4 fibrosis respectively (Figure 3).
- In the validation group, B-index predicted Brunt fibrosis stage with AUROCs of 0.740, 0.865, 0.967, 0.886. (Table 2).
- A B-index score of >1.9 had an overall diagnostic accuracy of 92.5% for prediction of presence of bridging fibrosis (Brunt≥3) with sensitivity of 83.3%, specificity 96.4%, positive likelihood ratio 23.3 and negative likelihood ratio of 0.17.

Figure 1. Illustration of central vein, portal tract and perisinusoidal regions in H&E stained image and SHG/TPEF image.

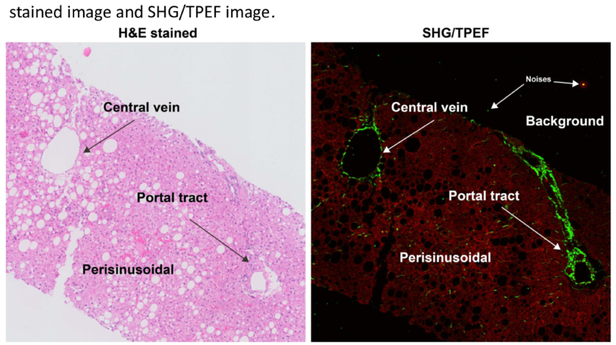

Figure 2. Illustration of specific collagen at central vein, portal tract and perisinusoidal regions.

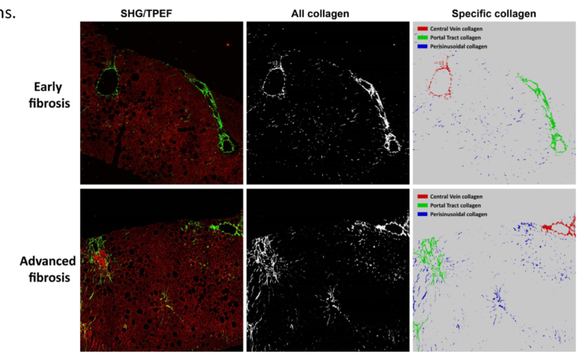

Table 1. Baseline characteristics of study cohort

	All subjects (n=83)	Training Group (n=43)	Validation Group (n=40)	*p*
Age (mean ± SD)	51.8 ± 11.7	52.2 ± 11.9	51.2 ± 11.6	0.704
Male gender	34 (41%)	16/43 (37.2%)	18/40 (45.0%)	0.509
Chinese ethnicity	64 (77%)	71.8%	92.3%	0.072
BMI (kg/m^2)	28.4 ± 6.9	30.8 ± 7.3	24.7 ± 4.2	*0.001*
HbA1c	6.9 ± 1.2	6.9 ± 1.3	6.8 ± 0.2	0.878
Albumin, g/L	42.7 ± 29.0	40 ± 4.6	46.3 ± 4.2	0.373
Bilirubin, µmol/L	19.6 ± 26.4	16.9 ± 16.7	22.8 ± 34.7	0.374
Alanine transaminase, U/L	80.9 ± 56.7	74.4 ± 37.1	89.0 ± 73.6	0.304
Aspartate transaminase, U/L	62.3 ± 44.3	56.5 ± 28.6	69.3 ± 57.7	0.250
Platelet count, x 10^9/L	220 ± 83	220 ± 76	220 ± 92	0.979
Prothrombin Time, s	10.6 ± 1.3	10.4 ± 0.7	10.7 ± 1.7	0.351
Creatinine, mmol/L	71.8 ± 24.7	73.8 ± 27.4	68.9 ± 20.4	0.484

Figure 3. Correlation of B-index with Brunt fibrosis grades

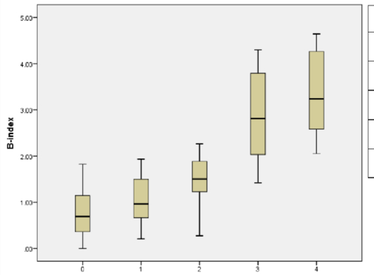

Table 2. AUROCs of B-index for prediction of Brunt fibrosis in training and validation groups

		Training Group (n=43)			Validation Group (n=40)		
Fibrosis Group	Brunt	AUROC	*p*	95% CI	AUROC	*p*	95% CI
Mild fibrosis	B1	0.895	<0.001	0.802-0.988	0.740	<0.001	0.578-0.902
Significant fibrosis	B2	0.982	<0.001	0.952-1.000	0.865	<0.001	0.719-1.000
Bridging fibrosis	B3	0.989	<0.001	0.965-1.000	0.967	<0.001	0.916-1.000
Cirrhosis	B4	0.964	<0.001	0.917-1.000	0.886	<0.001	0.780-0.991

CONCLUSIONS

This study demonstrates that the B-index (a unique SHG-based fibrosis index) provides highly reliable automated assessment of fibrosis in NAFLD patients. This index has a high specificity for advanced fibrosis, which is a clinically important sentinel time point in the management of NAFLD.

- The B index requires validation in larger cohorts from a multi-center study.
- The potential role of B-index for assessment of progression or regression of NAFLD fibrosis in longitudinal pre-and post treatment liver biopsies needs to be explored.
- Of interest is the role of B-index as a unique quantitative index for NAFLD fibrosis, correlating it to meaningful clinical outcomes rather than histological fibrosis grades.

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CONFLICT OF INTEREST

Ren YY is an employee of HistoIndex, Singapore Pte Ltd. All other authors have no conflicts of interest to declare.

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CONTACT INFORMATION

Dr. Jason Chang Pik Eu, MBBS, MRCP (UK)
Department of Gastroenterology & Hepatology, Singapore General Hospital
Email: *jason.chang@singhealth.com.sg*