Fibroblast Activity (PRO-C3) and Liver Stiffness Correlate with spatial distribution of Collagens in MASLD and ALD: Insights from Digital Pathology

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BACKGROUND

In patients with metabolic dysfunction associated steatotic liver disease (MASLD) and alcohol-related liver disease (ALD) Kleiner fibrosis stage provides limited information on the spatial distribution of collagen in the liver. Digital pathology provides a more advanced assessment of collagen localization; however, there is limited knowledge linking non-invasive tests to the spatial distribution of collagen.

Our aim was to explore the ability of digital pathology by second harmonic generation to evaluate fibrosis in ALD and MASDL as well as investigate the correlation between the spatial distribution of collagens and fibroblast activity (PRO-C3)

METHODS

- Liver biopsies and serum samples from 121 patients with MASLD enrolled at the University Medical Center Mainz, and 31 patients with ALD from the GALA-POSTBIO phase 2 trial conducted at the Centre for Liver Research, Odense University Hospital were evaluated.
- Second harmonic imaging (SHG) by Histoindex was used to quantitate hepatic fibrosis by qFibrosis (qFib) on unstained liver biopsies. qFib is a composite index combining collagen-related morphological parameters across different regions in the liver lobule.
- NordicPRO-C3™, a blood-based type III collagen formation marker of fibroblast activity was measured by ELISA and the ADAPT algorithm was calculated (PRO-C3, age, diabetes Y/N, platelets) and both correlated to the SHG analysis.
- Transient elastography (TE) was assessed by Fibroscan and correlated to the SHG analysis together with ALT and AST.

RESULTS

- Baseline demographics are seen in table 1 (MASLD cohort) and table 2 (ALD cohort).
- The Spearman correlation between PRO-C3 and qFib was similar in MASLD: (r=0.56, p>0.0001) and in ALD (r=0.55, p=0.001)(Fig 1 & 4)
- The ADAPT score (PRO-C3, diabetes, platelets, age) was highly correlated to qFib in MASLD (r=0.72, p>0.0001) and less in ALD (r=0.43, p=0.018). TE was correlated to qFib in MASLD (r=0.58, p>0.0001) and in ALD (0.46, p=0.010).
- The absolute amount of collagen across all regions (%SHG) correlated to PRO-C3 in MASLD (r=0.33, p=0.0002), and in ALD (r=0.54, p=0.002); ADAPT in MASLD (r=0.39, p= p>0.0001) and ALD (r=0.39, 0.032) as well as TE in MASLD (r=0.38, p<0.001) and ALD (r=0.51, p<0.004).
- No correlation between qFib or %SHG were seen for AST or ALT in MASLD and ALT in ALD, except for AST versus qFib (r=0.41, p=0.028) and AST versus %SHG (r=0.42, p=0.024) in ALD only.
- Finally, the spatial distribution of collagen correlated with PRO-C3, ADAPT, TE for portal tract (StrAreaPT, StrArea; StrLengthPT) however not to AST or ALT.

RESULTS- MASLD (F0-F4)

Demographics n=121

Table 1.

	Median (Q1, Q3)	n (%)
Age (Years)	54.0 (44.0, 50.0)	
BMI (kg/m²)	32.4 (29.4, 36.4)	
AST (U/L)	49.0 (38.0, 67.0)	
ALT (U/L)	73.0 (50.0, 109.0)	
FIB4	1.3 (0.9, 1.8)	
PRO-C3 (ng/mL)	11.5 (9.4, 16.6)	
ADAPT	5.4 (4.3, 6.8)	
TE(kPa)	8.2 (5.6, 12.6)	
qFibrosis value	1.7 (1.4, 2.4)	
Sex (male)		66 (54.5%)
Diabetes (no)		73 (60.8%)
Fibrosis stage		F0 (n=4, 3.3%); F1 (n=34, 28.1%); F2 (n=46, 38.0%); F3 (n=25, 20.7%); F4 (n=12, 9.9%)
qFibrosis stage		0 (n=11, 9.1%); 1 (n=25, 20.7%); 2 (n=44, 36.4%); 3 (n=29, 24.0%); 4 (n=12, 9.9%)

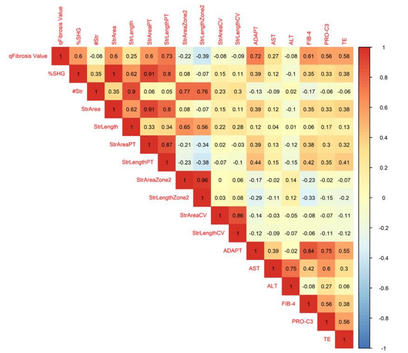


***Figure 1**. Heatmap of Spearman's rank correlations in MASLD patients (n=121)*

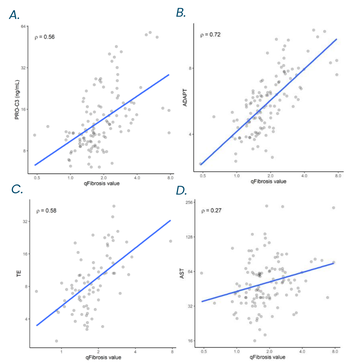


***Figure 2**. Scatter plots between A) PRO-C3 (ELISA), B) ADAPT, C) TE, or D) ALT and qFibrosis in MASLD patients*

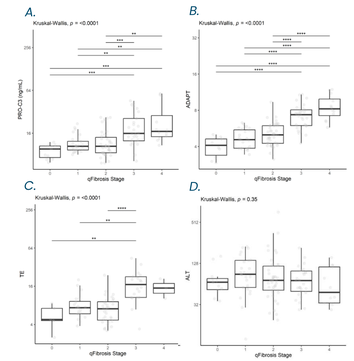


***Figure 3.** Plots of A) PRO-C3 (ELISA), B) ADAPT, C) TE, or D) ALT levels at qFibrosis stage 0-4.*

RESULTS – GALA-POSTBIO; ALD (F2-4)

Demographics n=31

Table 2.

	Median (Q1, Q3)	(n, %)
Age (Years)	64.0 (59.5, 68.5)	
BMI (kg/m²)	29.6 (24.6, 33.6)	
AST (U/L)	42.0 (34.0, 64.0)	
ALT (U/L)	33.0 (23.5, 57.5)	
FIB4	3.0 (1.8, 4.7)	
PRO-C3 (ng/mL)	22.6 (19.2, 28.3)	
ADAPT	8.1 (7.2, 9.0)	
TE (kPa)	21.1 (14.4, 32.0)	
qFibrosis value	3.4 (2.4, 5.0)	
Sex (male)		26 (83.9%)
Diabetes (no)		25 (80.6%)
Fibrosis stage		F2 (n=1, 13.2%); F3 (n=11, 35.5%); F4 (n=19, 61.3%)
qFibrosis stage		1 (n=2, 6.5%); 2 (n=5, 16.1%); 3 (n=9, 29.0%); 4 (n=15, 48.4)

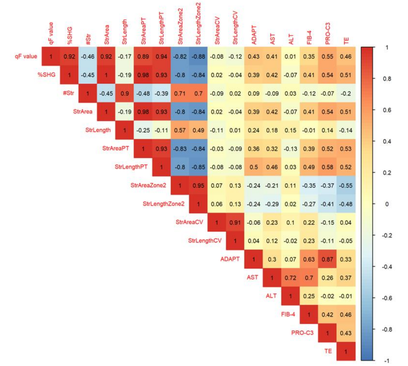


***Figure 4.** Heatmap of Spearman's rank correlations in ALD patients (n=31)*

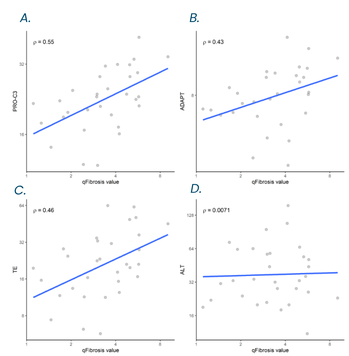
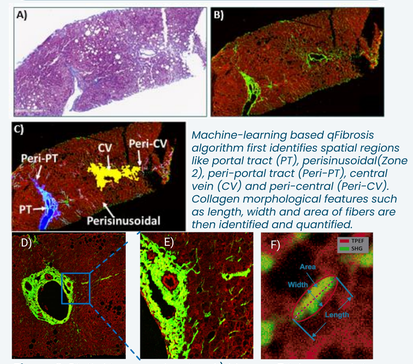


***Figure 5.** Scatter plots between A) PRO-C3 (ELISA), B) ADAPT, C) TE, or D) ALT and qFibrosis in ALD patients*

Histoindex analysis

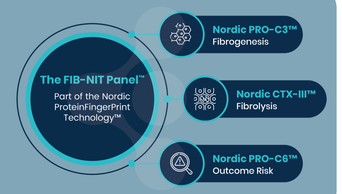


Machine-learning based qFibrosis algorithm first identifies spatial regions like portal tract (PT), perisinusoidal(Zone 2), peri-portal tract (Peri-PT), central vein (CV) and peri-central (Peri-CV). Collagen morphological features such as length, width and area of fibers are then identified and quantified.

***Figure 6**. Digital images showing A) Masson Trichrome-stain, B)SHG-scan and C) qFibrosis detection of PT: Portal Tract, CV: Central Vein. Evaluation of collagen parameters on (D) SHG/Two-photon Excitation Fluorescence (TPEF) image; (E) high magnification imaging of area denoted within the blue square in Figure 6D; (F) AI identification of string length (StrLength), string area (StrArea), string width (StrWidth).*

Key Messages

- **We observed correlations between spatial distribution of collagens and systemically assessed active fibrogenesis and liver stiffness in both MASLD and ALD.**
- **While similarities and differences were observed, these correlations were potentially influenced by etiology and sample size.**
- **These findings underscore the connection between biopsy-based digital pathology assessments and non-invasive tests for liver fibrosis.**



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