Spatial distribution of collagen in MASLD affects levels of N-terminal pro-peptide of type III collagen (PRO-C3)

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INTRODUCTION

- In MASLD hepatic fibrosis develops slowly. The NASH CRN scoring system uses a 5-tier semi-quantitative system to stage hepatic fibrosis.
- Little is known about the correlation of non-invasive tests in relation to the spatial distribution of collagen in the liver.
- We aimed to understand the relationship between HistoIndex digital quantitation and the biomarker PRO-C3, a marker of active fibrogenesis.
- Aim: To compare the correlation of blood-based PRO-C3 between continuously quantitated collagen in zonal regions, and the semi-quantitative NAS-CRN grading system.

METHODS

- In this retrospective analysis, unstained liver biopsies of 132 patients with MASLD were explored.
- 11 biopsies were excluded from unstained scanning due to sample inadequacy, and 13 more were subsequently excluded due to the PRO-C3 measurements going below lower limit of quantification (LLOQ).
- Second harmonic generation (SHG) microscopy was used to assess hepatic fibrosis (qFibrosis) on a continuous scale as previously been reported.[1]
- Specific collagen morphological features such as collagen string area (StrArea), length of collagen strings (StrLength) are identified by AI algorithm in histopathological regions including central vein (CV), portal tract (PT) and zone 2.
- PRO-C3 (ELISA), a type III collagen formation marker of fibrosis was measured and correlated to the spatial distribution of collagen.

RESULTS

Table 1. Demographics overview of 132 patients

Demographics	
Gender, male	52.7%
Age	54 [43.0 - 60.0]
BMI	32.2 kg/m² [28.7 - 37.0]
T2DM	
ALT	73.0 [50.0 - 115.7]
AST	50 [38 - 67]
GGT	109 [60.7 - 115.7]
A1c	6.0 [5.4 - 6.9]
Transient elastography	8.2 kPa [5.7 - 13.2]
Type 2 Diabetes	41.2%
Arterial hypertension	72.5%
Hypertriglyceridemia	32.8%
Obesity	BMI >30; 54.2%
Fibrosis stage	F0 (3.1 %), F1a-c (27.5%), F2 (35.1%), F3 (23.7%) and F4 (10.7%)

Table 2. Summary table of qFibrosis and PRO-C3 (ELISA) readouts for 108 subjects.

	Overall (N=108)
qFibrosis Value	
Median (Q1, Q3)	1.66 (1.34, 2.32)
Range	0.48 - 7.77
qFibrosis	
0	11 (10.2%)
1	24 (22.2%)
2	39 (36.1%)
3	23 (21.3%)
4	11 (10.2%)
PRO-C3 ELISA (ng/mL)	
Median (Q1, Q3)	11.00 (9.35, 16.25)
Range	6.10 - 57.70

- The median (Q1, Q3) qFibrosis value was 1.66 (1.34, 2.32) with a range from 0.48 – 7.77. The median (Q1, Q2) PRO-C3 (ng/mL) was 11.0 (9.4-16.2) with a range from 6.1 – 57.7. The Spearman correlation between PRO-C3 and qFibrosis was high (ρ=0.56, 95% CI [0.43, 0.67]). This was not seen for AST (ρ=0.27, 95 % CI [0.10, 0.43]), nor for ALT (ρ=-0.080, 95% CI [-0.25, 0.10]).

Figure 1. Scatter plots between qFibrosis value versus A) PRO-C3, B) AST, and C) ALT.

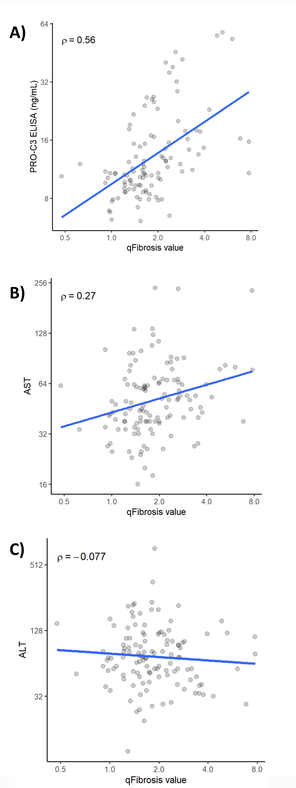

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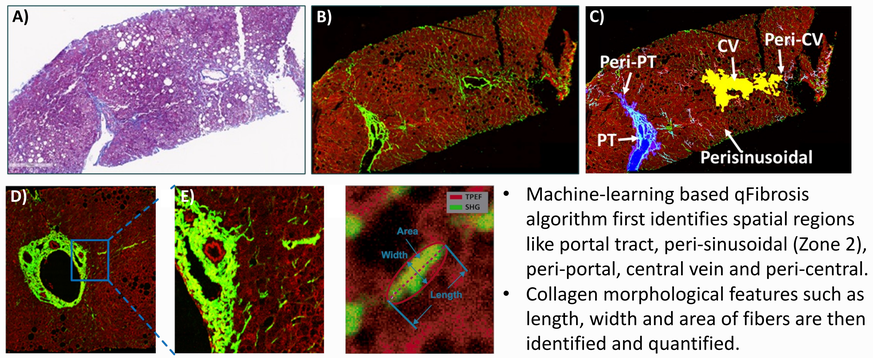
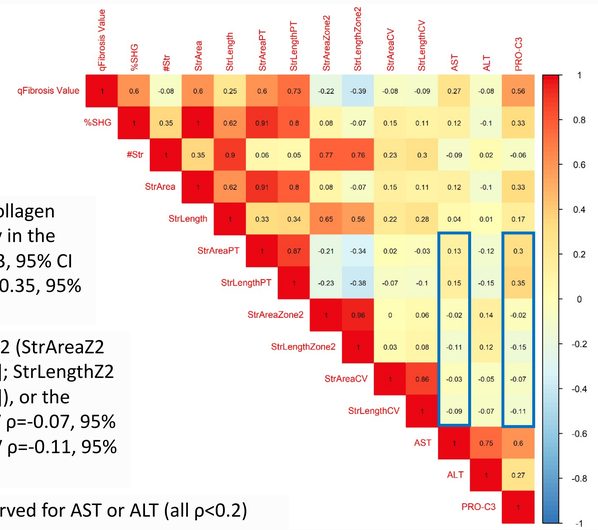
Figure 2. Digital images showing A) MT-stained, B) SHG-scanned and C) qFibrosis detection of PT: Portal Tract, CV: Central Vein. Evaluation of collagen parameters on (D) SHG/TPEF image; (E) high magnification imaging of area denoted within the blue square in Figure 2D; (F) AI identification of string length (StrLength), string area (StrArea), string width (StrWidth).



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

Figure 3. Heatmap of Spearman's rank correlation.



- When exploring the absolute amount of collagen across all regions (%SHG) the correlation with PRO-C3 was ρ=0.33, 95% CI [0.16, 0.48].
- The spatial distribution of collagen correlated with PRO-C3 only in the portal tract (StrAreaPT ρ=0.3, 95% CI [0.13, 0.46]; StrLengthPT ρ=0.35, 95% CI [0.18, 0.50]).
- It was not observed in zone 2 (StrAreaZ2 ρ=-0.02, 95% CI [-0.20, 0.16]; StrLengthZ2 ρ=-0.15, 95% CI [-0.32, 0.03]), or the central vein area (StrAreaCV ρ=-0.07, 95% CI [-0.25, 0.11]; StrLengthCV ρ=-0.11, 95% CI [-0.28, 0.07]).
- Weak correlations was observed for AST or ALT (all ρ<0.2)

CONCLUSION

- PRO-C3 is both a stage and an activity marker so it is not expected to correlate completely to assessments obtained from a biopsy section evaluated either by a pathologist or AI.
- Current analysis highlights the correlation of non-invasive collagen biomarkers with hepatic collagen is dependent on collagen spatial distribution.
- While the NASH-CRN system stresses changes of collagen in the peri-portal region, the current finding highlights that in blood-based biomarkers such as PRO-C3, spatial fibrosis distribution is relevant.
- Additional validation will be conducted on a larger dataset.

ACKNOWLEDGEMENTS

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REFERENCE

1) Liu F, et al. Hepatology 2020; 71: 1953–1966

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