Subgroup definition and monitoring of liver fibrosis evolution in patients with MASH bridging fibrosis based on objective measurements of septa parameters using digital pathology with artificial intelligence analyses

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Introduction

- Metabolic Dysfunction-Associated Steatohepatitis (MASH) with bridging fibrosis (stage F3) is a critical, yet very broad category, in steatotic liver disease encompassing subjects with potential progression to cirrhosis or regression to milder stages.
- Second harmonic generation/two-photon excitation fluorescence (SHG/TPEF) microscopy with artificial intelligence (AI) provides sensitive and objective quantification of liver fibrosis.

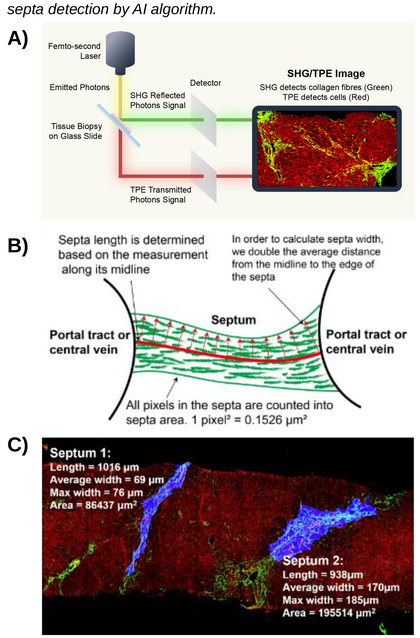
Aim

- Leveraging the advantages of SHG microscopy, a highly sensitive system for quantitative analyses of collagen fibrillar properties in unstained tissue, the present study aimed to identify subsets of the MASH F3 population by quantitative evaluation and definition of bridging septa.

Method

- Paired liver biopsies from 57 patients, all with bridging fibrosis, part of the FLIGHT-FXR trial (NCT02855164): placebo (PLB, n=17), tropifexor (TXR, n=40) were included.
- Unstained sections were analysed by SHG/TPEF microscopy with quantification of 12 septa parameters at baseline (BL) and end of treatment (EOT) biopsies. (Figure 1)
- Septum width measurement of 93 randomly selected septa was used to differentiate ***thick*** (mean width 167 µm) from ***thin*** septa (mean width 41 µm) with a cutoff of 88.5 µm, as determined by the Youden's index.
- The ratio between the thick septa area to total septa area per biopsy identified 2 subgroups: predominantly thin septa (F3a) (mean ratio 13%, range 0-48%) or predominantly thick septa (F3b) (mean ratio 71%, range 51-97%).

Figure 1. *(A) SHG/TPEF stain-free imaging platform.[1] (B) Schematic illustration of the septa area, length and width measurements in each liver specimen by SHG/TPEF. (C) Selected region of interest (ROI) of a SHG/TPEF image showing septa detection by AI algorithm.*



Results

Figure 2. *Selected region of interest (ROI) of Masson Trichrome (MT)-stained and SHG/TPEF images from consecutive sections showing (top row) an example of a thick septa and (bottom row) a thin septa.*

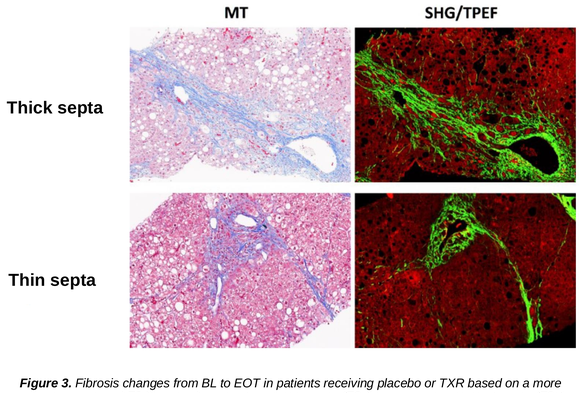
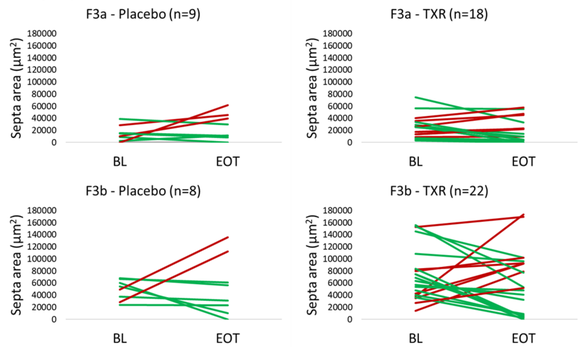


Figure 3. *Fibrosis changes from BL to EOT in patients receiving placebo or TXR based on a more granular staging of F3 fibrosis of predominantly thin septa (F3a) or predominantly thick septa (F3b). The lines indicate individual patients with either increase (**red line**) or decrease (**green line**) of the average septa area from BL to EOT.*



- Based on the analyses of SHG/TPEF images, the fibrous septa in liver specimens were categorized as progressive, regressive or intermediate septa according to the classification previously reported in patients with chronic hepatitis B.[2]
- **Thick (progressive) septa**: Broad, mostly (more than 50%) with loosely aggregated collagen fibres, irregular borders invading liver parenchyma, with moderate to marked cellular content. (Figure 2, top row).
- **Thin (regressive) septa:** Mostly (more than 50%) thin with densely compacted stroma, largely acellular, having sharp borders with liver parenchyma, some septa have broken collagen fibres. (Figure 2, bottom row)
- At BL, 27 of 57 F3 patients were categorised as F3a (PLB, n=9; TXR, n=18) and 30 were F3b (PLB, n=8; TXR, n=22). The average septa area in F3b patients was 3-fold larger (21820 ± 3383 µm²) than in F3a patients (6752 ± 7219 µm², p < 0.001). I**mportantly, septa area measurements revealed significant imbalance between those randomised to receive PLB or TXR (p = 0.027).** (Table 1)[3]

Table 1. *Median septa area in µm² (lower quartile, upper quartile) of the 2 subgroups of predominantly thin septa (F3a) and predominantly thick septa (F3b). Wilcoxon paired test is used to determine significance between BL vs EOT.*

		BL	EOT	p-value
F3a	Placebo	10524.4 (1329.4, 22015.5)	11474.6 (4123.7, 42553.8)	0.38
	Tropifexor	26046.6 (12326.3, 34220.4)	11977.5 (3619.2, 36068.4)	0.20
F3b	Placebo	51999.7 (30762.6, 65272.9)	43822.3 (13633.6, 99577.3)	0.74
	Tropifexor	66243.9 (39611.6, 90085.4)	52330.7 (6867.6, 93468.4)	0.78

- From BL to EOT, intra-stage changes F3a to F3b and vice versa were observed in 5 of 11 PLB and 10 of 28 TXR cases with unchanged F3 stage by the ordinal scoring
- The placebo cohort showed no consistent trend in septa area dynamics across F3a or F3b subgroups, while the TXR cohort, especially F3b subgroup, showed a notable reduction in the septa area, though not statistically significant. (Figure 3)

References:
1.Naoumov NV, Chng E. FMAI. 2024;2(2).
2.Sun Y et al. Hepatology. 2017 May;65(5):1438-1450.
3.Naoumov NV et al. Liver Int. 2024 Dec;44(12):3214-3228.

Conclusions

- Digital pathology with SHG microscopy provides enhanced granularity and precision in assessing MASH F3 fibrosis, allowing the detection of fibrosis improvement or worsening beyond whole-stage changes identified through ordinal scoring.
- The imbalance between patients randomized to the placebo and tropifexor groups highlights the advantages of digital pathology in clinical trials. Metrics such as average septa area and other septa parameters can improve patient selection, ensure balanced stratification during randomization, and support dose-response analyses in early drug development.
- The modest sample size (57 patients with MASH F3 fibrosis) and relatively short follow-up period in this study limit the ability to determine the probability of disease progression or regression based on objective subgroup separation within F3 stage. Future studies will evaluate the prognostic significance of septa characteristics, such as thickness, in relation to different clinical outcomes.

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