A deep exploration of bridging fibrosis evolution and individual septa parameters in NASH using quantitative second harmonic generation imaging reveals fibrosis changes in natural history and treatment-induced not seen with conventional histology

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INTRODUCTION

- Nonalcoholic steatohepatitis (NASH) with bridging fibrosis (stage F3) is a critical stage in the evolution of fatty liver disease, which has the highest incidence of liver-related events and all-cause mortality in the pre-cirrhotic NAFLD group[1].
- Second harmonic generation/two photon excitation fluorescence (SHG/TPEF) microscopy of unstained liver sections with artificial intelligence (AI) provides sensitive and reproducible quantitation of liver fibrosis.
- Using this novel approach, the present study aims to gain in-depth understanding of changes in liver fibrosis and individual septa parameters over time in a homogenous, well-characterised group of patients with NASH F3 fibrosis stage.

AIM

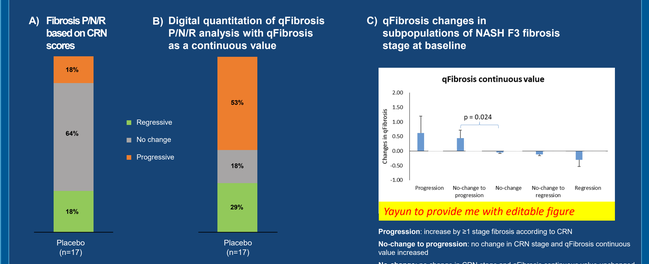
- To apply SHG/TPEF methodology with computer-assisted analyses for an in-depth, quantitative evaluation of changes in liver fibrosis and individual septa parameters in a homogenous, well-characterised group of patients with bridging NASH fibrosis (F3 stage).
- The objectives of this analysis were:
  1. To quantitatively assess and graphically present intra-stage changes of liver fibrosis from baseline (BL) to end of treatment (EOT)
  2. To compare progressive and regressive types of fibrous septa and quantitatively assess the changes in individual septa parameters from BL to EOT

METHOD

- This investigation is based paired liver biopsies from 57 patients [placebo, n=17) or tropifexor (TXR) [n=40], with biopsy-proven NASH, all with bridging fibrosis (F3 stage) according to the CRN scoring system at baseline (BL), who participated in the FLIGHT-FXR clinical trial (NCT02855164).
- Unstained liver sections from BL and end-of-treatment (EOT) were examined using SHG/TPEF microscopy. SHG/TPEF microscopy was used to assess liver fibrosis on a continuous scale (qFibrosis); these scores were also converted into categorical scores (qF0–qF4) using cut offs which have previously been reported.[2]
- Changes in liver fibrosis overall and in five different zones of liver lobules were quantitatively assessed by qFibrosis – a cumulative index based on measuring collagen features on a continuous scale.
- Radar maps were developed as a novel approach for assessing fibrosis changes in liver lobules. In addition, septa morphology – progressive or regressive septa and 12 individual septa parameters were analyzed at BL and EOT biopsies.

RESULTS

Figure 1. Digital quantification of overall liver fibrosis (qFibrosis) at BL and at the EOT reveals fibrosis regression in a greater proportion of patients than conventional microscopy in untreated patients with F3 NASH biopsies.

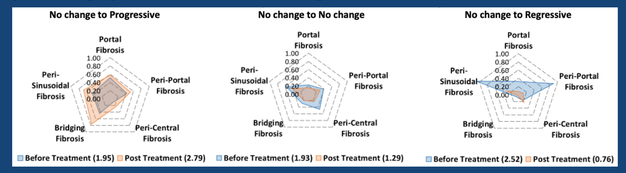


- Fig 1A and 1B: Assessment of liver fibrosis by qFibrosis continuous value showed an increased proportion of patients (29%) with fibrosis regression when compared with conventional histology (18%) for the placebo group.
- Fig. 1C: Taking into account both the NASH CRN scoring and digital quantitation readouts, patients were divided in 5 subgroups. qFibrosis provided clear separation between these 5 subgroups i.e., significantly greater fibrosis increase in the second subgroup [No change by (NASH CRN) with fibrosis progression (by qFibrosis)] compared to the consensus readout as “no change” by both methods (p=0.024).

Table 1. qFibrosis readout according to 5 different regions – Portal fibrosis, Peri-portal fibrosis, Zone 2 Perisinusoidal fibrosis, Peri-central fibrosis, and Bridging fibrosis in 3 representative ”No-change” cases according to NASH CRN.

	qFibrosis increased		qFibrosis unchanged		qFibrosis decreased	
	BL	EOT	BL	EOT	BL	EOT
Portal fibrosis	0.52	0.58	0.22	0.16	0.32	0.08
Peri-portal fibrosis	0.43	0.52	0.39	0.30	0.89	0.12
Peri-central fibrosis	0.11	0.24	0.46	0.23	0.17	0.23
Bridging fibrosis	0.44	0.77	0.28	0.19	0.07	0.02
Peri-sinusoidal fibrosis	0.45	0.68	0.57	0.41	1.08	0.30
Total Weighted Score	**1.95**	**2.79**	**1.93**	**1.29**	**2.52**	**0.76**

Figure 2. qFibrosis readout according to 5 different regions presented as a radar map to provide a graphical view of fibrosis changes in 5 areas of liver lobule.



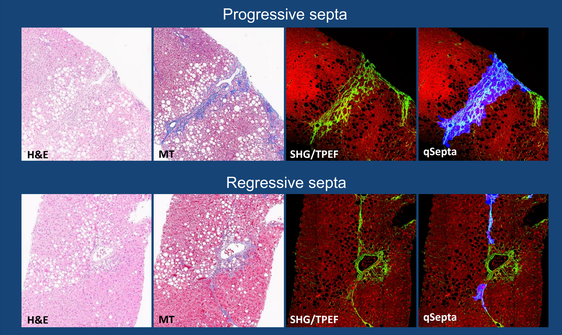
- Fig. 2 (from L to R): Radar maps clearly visualised the different patterns in fibrosis dynamics in 3 representative cases who were considered as “No Change” by the NASH CRN, while qFibrosis result in each of those cases showed either fibrosis progression, no change or regression.
- Table 1: In the fibrosis progression case, the overall qFibrosis increased from 1.95 (BL) to 2.79 (EOT), while in the fibrosis regression case, qFibrosis decreased from 2.52 (BL) to 0.76 (EOT).

Table 2. Comparison of Regressive and Progressive septa from F3 biopsies in FLIGHT-FXR clinical trial (NCT02855164).

No.	Septa parameters	Progressive septa N = 43, mean	Regressive septa N=50, mean	p value
1	Septa Area	234638.21	27002.33	<0.001
2	Cellular/acellular	0.75	0.56	0.082
3	Cellular/Collagen	1.27	0.93	0.169
4	Septa length	947.27	543.95	<0.001
5	Septa width	167.45	40.88	<0.001
6	Intersection Septa	2475.00	262.00	<0.001
7	Number of Thick Fiber Septa	64.00	5.00	<0.001
8	Number of Thin Fiber Septa	3016.00	344.50	<0.001
9	Thick/Thin Septa ratio	0.02	0.02	0.420
10	Aggregated Septa	80490.42	8730.77	<0.001
11	Distributed collagen within septa	2218.02	407.71	<0.001
12	Aggregated/Distributed collagen within septa	36.09	26.11	0.228

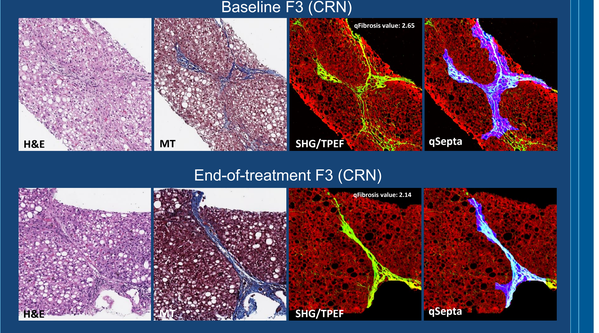
- To compare the numerical readouts of 12 individual septa parameters in progressive and regressive septa, as previously defined[3], 93 septa were randomly selected from 25 baseline liver biopsies
- Table 2: For 8 of 12 septa parameters there was highly significant difference (p<0.001) between progressive and regressive septa. E.g., area, length, width, number of intersections, number of thin and thick fibres, aggregated septa and distributed collagen fibres within septa.

Figure 3. Example of progressive and regressive septa with conventional staining method and digital SHG microscopy of the unstained liver tissue.



- Figure 3: Quantitative differences between progressive and regressive septa in Table 2 is reflected visually by the conventional staining methods versus SHG microscopy.

Figure 4. Representative case with no change in fibrosis stage (NASH CRN) but with fibrosis regression (qFibrosis).



- Figure 4: Representative case showing no change according to CRN, but with fibrosis reduction by qFibrosis is reflected visually. This illustrates the granularity of digital quantitation in characterising the direction of fibrosis dynamics with progression or regression.

CONCLUSIONS

- SHG/TPEF microscopy with AI provides greater granularity and precision in assessing fibrosis dynamics in NASH patients with bridging fibrosis
- It can reveal worsening or improvement undetectable by conventional microscopy, enhancing the understanding of pathogenesis and treatment response.
- The clinical relevance of AI digital measurements of the NASH features, especially for liver fibrosis progression or regression, will have to be established in future studies in relation to liver-related clinical outcomes.

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