

INTRODUCTION

- At present, there are no approved therapies for NASH, and bariatric surgery is indicated in selected patients with severe obesity as an intervention of choice for massive and durable weight loss and metabolic improvement, including NAFLD.
- The advent of AI-assisted digital pathology allows for the automated and quantitative staging/ grading of liver injury in NASH as compared to semi-quantitative traditional histological reads.

STUDY AIM

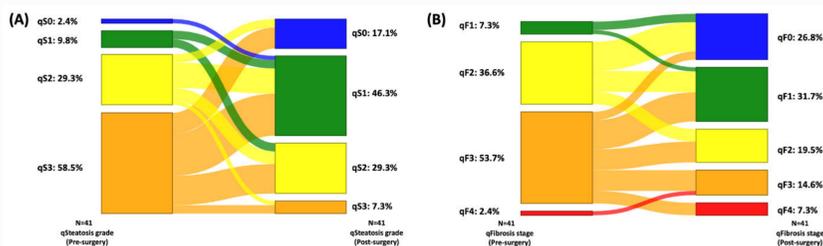
- To examine the potential of second harmonic generation/two photon excitation fluorescence (SHG/TPEF)-based technology to detect histological changes and patterns of fibrosis and steatosis changes in response to bariatric surgery (sleeve or RYGB) on patients with severe NASH or liver fibrosis.

METHODS

- Unstained paired liver sections (pre- and >1-year post-surgery) from 41 patients with obesity and significant liver injury (SAF activity grades 3 or 4 or NASH CRN fibrosis stages 3 or 4)¹ were examined using SHG/TPEF microscopy.
- SHG/TPEF microscopy was used to assess liver fibrosis (qFibrosis) and steatosis (qSteatosis) on a continuous scale; these quantitative values were also converted into categorical scores (qF0–qF4; or qS0–qS3) using cut offs which have previously been reported.²
- Changes in liver fibrosis in five different zones of liver lobules were quantitatively analyzed pre- and post-surgery by qFibrosis and presented as a radar plot to further understand differential changes in the spatial distribution of liver fibrosis.

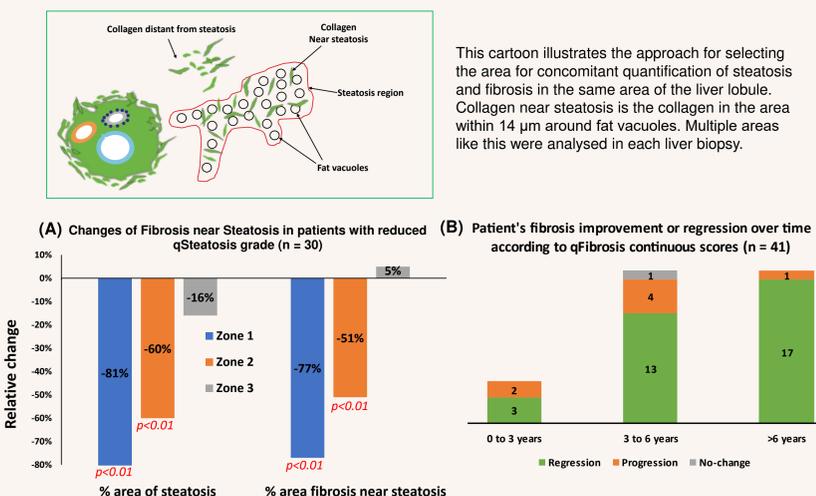
RESULTS

Figure 1. Digital quantification of steatosis (qSteatosis panel A) and liver fibrosis (qFibrosis panel B) before and after bariatric surgery.



- The distribution of qS-grades in 41 patients was 0: 2%; 1: 10%; 2: 29%; 3: 59% and that of qF-stages was 1: 7%; 2: 37%; 3: 54% and 4: 2%. Fibrosis regression according to qF-stage was seen in 71% (29/41), stabilization in 22% (9/41) and progression in 7% (3/41) of patients.
- The proportion of qF3/qF4 dropped from 56% pre-operatively to 22% at follow-up and
- The change in qF continuous score documented a more dynamic level of fibrosis changes with only 1 patient (2%) displaying stabilization while 81% (33/41) had improved fibrosis and 17% (7/41) worsened, showing that hepatic lesions continue to improve with the length of follow-up.
- Using a continuous scoring approach, the qFibrosis and qSteatosis continuous values documented a higher proportion of patients with regression when compared with their corresponding ordinal scores, demonstrating the limitation of an ordinal system.
- While patients with qS score reduction had fibrosis regression, those with stable or increased qS score had persistent fibrosis and steatosis in zones 2 and 3.
- The SAF activity readouts and NASH CRN scoring for liver fibrosis were unavailable at the time of analysis.

Figure 2. Co-localisation analysis of qSteatosis and qFibrosis as a result of bariatric surgery.



- Fig 2A: Patients with reduced qSteatosis showed a marked 50–80% (p<0.01) reduction of steatosis area along with concomitant reduction of fibrosis across the Zones 1 and 2 of the liver lobule regions.
- Patients with no-change or increased qSteatosis grade showed concomitant reduction in Zone 1 (no stats significance), and increased fibrosis in Zones 2 and 3 (Data not presented in poster).
- Fig 2B: Using qFibrosis continuous scores, we observe a clear trend of increasing number of patients with fibrosis regression over time. At the longest interval of >6 years between the biopsies taken at surgery and follow-up biopsy, 17 out of 18 patients showed fibrosis improvement.
- A similar trend is observed for steatosis over the same intervals, with 14 out of 18 patients showing steatosis improvement for the interval of >6 years (Data not presented in poster).

Figure 4. Septa analysis for (A) area, (B) length, and (C) width of patients reveals septa regression post-bariatric surgery. Representative SHG/TPEF region of interest of (D) before bariatric surgery, and (E) after bariatric surgery from the same patient.

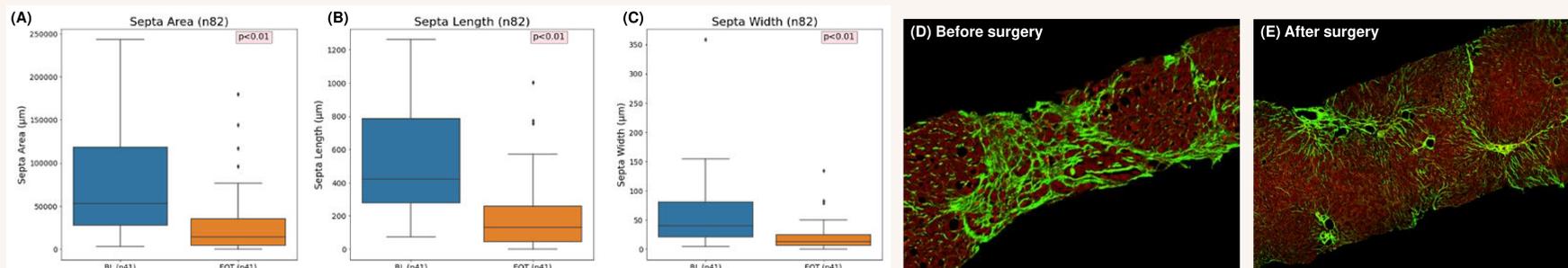
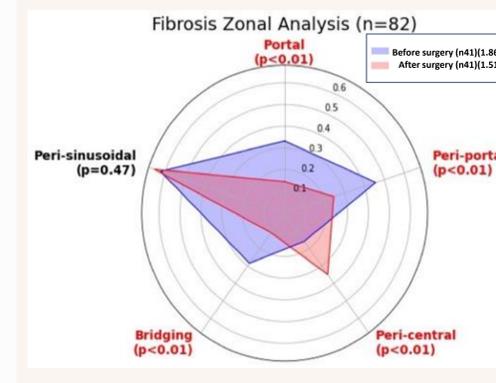


Figure 3. qFibrosis readout from 5 different regions presented as a radar map to provide a graphical view of fibrosis changes in 5 areas of liver lobule.



- Fig 3: Quantitative readout of fibrosis area in each zone is normalized to 0-1 and the summation gives the total weighted score of 1.86 pre-surgery and 1.51 post-surgery, indicating an overall reduction in fibrosis in 41 subjects.
- Radar maps clearly visualized the different patterns in the overall fibrosis dynamics in 41 subjects where statistically significant reduction is seen in the portal tract, peri-portal and bridging fibrosis regions.

- Fig 4A – C: Regression was observed for overall septa area, length and width for all 41 subjects who underwent bariatric surgery (p<0.01).
- Figures 4E, F: SHG/TPEF image of a selected region of interest clearly showing septa regression of a subject before and after bariatric surgery. Thinning of the septa can be observed.

CONCLUSION

- SHG/TPEF microscopy with AI provides greater granularity and precision in assessing fibrosis dynamics in patients who have undergone bariatric surgery.
- Additional insights include co-localization analyses detecting patterns and kinetics of fibrosis improvement in relation to steatosis regression.
- SHG/TPEF AI-DP provides a more thorough documentation of liver injury improvement post-bariatric surgery and has the potential to reveal fibrosis worsening or improvement undetectable by conventional microscopy, enhancing the understanding of the histological impact of bariatric surgery in patients with advanced fibrosis.
- Pathologist's re-reads of fibrosis and steatosis on the same slides are underway.

REFERENCES

- Pais R et al. Hepatology 2022;76:456-468
- Liu F, et al. Hepatology 2020; 71: 1953–1966

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