FRI-518

Hepatic fat and liver volume reductions – impact on non-alcoholic steatohepatitis trials and potential solutions using concomitant fibrosis with ballooning with fibrosis

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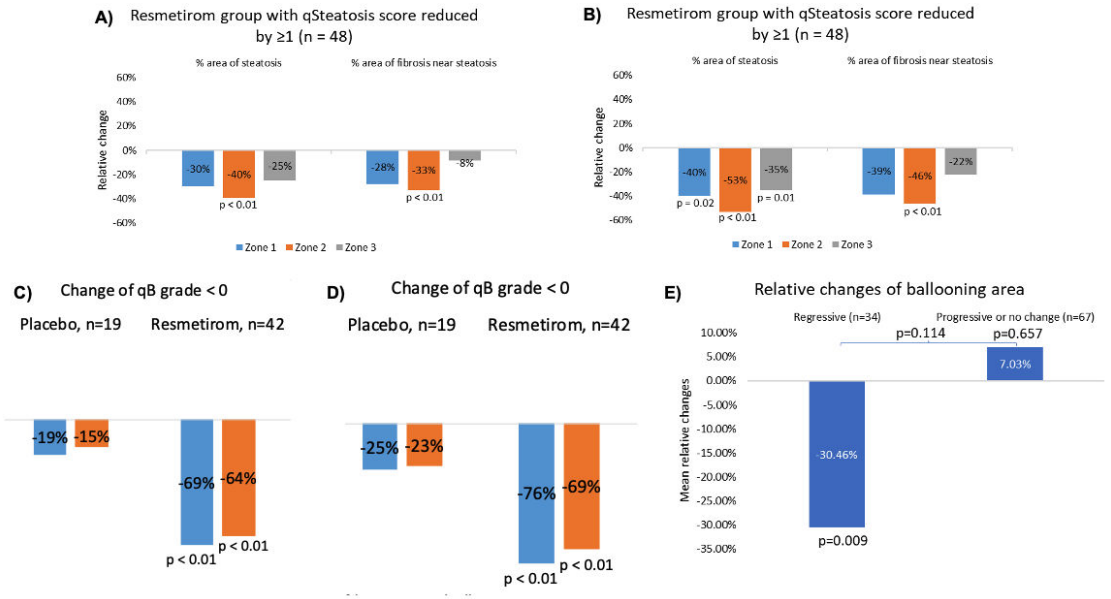
Background and Aims: Experimental treatment of non-alcoholic steatohepatitis (NASH) leads to reduction of hepatic fat and liver volume (LV) as assessed by Magnetic Resonance Imaging-Proton Density Fat Fraction (MRI-PDFF). The impact of hepatic fat and LV reduction on histological fibrosis interpretation using the CRN system remains unexplored. We propose analyzing the concomitant changes of qFibrosis (qF) with qSteatosis (qS) and qBallooning (qB) in zonal regions to evaluate the impact of hepatic fat and LV reduction on fibrosis changes.

Method: NASH patients were included from two phase 2b studies: 24-week study of Aldafermin (NCT02443116) and 36-week study of Resmetirom (NCT02912260). Steatosis correction (SC) was done by subtracting the steatosis area as detected by qS from total tissue area followed by an analysis of zonal fibrosis in the respective zones 1, 2, and 3. Concomitant fibrosis with drug-induced steatosis and ballooning changes were evaluated by co-localization of qF changes around qS and qB, respectively.

Results: qF continuous measures on the phase 2 Aldafermin study revealed 54% fibrosis regression in the treated group versus 19% in placebo group (p=0.007). With SC, zonal qF assessment showed trends of dose-dependent fibrosis reduction in portal, periportal (p=0.02) and zone 2 regions. In the Resmetirom study where the treated group had markedly reduced LV, LV correction was applied and there was a greater reduction in concomitant fibrosis, as well as significant zonal steatosis reduction across all zones (Figure 1A, 1B). In contrast, the impact of LV is negligible on concomitant qB/qF. Further qB analysis revealed an association between 1-stage fibrosis improvement with a decrease in qB area from baseline to end-of-treatment (Figure 1C). Using cut-off of -30.46%, the performance for predicting 1-point reduction was 50% sensitivity, 58% specificity with 39% negative predictive value and 68% positive predictive value.

Conclusion: Results from this proof-of-concept analysis highlights the impact of hepatic fat reduction on fibrosis regression in NASH. The impact of SC and LV correction is great on the concomitant fibrosis around steatosis, but minimal on the concomitant fibrosis around ballooning. Therefore, concomitant analyses with digital pathology can augment the interpretation of the mechanism of action of drugs in NASH as well as allow for a better understanding of the impact these drugs have on histopathology and should be considered in future trials. Validation with clinical outcomes is ongoing.

Figure:



(A), (B) Colocalization of steatosis and fibrosis changes within liver lobule in patients with reduced qS, without and with LV correction, respectively. (C), (D) Colocalization of ballooning and fibrosis changes within liver lobule in patients with reduced qB, without and with LV correction, respectively. (E) Association of 30.46% decrease (relative change) in qB area with 1-stage fibrosis improvement.

Hepatic fat and liver volume reductions – Impact on non-alcoholic steatohepatitis trials and potential solutions using concomitant fibrosis with ballooning with qFibrosis

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INTRODUCTION

- Experimental treatment of non-alcoholic steatohepatitis (NASH) leads to reduction of hepatic fat and liver volume (LV) as assessed by magnetic resonance imaging-proton density fat fraction (MRI-PDFF). The impact of hepatic fat and LV reduction on histological fibrosis interpretation using the CRN system remains unexplored.
- Assessment of histological features that stage NASH fibrosis are may be impacted in the setting of decreased steatosis and liver volume reduction following therapeutic intervention.
- Second harmonic generation/two photon excitation fluorescence (SHG/TPEF) microscopy of unstained liver sections with artificial intelligence (AI)-based algorithms such as qFibrosis can incorporate normalization procedures to account for steatosis area and liver volume reduction, thereby improving the detection of fibrosis changes.

AIM

- The aim of this post hoc analysis was to apply SHG/TPEF methodology with computer-assisted analyses to gain an in-depth understanding of the impact of hepatic fat and LV reduction on liver fibrosis regression, particularly on fibrosis concomitant to treatment-induced steatosis and ballooning changes.

METHOD

- This investigation is based on paired liver biopsies from two phase 2b studies:
  - 24-week study of Aldafermin (NCT02443116)
  - 36-week study of Resmetirom (NCT02912260)
- Unstained liver sections from BL and end-of-treatment (EOT) liver biopsies were examined using SHG/TPEF microscopy.1
- Steatosis correction (SC) was done by subtracting the steatosis area as detected by qS from total tissue area followed by an analysis of zonal fibrosis in the respective zones 1, 2, and 3.
- Resmetirom-mediated changes of collagen fibers in relation to steatosis and ballooning changes were evaluated by quantitatively measuring fibers in the immediate vicinity of fat vacuoles and ballooned hepatocytes, respectively.

RESULTS

COHORT 4: 24-Week Phase 2 Study of Aldafermin

- 78 patients with NASH randomly assigned (1:2) to groups given placebo (n=25) or Aldafermin 1 mg (n=53) daily for 24 weeks.
- Figure 1B: qF continuous measures revealed 54% fibrosis regression in the treated group versus 19% in placebo group (p=0.007).
- Significant hepatic fat reduction, and trend for both fibrosis improvement and NASH resolution were observed for COHORT 4 study.

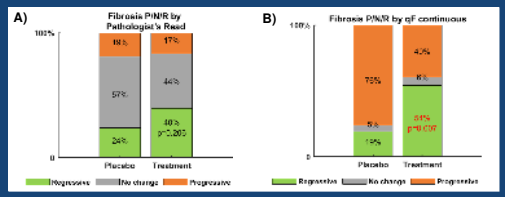

Figure 1: Progression/No change/Regression (P/N/R) plots according to (A) CRN versus (B) qFibrosis continuous value.

- With **steatosis correction**, zonal qF assessment showed trends of fibrosis reduction in portal, periportal (p=0.02) and zone 2 regions. (See Figure 2 below)

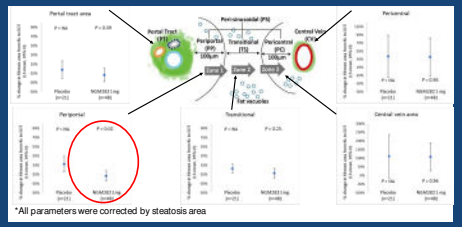

Figure 2: Zonal fibrosis assessment using steatosis tissue area correction for COHORT 4 study. Statistically significant fibrosis improvement in peri-portal zone is observed (circled red).

Phase 2 36-Week Study of Resmetirom in Patients with NASH

- Retrospective analysis was conducted on 102 paired samples. Based on the liver volume reduction measured on serial MRI-PDFFs, corrections of qFibrosis were made for liver volume reduction.
- Figure 3A: In Resmetirom-treated patients with reduced qSteatosis score, we observed statistically significant steatosis reduction with concomitant fibrosis improvement in Zone 2.
- Figure 3B: Reduction in concomitant fibrosis in treated patients showed more consistent trends post-liver volume correction, with significant zonal steatosis reduction across ***Zones 1, 2 and 3***.

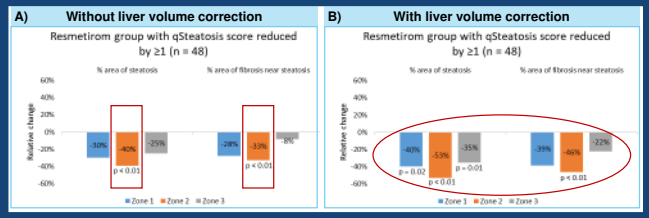

Figure 3: qFibrosis and qSteatosis colocalization analysis showing consistent fibrosis reduction with steatosis in treated group with (A) without LV correction, and (B) with LV correction.

- Figure 4: In the subset of patients who had a reduction in qB grade, there was a greater degree of reduction in concomitant fibrosis near ballooning in the Resmetirom group.
- In contrast to Figure 3, fibrosis changes concomitant to ballooning is **less sensitive** to liver volume correction (denoted by red boxes).

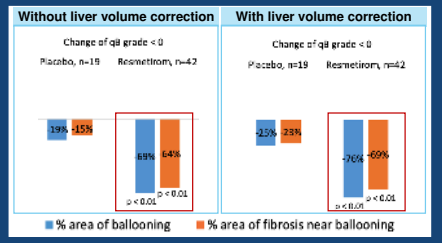

Figure 4 (left): qFibrosis and qBallooning colocalization analysis with and without LV correction (only patients with improvement in qB grade shown here).

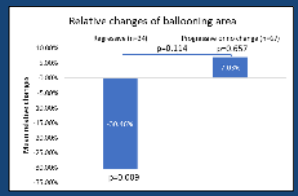

Figure 5: Correlating the area of ballooned hepatocytes with a 1-point reduction in fibrosis.

Cut-off value for relative change of ballooning area	Validation results on COHORT 4
Cut-off = -30.46%	
Sensitivity	50%
Specificity	58%
Positive predictive value (PPV)	39%
Negative predictive value (NPV)	68%

Table 1: The performance of ballooning area for predicting 1-point reduction was 50% sensitivity, 58% specificity with 39% PPV and 68% NPV.

- To further explore how AI in digital pathology can provide potential solutions in efficacy evaluation for NASH studies, we investigated the association between a quantitative change in ballooning to a semi-quantitative score.
- Figure 5: Further qB analysis to examine the correlation of ballooning reduction with fibrosis changes revealed an association between 1-stage fibrosis reduction with a relative change in qB area.
- (Data not shown) Similar observations were also seen for number of ballooned hepatocytes, as well as area of collagen around ballooned hepatocytes.
- The performance of applying -30.46% cut-off for relative change in qB area to predict 1-point reduction is shown in Table 1.

CONCLUSIONS

- Results from this proof-of-concept analysis highlights the impact of hepatic fat reduction on fibrosis regression in NASH. The impact of SC and LV correction is great on the concomitant fibrosis around steatosis, but minimal on the concomitant fibrosis around ballooning.
- Concomitant analyses with digital pathology can potentially augment the interpretation of the mechanism of action of drugs in NASH as well as allow for a better understanding of the impact these drugs have on histopathology and should be considered in future trials.
- Validation with clinical outcomes is ongoing.

REFERENCES

1. Liu F, Goh GBB, Tiniakos D, Wee A, Leow WQ, Zhao JM, et al. qFIBS: an automated technique for quantitative evaluation of fibrosis, inflammation, ballooning, and steatosis in patients with nonalcoholic steatohepatitis. Hepatology 2020;71:1953–1966

ACKNOWLEDGEMENTS

All authors participated in the development of this poster and approved the final poster for presentation.

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