

Integrating AI-Based Fibrosis Staging and Pattern Analysis in a Translational Mouse Model of MASH

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Background

Histopathological fibrosis staging is a key endpoint in preclinical drug development for MASH. However, the MASH CRN (Kleiner) system assigns one global stage per biopsy, potentially obscuring subtle treatment-related collagen remodelling and tissue heterogeneity.

We developed an automated deep learning-based digital imaging analysis pipeline (GHOST) for simultaneous scoring and quantification of fibrosis architecture in mouse models of MASH.

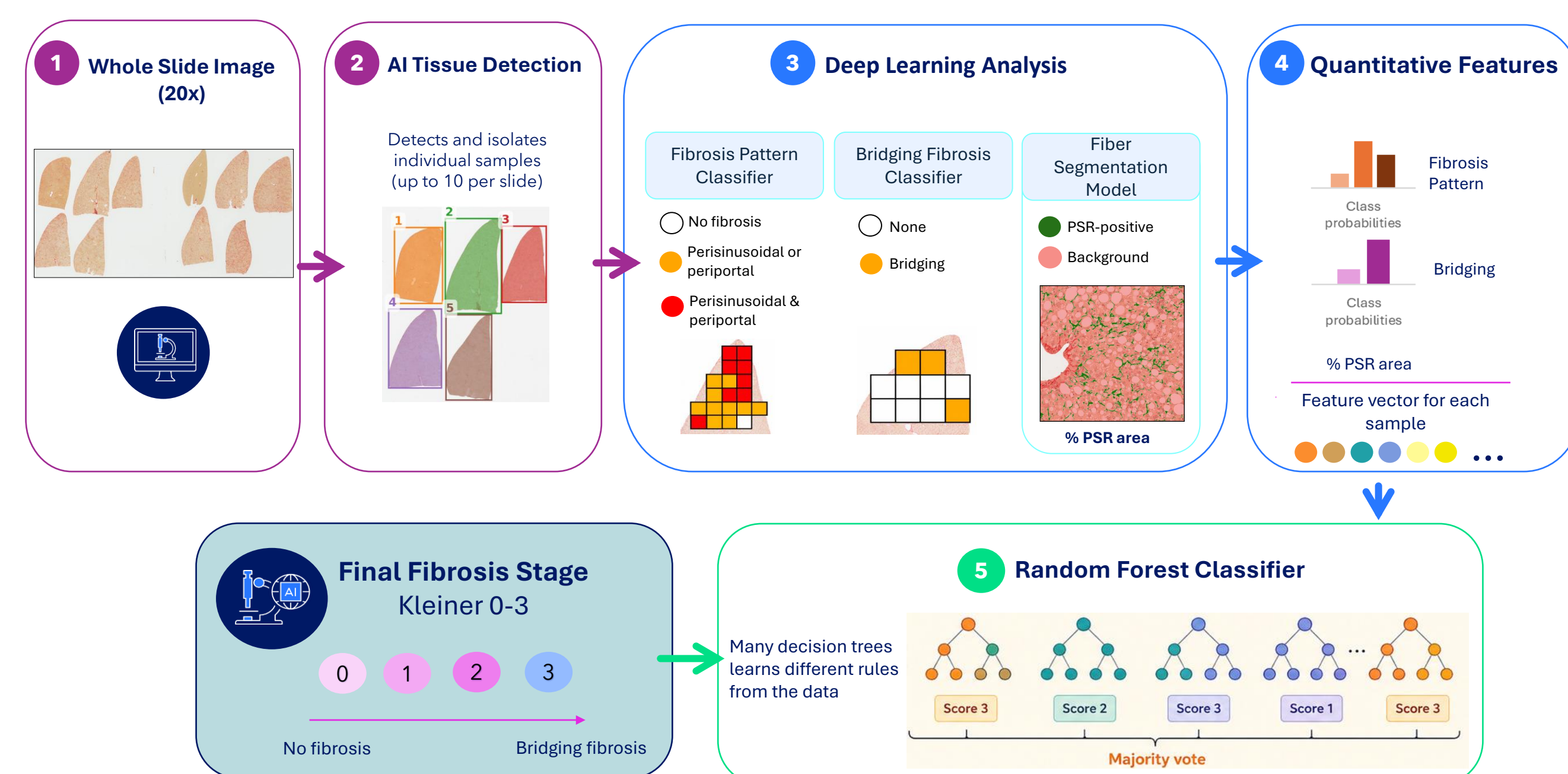
Methods

The deep learning-based GHOST application was validated using PSR-stained liver sections from 2,321 GAN DIO-MASH mouse biopsies scanned at 20X. The GHOST workflow included:

- AI-based tissue detection to identify individual biopsies.
- Subdivision of each biopsy into small image regions (tiles).
- Three CNN-based analyses:
 - Fibrosis pattern detection.
 - Bridging fibrosis classification.
 - PSR-positive collagen fiber segmentation.
- Aggregation of tile-level outputs into biopsy-level quantitative features.
- Random Forest classification to predict Kleiner fibrosis stage.

GHOST was applied to characterize fibrosis outcomes after long-term treatment with clinically relevant drugs (semaglutide, efruxifermin, resmetirom, tirzepatide) or dietary intervention (chow reversal) in the translational GAN DIO-MASH mouse model.

1 GHOST workflow



2 High fibrosis staging performance

Cohen's kappa 0.85

Manual score \ GHOST score	0	1	2	3
0	67	6	1	0
1	6	23	17	0
2	0	5	193	14
3	0	0	23	110

Figure 2. Validation of AI-based Kleiner fibrosis staging using GHOST in GAN DIO-MASH mice. Confusion matrix showing agreement between manual Kleiner fibrosis stages (0-3) and GHOST-predicted stages in an independent validation dataset of 465 PSR-stained liver biopsies from GAN DIO-MASH mice. Rows represent manual scores and columns represent GHOST predictions. Color intensity indicates the number of biopsies in each category. GHOST demonstrated high agreement with manual scoring (linear weighted Cohen's $\kappa = 0.85$), with most misclassifications occurring between adjacent fibrosis stages.

3 Sub-biopsy fibrosis pattern analysis

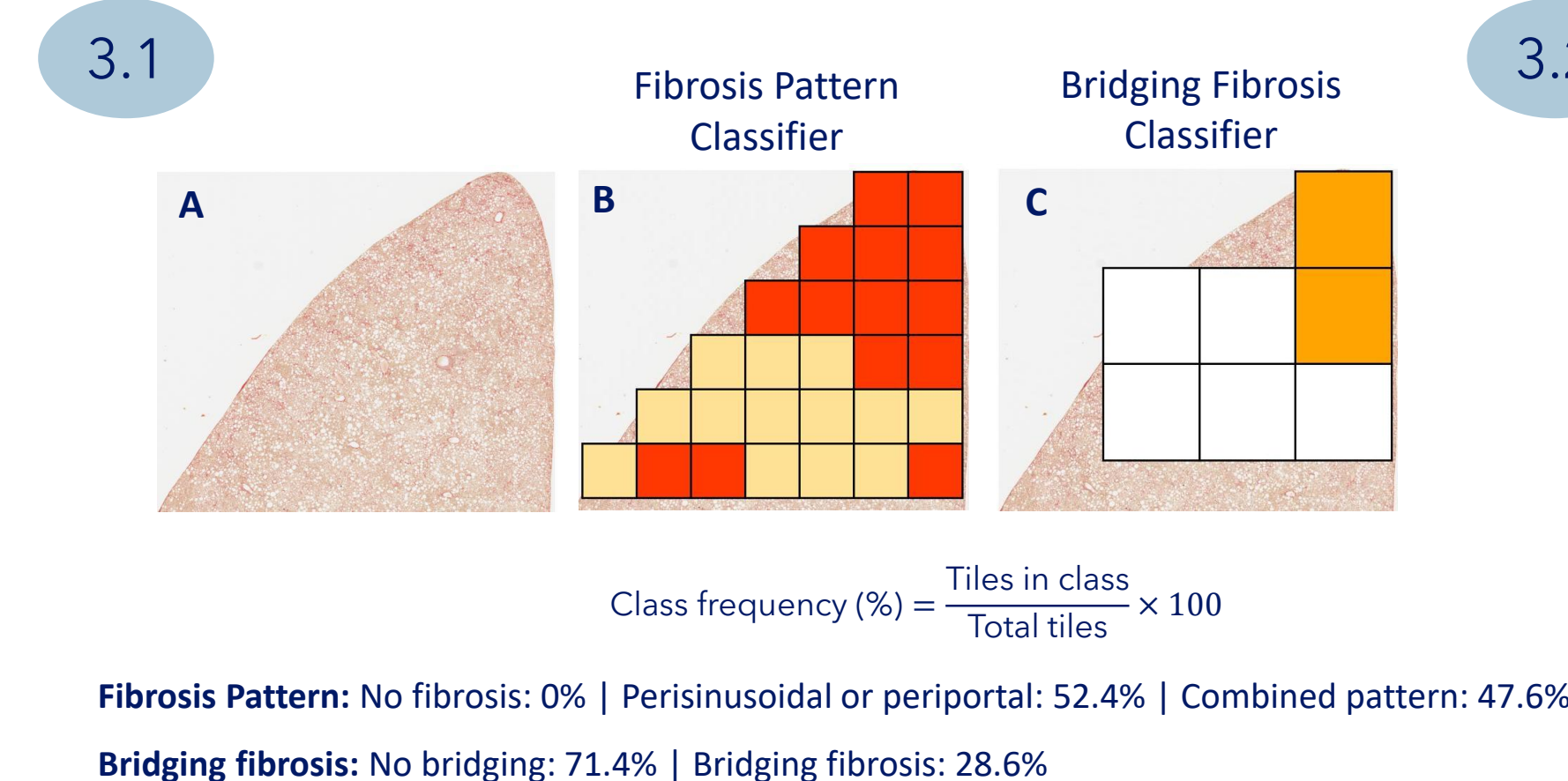


Figure 3.1. Quantitative fibrosis pattern analysis at single-tile level. (A) Representative PSR-stained liver biopsy. (B) Fibrosis pattern classification, where individual image tiles are assigned to one of three classes: no fibrosis, perisinusoidal or periportal fibrosis, or combined perisinusoidal and periportal fibrosis. (C) Bridging fibrosis classification, distinguishing tiles with and without bridging fibrosis. For each classifier, the percentage of tiles assigned to each class is calculated relative to the total number of tiles analyzed in the biopsy.

Figure 3.2. AI-Based Quantification of Periportal and Sinusoidal Fibrosis. Scanned PSR-stained slides (A) are analyzed in several steps: Each sample is analyzed using an additional deep learning model that detects portal triads (B, blue) and central veins (B, green). Periportal zone is defined as 100 μm around the portal triads (figure B, yellow). By postprocessing, the PSR detection (C) is divided into periportal (D, arrow) and sinusoidal fibrosis (D, asterisk) fractions.

4 Enhanced resolution of fibrosis outcomes in the translational GAN DIO-MASH mouse model of biopsy-confirmed MASH

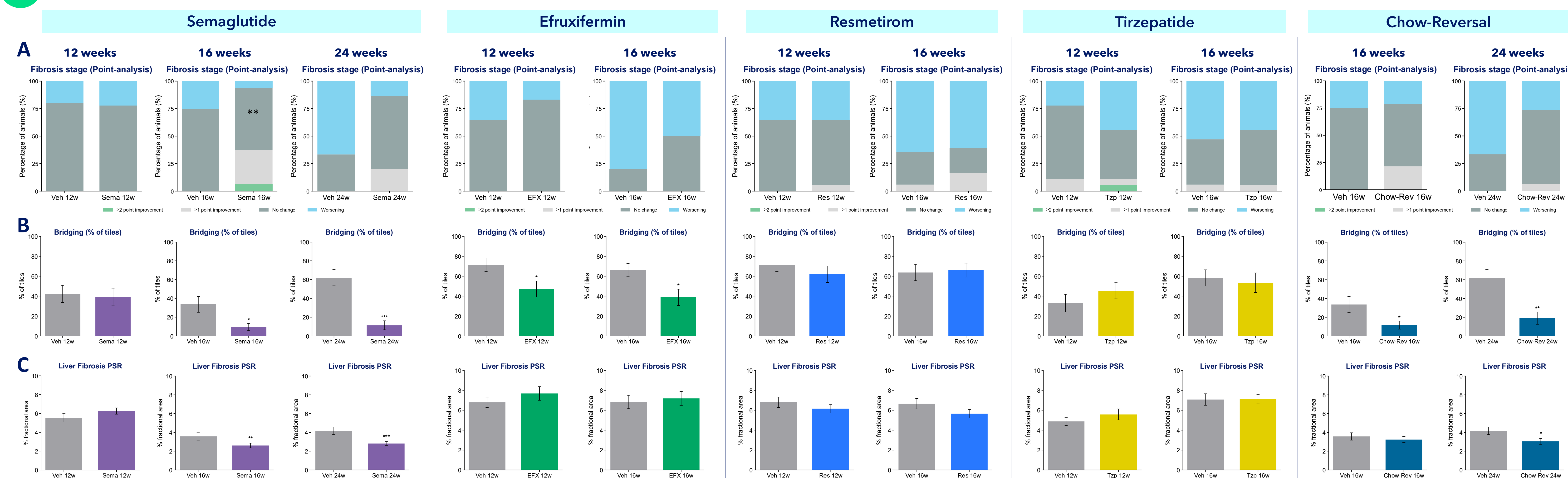


Figure 4. Treatment-associated changes in fibrosis stage, fibrosis pattern, and PSR-positive area across GAN DIO-MASH mouse studies. GHOST was applied to multiple treatment studies evaluating semaglutide (30 nmol/kg, sc. q.d.; 12, 16, or 24 weeks of treatment), efruxifermin (EFX; 1 mg/kg, s.c., q.w. for 12 weeks or b.w. for 16 weeks of treatment), resmetirom (3 mg/kg, p.o., q.d.; 12 or 16 weeks of treatment), tirzepatide (10 nmol/kg, s.c., q.d.; 12 or 16 weeks of treatment), and chow reversal (change to chow feeding for 16 or 24 weeks). For each study and time point, treatment groups were compared with their corresponding vehicle controls. Fibrosis stage changes are presented as the percentage of animals showing improvement, no change, or worsening relative to baseline biopsy scores (panel A). Bridging fibrosis is expressed as the percentage of biopsy tiles classified as bridging-positive by GHOST (panel B), while PSR-positive area is expressed as the percentage of analyzed tissue area (panel C). Additional tile-based analysis of perisinusoidal and periportal patterns was performed (data not shown). Data are shown as mean $\pm$ SEM. Statistical comparisons between vehicle and treatment groups were performed using Fisher's exact test for fibrosis stage and a one-factor linear model for bridging fibrosis and PSR-positive area. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to corresponding vehicle group.

Conclusion

- GHOST provides unbiased, automated and clinically interpretable Kleiner fibrosis staging with high agreement to manual scoring
- The relatively large liver biopsies from GAN DIO-MASH mice enable direct comparison of fibrosis outcomes at the whole-biopsy and sub-biopsy levels
- Tile-based sub-biopsy analysis quantifies fibrosis architecture beyond a single global stage
- Tile-based sub-biopsy analysis is more sensitive than whole-biopsy staging and fibrosis quantification for detecting antifibrotic responses to clinically relevant therapies

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