Efficacy and reproducibility of the AI-assisted Gubra Histopathological Objective Scoring Technique (GHOST) in preclinical rodent models of fibrosing NASH

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Background & Aim

Drug efficacy testing in animal models of NASH should include clinical primary endpoint assessment for histopathological NAFLD Activity Score and Fibrosis Stage/Ishak Fibrosis Score. Manual histopathological scoring are prone to inter- and intra-observer variability which can significantly influence reproducibility of results.

To enable objective and unbiased histopathological assessment in liver biopsies from mouse models of NASH, we developed and validated **Gubra Histopathological Objective Scoring Technique (GHOST)**, an automated deep learning-based digital imaging analysis pipeline for the NAFLD Activity Score and fibrosis staging/scoring system.

Methods

Liver biopsies were obtained from two industry-standard rodent models of NASH:

- GAN diet-induced obese (GAN DIO-NASH) mouse
- Choline-deficient L-amino acid defined high-fat diet (CDAA-HFD) rat

Automated GHOST analysis was performed on HE and PSR stained sections and validated against manual scoring:

- NAFLD Activity Score (NAS):
  - Steatosis (score 0-3)
  - Lobular inflammation (score 0-3)
  - Ballooning degeneration (score 0-2)
- Fibrosis stage (score 0-4)
- Ishak score (score 0-6)

Corresponding quantitative histomorphometrics:

- Density of hepatocytes with lipid droplets
- Number of inflammatory foci
- Ballooning cell index
- Fractional area of periportal and perisinusoidal fibrosis

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1 NAFLD Activity Score using GHOST

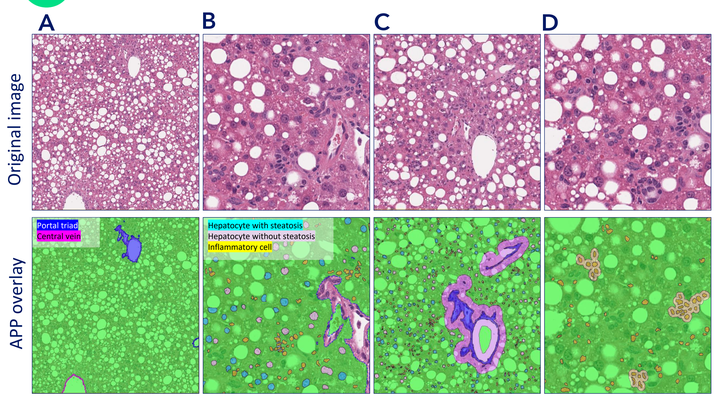

Figure 1. GHOST-based NAFLD Activity Score (NAS). (A) Portal triads and central veins were detected using deep learning (10X). **(B)** Deep learning detected nuclei of hepatocytes with steatosis, hepatocytes without steatosis, and inflammatory cells (20X). **(C)** Post-processing excludes periportal inflammation. **(D)** Post-processing converted clusters of ≥4 inflammatory cells into foci. Scores were calculated based on simple threshold.

2 Fibrosis Staging using GHOST

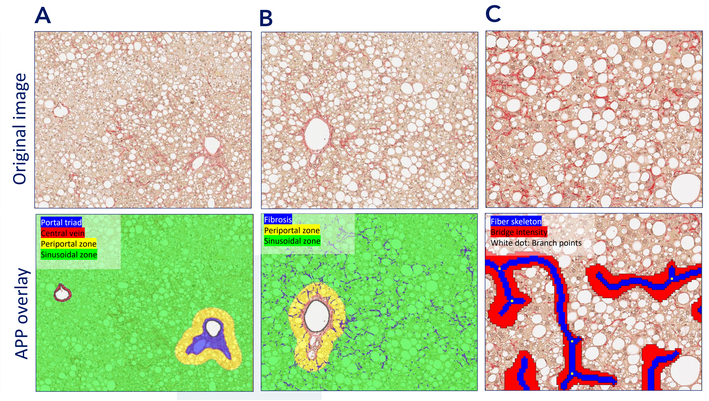

Figure 2. GHOST-based fibrosis score. (A) Portal triads and central veins were detected using deep learning (post-processing creates a periportal zone of 100 µm). **(B)** Fibrosis was detected using the linear Bayesian image analysis method in the periportal and sinusoidal zones, and different measures of collagen fiber fragment size and shape was used to predict bridging. **(C)** Bridging was also detected using the Threshold image analysis method based on a polynomial local linear filter feature.

3 Ishak Fibrosis Score using GHOST

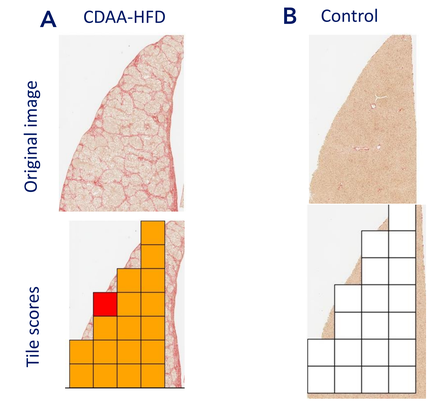

Figure 3. GHOST-based Ishak fibrosis score. (A) PSR-stained liver section from CDAA-HFD rat. **(B)** PSR-stained liver section from age-matched control. In the top panel the original image is shown. In the lower panel images are divided into squares and classified using convolutional neural network (CNN) analysis. Output of the CNN analysis was used in a machine learning algorithm to train the AI to predict fibrosis. Boxes of different colours have been given different Ishak scores : White=0, orange=5 and red=6, .

4 Agreement between GHOST and manual scoring

NAFLD Activity Score

Manual score \ GHOST score	0	1	2	3	4	5	6	7
0	12	9	2	0	0	0	0	0
1	1	3	4	0	0	0	0	0
2	0	2	10	0	0	0	0	0
3	0	0	2	12	4	0	0	0
4	0	0	3	11	12	3	0	0
5	0	0	0	9	15	80	45	0
6	0	0	0	0	4	21	64	0
7	0	0	0	0	0	0	4	1

Fibrosis Stage

Manual score \ GHOST score	0	1	2	3
0	61	7	0	0
1	6	53	23	0
2	0	16	176	22
3	0	0	29	144

Ishak Fibrosis Score

Manual score \ GHOST score	0	1	2	3	4	5	6
0	11	1	0	0	0	0	0
1	2	1	0	0	0	0	0
2	0	0	2	3	1	0	0
3	1	0	6	8	1	0	0
4	0	0	0	1	9	2	0
5	0	0	0	0	4	8	10
6	0	0	0	0	1	3	20

Cohen's Kappa

NAFLD score	Fibrosis stage	Ishak score
0.72	0.84	0.82

5 Corresponding histomorphometric variables by GHOST analysis

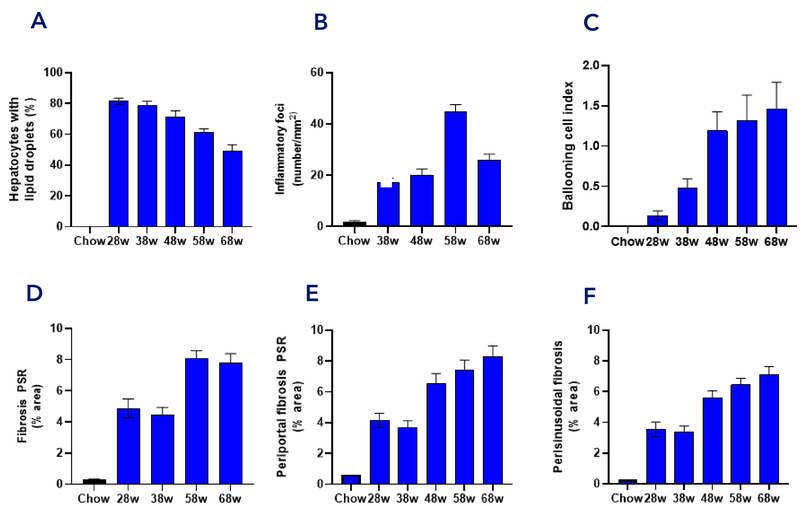

Figure 5. GHOST-based histomorphometrics on scoring variables. (A) Percentage of hepatocytes with lipid droplets relative to total hepatocyte counts (mean ±SEM) **(B)** Number of inflammatory foci pr mm² (mean ±SEM). **(C)** Ballooning cell index. **(D)** Percentage of area with fibrosis in section (mean ±SEM). (E) Percentage of area of periportal fibrosis in the section (mean ±S EM). **(F)** Percentage of area of sinusoidal fibrosis in the section (mean ±SEM).

Conclusion

- GHOST shows high agreement with manual scoring by expert histopathologist in industry-standard rodent models of NASH
- GHOST provides fast, accurate and reproducible histopathological scoring
- GHOST enables quantitative analysis of scoring-derived variables
- GHOST is highly applicable for assessment of drug effects on clinical histopathological hallmarks in rodent models of NASH

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