# The Complexity of Ballooned Hepatocyte (BH) Identification: Time to Rethink Trial Endpoints for Nonalcoholic Steatohepatitis?

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## INTRODUCTION

- Hepatocyte ballooning is a key feature discriminating nonalcoholic steatohepatitis (NASH) from steatosis (NAFL).
- High inter/intra-observer variation in ballooning measured has been reported.
- Reliable identification of ballooning is crucial for patient enrollment and drug efficacy evaluation.
- There is a pressing need for reproducible, objective and standardized evaluation of hepatocyte ballooning.
- Artificial intelligence (AI)-based approach may provide a more reliable way to assess the range of injury recorded as “hepatocyte ballooning” as a clinical trial endpoint by expert hepatopathologists.

## MATERIALS AND METHODS

- Liver biopsies were obtained from the Seladelpar (NCT03551522) and Resmetirom phase 2 trials (NCT02912260).
- Digitized NAFLD H&E slide images were independently reviewed by 9 expert liver pathologists on two separate occasions
- Each pathologist marked every ballooned hepatocyte (Phase 1) and later provided an overall NAFL/NASH assessment (Phase 2).
- Inter-observer variation was assessed, and a consensus atlas of ballooned hepatocytes was used to train second harmonic generation/two-photon excitation fluorescence (SHG/TPE) imaging-based AI to detect ballooning.

## RESULTS

**Figure 1.** (Left) Heatmap showing number of ballooned cells observed (Dark blue is 0 ballooned cells, through to red) in comparison to ‘non-NASH NAFL’ vs. ‘NASH’ diagnostic call by slide/ pathologist. (Right) Semi-Quantitative Ballooning Score (SQBS) (0-2) by slide and pathologist. (SQBS Ballooning 0: <5 cells circled; 1: 5-75; 2: > 75).

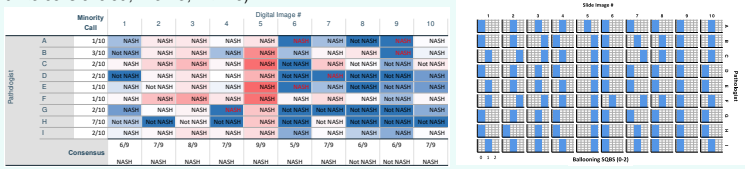


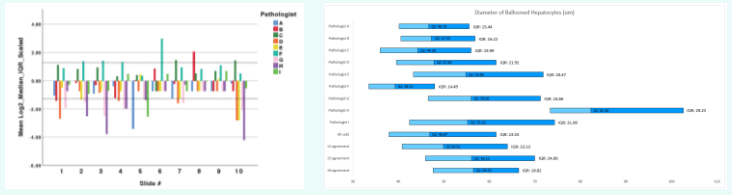
Figure 1 (left):

- Text in red denotes a NASH diagnosis call by a pathologist despite previously reporting no ballooned hepatocytes present at Phase 1.
- No cases for which all pathologists agreed that NASH was absent.
- Kappa value of 0.127 (95%CI 0.024-0.230, P=0.016) for agreement of a NASH diagnosis

Figure 1 (right):

- Using SQBS categories, the level of inter-observer agreement between pathologists remained ‘fair’ (kappa 0.291, 95%CI 0.210-0.371, p<0.0005).

**Figure 2.** (Left) Scaled Count of Cells Circled by Slide and Pathologist. (Right) Ballooned hepatocyte diameter by pathologist.



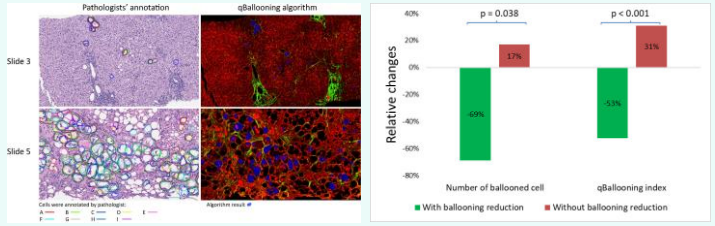
- Individual pathologists consistently tended to identify greater or lesser numbers of ballooned cells.
- Pathologists who considered more cells to be ballooned adopted a more permissive, lower, cell-diameter threshold (pathologists F 39.31 ± 14.49µm and C 33.28 ± 19.99µm) vs. those who identified the least cells to be ballooned (pathologist H 82.30 ± 29.23µm), p<0.001.

**Table 1.** Use of the Histological ‘Ground Truth’ Atlas to tune the qBallooning2 Algorithm. **Relative to majority consensus of ≥5-pathologists. § Based on an estimated mean 8,150 hepatocytes per digital image from nuclear counting and shown for completeness.*

| qBallooning2 training-set cell-selection criteria | Number of ballooned cells identified by Pathologists | Number of ballooned cells identified by qBallooning2 | Overlap between qBallooning2 and majority consensus of ≥5-Pathologists | Positive Predictive Value Proportion of ballooned cells called by qBallooning2 are 'True Positive' * | False Discovery Rate Proportion of ballooned cells called by qBallooning2 are 'False Positive' * | True Positive Rate (Sensitivity) Proportion of ballooned cells identified by qBallooning2 * | False Negative Rate Proportion of ballooned cells missed by qBallooning2 * | Estimated True Negative Rate (Specificity) *§ |
|---|---|---|---|---|---|---|---|---|
| Agreement of any 1 pathologist | 1188 | 346 | 54 | 54/346 (16%) | 292/346 (84%) | 54/133 (41%) | 79/133 (59%) | >99% § |
| Agreement of any 2 pathologists | 481 | 250 | 51 | 51/250 (20%) | 199/250 (79.6%) | 51/133 (38%) | 82/133 (62%) | >99% § |
| Agreement of any 3 pathologists | 284 | 170 | 37 | 37/170 (22%) | 133/170 (78.2%) | 37/133 (28%) | 96/133 (72%) | >99% § |
| Agreement of any 4 pathologists | 188 | 114 | 25 | 25/114 (22%) | 89/114 (78%) | 25/133 (19%) | 108/133 (81%) | >99% § |
| Agreement of any 5 pathologists | 133 | 88 | 22 | 22/88 (25%) | 66/88 (75%) | 22/133 (17%) | 111/133 (83%) | >99% § |
| Agreement of any 6 pathologists | 86 | 59 | 16 | 16/59 (27%) | 43/59 (73%) | 16/133 (12%) | 117/133 (88%) | >99% § |
| Agreement of any 7 pathologists | 59 | 40 | 15 | 15/40 (38%) | 25/40 (62.5%) | 15/133 (11%) | 118/133 (89%) | >99% § |
| Agreement of any 8+ pathologists | 26 | 24 | 5 | 5/24 (21%) | 19/24 (79%) | 5/133 (4%) | 128/133 (96%) | >99% § |

- The SHG/TPE “qBallooning2” algorithm was optimized to detect the ballooned cells for each level of inter-observer concordance
- Comparing the qBallooning2 index to cells identified by expert pathologist consensus as a reference standard, hepatocyte ballooning could be detected with 17-73% specificity and 5-57% sensitivity
- Based on the performance analysis shown in Table 1, the “qBallooning2” algorithm that had been optimized based on concordance of ≥5-pathologists was selected for further study.

**Figure 3.** (Left) Two examples showing the BH identification by pathologists and qBallooning index. (Right) The AI algorithm reading from a separate drug trial, showing agreement with the study pathologist’s interpretation




- Pilot data demonstrates that qBallooning index has the capacity to detect change in ballooning
- qBallooning performance may be improved by further refinement and validation will be required before implementation.

## CONCLUSIONS

- Substantial divergence in BH identification amongst expert liver pathologists suggests ballooning is a spectrum, too subjective for its presence or absence to be unequivocally determined.
- By digitized slide evaluation, 9 expert liver pathologists had poor agreement on the exact numbers of BH per slide.
- By separate, blinded evaluation, diagnostic categorization of NAFL vs NASH likewise did not always correlate with presence of BH in the slides.
- An AI algorithm, based on consensus cells of ≥ 5 pathologists was created and successfully quantified change in BH in a separate clinical trial.
- BH result from complex alterations to hepatocytes; and variable appreciation by pathologists, including cell size.
- A consensus atlas of cells may be used to train AI for assessment of efficacy of therapy as well as understanding of etiology.
- A second phase of this work is planned for consensus amongst pathologists.

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