Evaluation of performance of a cellular profiling technique for quantification of inflammation and steatosis in liver biopsies of patients with MASH

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Introduction & Aim

The gold standard for assessment of liver inflammation and steatosis is through pathologist analysis of MASH biopsies. Using a new AI cellular detection, classification, and quantification method trained on pathologist annotations of individual cells and validating the results on pathologist classification of the overall tissue we can generate a quantitative continuous score based on single cell analysis. This is an asset to R&D, diagnosis, as well as addressing limitations of current histological nomenclatures and methods (doi:10.1002/hep.32475).

Methods

- This retrospective study included 87 NASH liver biopsies diagnosed by a pathologist for histologic assessment of lobular inflammation grades of 0 (N=7), 1 (68), and 2 (12), and steatosis grades of 0 (N=2), 1 (40), 2 (30) and 3 (14).
- Quantitative image analysis of these biopsies stained with H&E detects cell nuclei (~ 20k per biopsy) and quantify their morphometric and local surroundings in 86 parameters.
- A subset of 18 images (~260K nuclei) was used to develop a machine learning (ML) model using 34.8K annotations. This includes steatotic hepatocytes (7.3k), normal hepatocytes (12k), inflammatory cells (all kinds, 7.8k), liver specialized cells (all kinds, 5.4k), and “debris” (degraded cellular bits, 2.3k).
- Once the cells were classified, the density (count /mm²) and relative cell count (%) for each cell type were calculated.
- Macro-steatosis Area Ratio was calculated through vacuole detection as a secondary method (the first being comparison of the cell detection to pathologist definition).
- Clusters of inflammatory cells were segmented into “small”, “medium”, and “Large” foci.

Results

ML Model Performance (5 classes)

<table>
<thead>
<tr>
<th>Class</th>
<th>Samples (n)</th>
<th>Accuracy (%)</th>
<th>Error (%)</th>
<th>F1 (%)</th>
<th>Validated (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflamatory cells</td>
<td>7865</td>
<td>73.4%</td>
<td>19.7%</td>
<td>59.9%</td>
<td>1935</td>
</tr>
<tr>
<td>Hep Nuclei w/o Fat</td>
<td>12038</td>
<td>91.1%</td>
<td>11.7%</td>
<td>80.4%</td>
<td>3793</td>
</tr>
<tr>
<td>Hep Nuclei w Fat</td>
<td>7376</td>
<td>74.4%</td>
<td>14.8%</td>
<td>63.4%</td>
<td>2338</td>
</tr>
<tr>
<td>Debris</td>
<td>2357</td>
<td>82.8%</td>
<td>15.3%</td>
<td>70.1%</td>
<td>726</td>
</tr>
<tr>
<td>Specialized cells</td>
<td>5467</td>
<td>65.2%</td>
<td>28.0%</td>
<td>47%</td>
<td>1498</td>
</tr>
</tbody>
</table>

- The cell classification accuracy (error) of the ML model ranged from 65.2% (28%) for specialized cells to 91.1% (11.7%) for inflammatory cells.
- The steatotic hepatocytes count ratio (detected from ML from pathologists’ annotations) correlates well with Steatosis Area Ratio (R²=0.5889).
- The Inflammatory cell ratio (% of cells that are inflammatory) corresponds well with the inflammation grades (p 0v1=0.03, p 0v2=0.05, p 0v3=0.015) count of small inflammation foci clusters (p 0v1=0.053, p 0v2=0.015) moderately corresponds to the histological grades, which is attributed to the histological definition of inflammatory clusters and their assessment.

Conclusions

- Quantitative digital pathology can automatically generate tissues panels from H&E-stained biopsies.
- While the scores extracted from these tissue panels moderately correspond to NASH-CRN histological stages, they present the benefit of being quantitative and translational, and not sensitive to liver tissue variation due to swelling, fat invasion, or other various artifacts.

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