Assessment of Compound-Induced Hepatocellular Hypertrophy in Rats Through Deep Learning-based Quantitative Analysis

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INTRODUCTION

In preclinical studies, hepatocellular hypertrophy is frequently encountered in rodent liver histology. The possible challenges with traditional approaches to interpreting hypertrophy, apart from being time consuming, is that they may lack uniformity, and could be influenced by scale shifts and subjectivity. Variation in defining treatment-related hypertrophy exists across studies due to factors like inter-pathologist variation, animal strains, age, and sex.

Interpretation of cell hypertrophy and its significance is based on the comparison of the control (non-treated) and the treatment group samples and correlating the histologic results to liver weight . Previously, image analysis applications have been trained to detect cell hypertrophy by annotating cells to a normal or hypertrophied class. Thus, image analysis applications with a predefined cutoff between normal and hypertrophied cells may generalise poorly between different study sets. The objective of this study was to develop an artificial intelligence (AI) model, using convolutional neural networks (CCN), capable of quantifying hepatocyte area, without a fixed hypertrophy cutoff, in HE-stained whole slide images tissue.

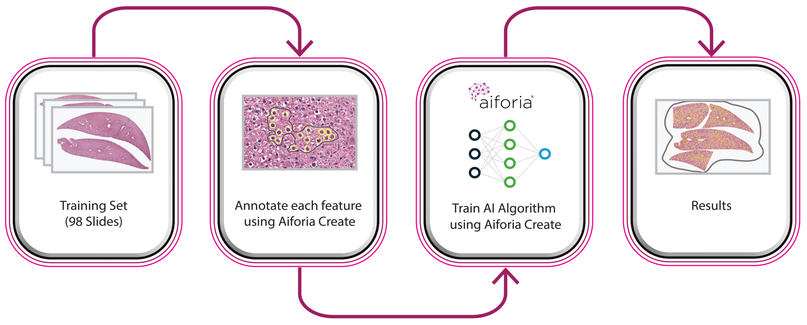

Figure 1. The AI model training and performance evaluation process.

MATERIALS AND METHODS

A supervised AI model was created with Aiforia® Create, trained on 98 digital images from HE stained rat liver tissue sections (Overview, Figure 1). Three CNNs were employed with the first layer segmenting tissue from the background. The second segmenting the different microanatomical structures, including parenchyma, portal tracts and central veins. The third CNN was used for object detection and instance segmentation of hepatocytes to provide individual hepatocyte metrics of and quantification of number, circumference and area. The AI was trained to recognize only those hepatocytes that include nuclei within the tissue sections.

After verification, the model was used to analyse 33 Rat liver tissue sections from a study with a rat group treated with Carbamazapine which is a compound known to induce hepatocellular hypertrophy (30, 100 & 300 mg/kg), producing data for correlation analysis. Comparison of the hepatocyte metrics between treatment groups, and between scores (DG Grade; slight, mild, moderate and severe) assigned by a board-certified pathologist were undertaken.

The hepatocytes were assigned to a zone by their distance to the classified portal tracts and central veins. The spatial metrics used to measure hepatocyte hypertrophy distance are adjustable, allowing for the customization of analysis. From internal discussions this was defined as ≤150um. Those hepatocytes were therefore defined as centrilobular or periportal with all remaining hepatocytes were defined as midzonal (Figure 2).

Individual hepatocyte cell area and circumference were calculated for each zone in each slide. The mean and median cytoplasmic area and circumferences per slide was calculated for each zone. Statistical analysis was performed and Pearson's coefficients of determination (R2) were calculated for centrilobular, periportal and midzonal areas between DG Grade, dose group, mean and median hepatocyte area, and mean and median circumference.

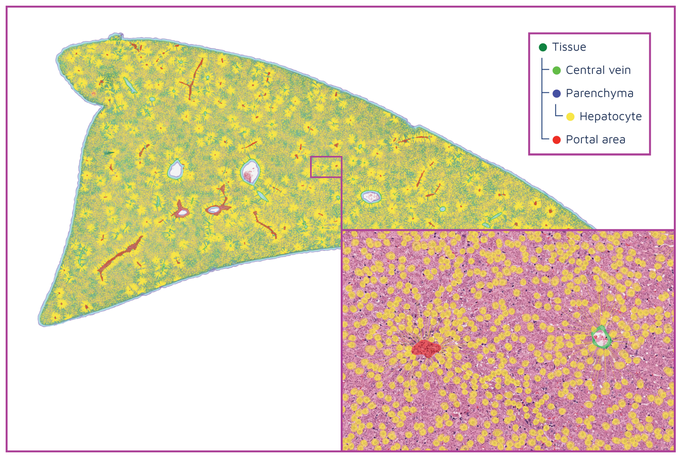

Figure 2. An example of the fully classified HE stained liver tissue and model tree structure (Tissue and Parenchyma and Hepatocyte results not shown). Portal structures segmented in red and centrilobular areas segmented in green and spatial distances to hepatocytes ≤150um in yellow. **Inset:** Close up view including object detection and instance segmentation of individual hepatocytes in yellow.

RESULTS

The AI model demonstrated high accuracy, in discerning cell hypertrophy variances in the dose groups, compared with the pathologists grade. As expected, this change was most evident in the centrilobular zone. However, also in the other zones there was a trend of increase of cytoplasmic area in animals with increases in carbamazepine dosing (Figure 3).

The R2 values for centrilobular values were found to be most significant and all above 0.67 with the strongest correlation between grading of the pathologist and hepatocyte mean area 0.78, (Figure 4) but with a moderate overlap of cell sizes ranging over multiple grades. As expected there was a poorer correlation of hepatocyte correlation coefficients for grading of the pathologist and dose groups against hepatocyte size and circumference (mean and median) for both periportal and midzonal areas (below 0.5 and 0.4 respectively).

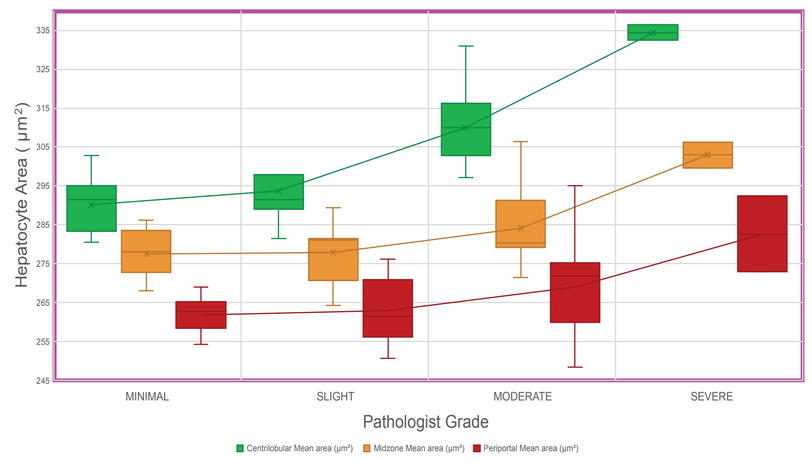

Figure 3. A graph comparing mean hepatocyte area for each segmented zone compared with the pathologist grade.

CONCLUSIONS

We trained the AI model to differentiate between tissue components, to measure hepatocyte cytoplasmic area and circumference. Spatial analysis provides the distribution of hypertrophic cells within the tissue microenvironment, quantifying the degree of hypertrophy in periportal, midzonal and centrilobular locations.

The AI model correctly established predominantly centrilobular hypertrophy within the Carbamazipine study with a significant correlation between centrilobular mean and median areas, alongside hepatocyte circumferences, and pathologist observations and grading, and facilitated the establishment of cutoffs.

The AI model provides a reliable and rapid solution to quantify hypertrophic liver changes, aiding in understanding compound-induced pathology. It accurately interprets hepatocyte hypertrophy, including zonal distribution. Integrating AI into pathological workflows is expected to enhance diagnosis accuracy and repeatability for pathologists. Furthermore, automated image quantification can help perform routine evaluations in a reproducible manner with high throughput.

Liver weight and control group data was not available in this preliminary study. Future research should attempt, using a much larger study group, including control data and liver weight, to further explore the ability of our AI model to calculate and segment liver hypertrophy.

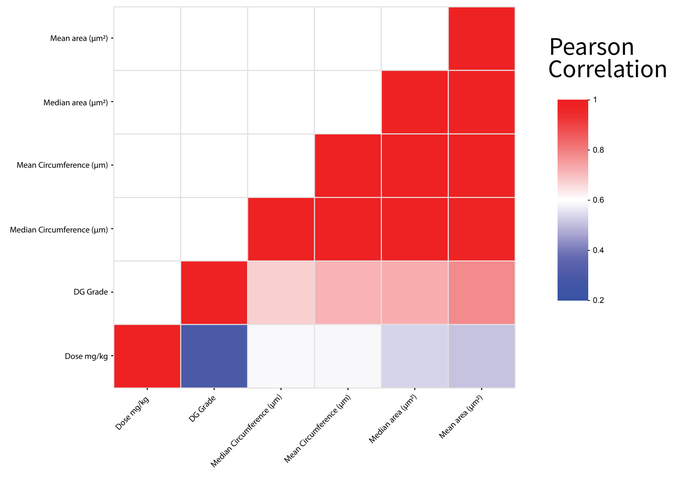

Figure 4. A correlation matrix heatmap demonstrating the Pearson-Correlation coefficients between the mean and median hepatocyte area and circumferences, pathologists grade and Carbamazipine dose.

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