

Quantitative evaluation using Halo AI in a MASH model using PXB-Mouse

PXB-Mouseを用いたMASHモデルにおける, Halo AIを用いた定量評価

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Background

Metabolic dysfunction-associated steatohepatitis (MASH) is a liver disease characterized by steatosis, inflammation, hepatocyte ballooning, and fibrosis arising from such as obesity, diabetes, hypertension, and dyslipidemia. However, currently, there are no approved therapeutic agents for MASH, and it is considered there is no animal model that appropriately represents the complexity of human MASH disease. Therefore, we developed a MASH model using Chimeric mice with humanized livers (PXB-Mouse[®]), and liver steatosis and fibrosis were evaluated using HALO AI.

Methods

- ✓ **Animal:**
 - PXB-mouse[®] (PhoenixBio Co., Ltd.), Male, 18–22 weeks old
- ✓ **Diet:**
 - CRF-1: Oriental Yeast Co., Ltd.
 - CDAA: The choline-deficient L-amino acid-defined 50kcal% fat containing diet (patented by Mediford Corporation).

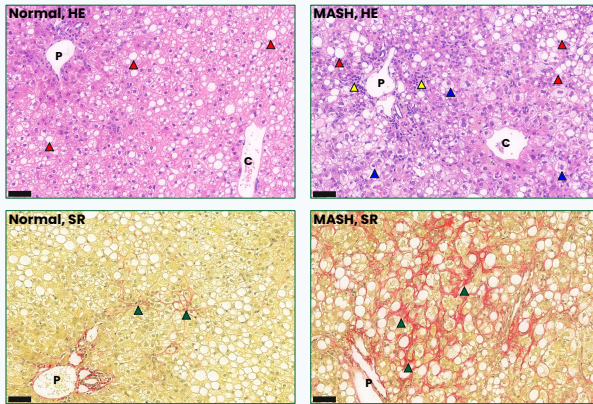
Group design

Group	Diet	Period	Number of animals
Normal	CRF-1	84 days	3
MASH	CDAA		5

- ✓ **Preparation of paraffin sections**
 - Paraffin blocks were prepared from formalin-fixed liver (left lateral lobe) and then sectioned.
- ✓ **Stain:**
 - Hematoxylin and eosin stain (HE)
 - Sirius Red stain (SR)
- ✓ **Quantitative evaluation analysis:**
 - Image analysis software: HALO[®] and HALO[®] AI (Indica Labs Inc.)
 - Classifiers: DenseNet V2 (HALO[®] AI)
 - Analysis: Area Quantification module (HALO[®], Fibrosis area, SR)
 - Vacuole Quantification module (HALO[®], Vacuolation area, HE)
 - Data: Proportion of fibrosis or vacuolation area in the analyzed area (%)

Results

Qualitative evaluation (Microscopic Examination)



Histopathological findings in the liver

Group	N	Fibrosis [*]	Steatosis	Lobular Inflammation	Ballooning hepatocyte
Normal	(3)	1.0 ± 0.0	2.3 ± 0.3	0.7 ± 0.3	0.0 ± 0.0
MASH	(5)	2.0 ± 0.0	3.0 ± 0.0	0.6 ± 0.2	1.0 ± 0.0

Score: 0, none; 1, minimal; 2, mild; 3, moderate; 4, marked.

Each value represents the mean ± S.E. of the histopathology score.

N: number of animals

*: Evaluated by SR

▲: Hepatocytes with large sized lipid droplets

▲: Ballooning hepatocytes and Mallory-Denk-body-like aggregates

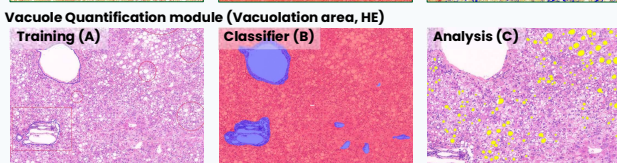
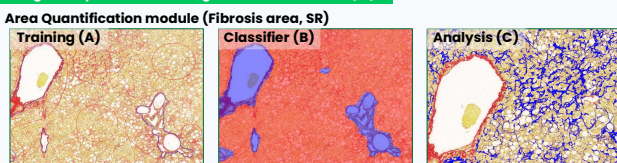
▲: Inflammatory cell, ▲: Fibrosis, C: Central vein, P: Portal area

Bar: 50 μm

This model was judged to be established as a MASH model.

Quantitative evaluation analysis

Image analysis method using Halo[®] and Halo[®] AI (AI)



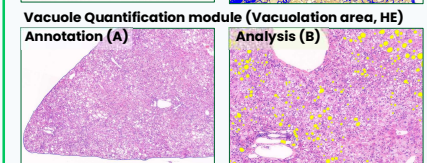
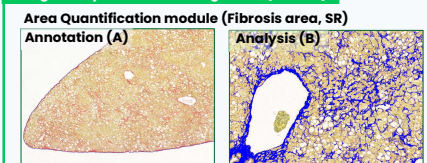
- A. The DenseNet V2 which is the classifier algorithm of HALO AI, was trained by drawing about many annotations for hepatocyte areas (red frame) and blood vessel/portal areas (blue frame).
- B. After completing the training, applied the classifier to images. It was confirmed that hepatocyte areas (red mask area) and blood vessel/portal areas (blue mask area) were classified.
- C. Using each module, the fibrotic areas (blue) or the large vacuoles (yellow) in hepatocytes were measured in the hepatocyte areas defined by a classifier trained with HALO AI.

Image analysis data

Group	N	Fibrosis area (%)		Vacuole area (%)	
		Non-AI	AI	Non-AI	AI
Normal	(3)	1.2 ± 0.1	0.6 ± 0.0	4.4 ± 0.8	4.5 ± 0.8
MASH	(5)	7.6 ± 0.8	6.9 ± 1.0	7.0 ± 0.7	7.1 ± 0.7

Each value represents the mean ± S.E. of the image analysis data
N: number of animals

Image analysis method using Halo[®] (Non-AI)



- A. The liver boundary was annotated (Blue line), and the annotated area was defined as the analysis area.
- B. Using each module, the fibrotic areas (blue) or the large vacuoles (yellow) were measured.

Summary

1. Quantitative evaluation showed a greater difference between normal and MASH groups than qualitative evaluation.
2. Non-AI approach: Vascular walls were recognized as fibrosis; some vascular/bile duct lumina were recognized as vacuoles (False positives).
3. HALO[®] AI successfully eliminated false positives by excluding vascular walls and vascular/bile duct lumina.

Conclusion

- ✓ The HALO[®] AI classifier algorithm improved image analysis accuracy by eliminating false positives observed in non-AI analysis.
- ✓ HALO AI-based pathological analysis is effective for evaluating this MASH model.

COI Disclosure Information

Takafumi Oshikata

In connection with this presentation, there is no COI to be disclosed with any companies.

本演題に関連して開示すべき利益相反はありません。