**Abstract**

Non-alcoholic steatohepatitis (NASH), the most common chronic liver condition in Western populations, is characterized by tissue damage and inflammation which leads to build-up of fat and fibrosis in the liver. We test if in vivo imaging techniques can be used to detect, visualize, and quantify disease progression in a mouse NASH model. The model is established by refeeding (p.) injections of carbon tetrachloride (CCl4, mixed in corn oil) to NASH mice for 1 week, inducing total lesions at 0.4 mg/kg. CCl4 is known to cause liver inflammation, and long-term exposure of CCl4 in conjunction with high fat diet (HFD) leads to liver steatosis and fibrosis.

Three types of imaging modalities were tested in this study: (1) Fluorescence (FL1): 3D planar and 3D tomography imaging to detect liver inflammation using specific IVIS®-enhanced imaging probes (GFP and RFP). PerkinElmer Inc.; (2) Ultrasound: shear wave elastography (SWE) to detect changes in liver stiffness; and (3) MicroCT: histological assessment to measure liver density changes associated with steatois/and fibrosis.

**Histological validation of NASH development in the HFD-CC4 mice**

Three groups of mice were used in this study: (1) Control mice received saline injections and were maintained on regular diet; (2) HFD mice received saline injections but were maintained on high fat diet (Teklad custom diet TD.03018, Envigo). (3) HFD-CC4 mice received CCl4 injections and were maintained on high fat diet for NASH development.

Fluorescence imaging can detect early liver inflammation in HFD-CC4b mice in two weeks of NASH development. SWE imaging showed that, three weeks after CCl4 induction, the HFD-CC4 mice started to develop liver stiffness, indicating fat accumulation and fibrosis. Interestingly, this phenomenon reversed after two weeks, and HFD-CC4 mice became stiffer, suggesting extensive tissue fibrosis. The longitudinal assessment of liver density in the NASH mice also supports the SWE findings. The HFD-CC4b mice showed lower HU readings at week 3. However, at week 12, HFD-CC4 mice showed recovery in HU, suggesting development of fibrosis in mice. These results demonstrate the potential of non-invasive and quantitative analysis of NASH disease progression in animal models.

**Fluorescence imaging of liver inflammation at the early stage of NASH**

Fluorescence imaging of liver inflammation during the initial stage of NASH. Two weeks after NASH development, mice received i.v. injections of a fluorescent agent cocktail of IVIS-Dye-680 (750, IVISense MP7 750, IVISense Transferrin Receptor 750 (JAM750, PerkinElmer Inc.). This cocktail can detect cell death, inflammation, and iron metabolism changes in the body. Another imaging modality was used to detect and visualize vascular leakiness in inflamed tissues. The day after probe injection, liver fluorescent signals were measured using the (A) IVIS® Spectrum (PerkinElmer Inc.) and (B) IVIS® 4000 system (PerkinElmer Inc.). P<0.05, P<0.01, ***P<0.001, ****P<0.0001; test compared with Ctrl.

**Conclusion**

This study demonstrates that a multimodal in vivo imaging approach can be used to non-invasively detect, visualize, and quantify disease progression in animal NASH models. Three imaging modalities were tested, and each has its unique strength for detection of certain aspects of NASH. (1) Fluorescence imaging (FL1) was used to study the formation of liver inflammation, making early detection of liver inflammation possible. (2) Ultrasound imaging can visualize, detect, and quantify subtle tissue changes in soft tissue without the need of contrast agent or probe. On this same NASH model, the shear wave elastography (SWE) mode can detect early reduction of tissue stiffness due to fat accumulation and fibrosis. (3) MicroCT imaging can visualize, detect, and quantify subtle tissue changes in soft tissue without the need of contrast agent or probe. On this same NASH model, the shear wave elastography (SWE) mode can detect early reduction of tissue stiffness due to fat accumulation and fibrosis. Interestingly, as the mice exposed to the chronic inflammatory condition induced by CCl4, SWE imaging revealed a significant gradual increase in liver stiffness due to fibrosis or possible cirrhosis at the later stage. The B-mode can be used to assess fat accumulation in the liver, as a higher fat content results in a higher grayscale intensity reading. (MicroCT) can also measure changes in liver texture by assessing the elastic stiffness. However, since it requires the use of ionizing radiation, microCT is not suitable for repeated measurements.

This multimodal strategy provides a novel approach for the non-invasive visualization and quantitative measurement of NASH disease progression. As each technology aims to visualize different aspects of the disease, this multimodal imaging strategy should facilitate in vivo assessment of potential NASH treatments in preclinical models.