Determination of Biomarkers in Liver Disease by In vivo and Ex vivo NMR

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INTRODUCTION Chronic liver diseases, in particular non alcoholic fatty liver disease and steatohepatitis(NAFLD/NASH), is becoming prevalent in association to obesity. NAFLD is characterized by excessive fat accumulation in the liver, while NASH refers to a hepatic disease where fat accumulation is complicated by hepatic inflammation, liver cell injury and liver fibrosis. The pathogenic mechanism and early diagnoses of NAFLD is poorly established in humans and this currently pose a major public health concern. The gold standard to reach the diagnosis of chronic liver disease relies on liver biopsy. The overall aim of this project is to identify biomarkers by in vivo and ex vivo NMR that can accurately determine patients at risk of severe liver disease and to assess the potential impact of treatment strategies. Metabolomics is established as an investigational tool that provides rich information on metabolic disturbances in human disease and in this sub-study, we make use of High-Resolution Magic Angle Spinning (HR-MAS) NMR[1] spectroscopy which is a powerful analytical tool for investigating the metabolic state of an intact tissue.

AIM We aim at determination of metabolic and lipid biomarkers from liver biopsies of NAFLD and NASH patients.

METHOD As an initial step we are currently utilizing control liver tissues obtained from pig. This was prepared for HR-MAS spectroscopy to generate a measurement protocol aimed at facilitating a comprehensive determination of lipid and metabolic profiles and physical properties, and in the future will be used to study liver biopsies from patients with liver diseases. Different NMR methods include 1D NMR with T2 filter for small molecules, diffusion weighted spectra for lipids, and more advanced methods for determination of physical properties including T1- and T2- determination, diffusion-constant determination (with different diffusion times Δ), and spinning speed variation. The metabolomical analyses, spectral analyses and metabolite identification of the acquired NMR data, will be correlated to biochemical and physiological results and will allow a comparison of metabolite and lipid compositions between ex vivo and in vivo methods.

RESULTS The measurement protocol was established and we will use it for the samples from patient biopsies. 1D and 2D HR-MAS NMR spectra were acquired from pig liver, indicating numerous signals corresponding to lipids and metabolites. With the help of databases and own reference spectra, numerous resonances could be assigned to specific metabolites. By applying different spectral filters, we successfully separated lipids from small molecules metabolites. Additionally, we determined the diffusion coefficients of lipids, providing insight on lipids mobility in the tissue microenvironment. To understand the organization and structural properties of the lipid deposits, we aim at calculation of lipid droplet sizes by using diffusion NMR applying different diffusion times.

DISCUSSIONThe separation of the lipid signals from the metabolite signals allows a detailed analysis of their individual contributions in liver metabolism. The result from the ex vivo and in vivo data will be correlated with findings from liver pathology results to accurately identify a non-invasive biomarker which can be used as drug target in chronic liver diseases.

[1]Diserens G, Vermathen M, Zurich MG, Vermathen P. Longitudinal investigation of the metabolome of 3D aggregating brain cell cultures at different maturation stages by (1)H HR-MAS NMR. Anal Bioanal Chem 2018;410:6733-