(10 mg/kg) + empagliflozin (10 mg/kg). After 8 weeks, mice were sacrificed and subjected to blood measurements, and tissues for RNA isolation, lipid measurements and histology.

**Results:** Ezetimibe, empagliflozin, and combination therapy significantly reduced liver steatosis. However, the histological NAFLD activity score (NAS) was most improved in the ezetimibe/empagliflozin group (0.667) than in the ezetimibe group (2.0, P = 0.032) or empagliflozin group (3.33, P = 0.043). Hepatic lipid contents were also significantly lower in the ezetimibe/empagliflozin group compared to other groups. Hepatic expression of lipogenesis genes such as FAS (Fatty Acid Synthase), ACC1 (Acetyl-CoA carboxylase 1) were significantly decreased in the ezetimibe/empagliflozin group. For in vitro study of ezetimibe and/or empagliflozin, we utilized murine liver organoids and provided them with fatty acid to induce hepatic steatosis. Lipid accumulation was observed in liver organoids treated with fatty acid compared with control. When liver organoids with fatty acid were also treated with ezetimibe and/or empagliflozin, lipid accumulation was most diminished in the ezetimibe/empagliflozin group by measuring fluorescence intensity.

**Conclusion:** Our data suggested that combined administration of empagliflozin and ezetimibe can additively improve NAFLD by decreasing lipogenesis. These results provide new insight into pathogenesis and strategies for treatment of the NAFLD.

**SAT027**

**Deviations of the peripheral blood and intrahepatic immune cell landscape between NAFLD patients and healthy controls**

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**Background and Aims:** Multiple factors are involved in the pathogenesis of NAFLD, but the exact immunological mechanisms that cause inflammation and fibrosis of the liver remain enigmatic. Several immune cells including cytotoxic T cells, Th17 cells, regulatory T cells (Tregs), mucosal associated invariant T (MAIT) cells, γδT cells, iNKT cells and natural killer (NK) cells have been found as being altered in the local inflammation of NAFLD patients. However, the significance and directionality of these differences are still controversially discussed, especially with respect to human NAFLD.

**Method:** In this study, we present 16-color flow cytometric data of a cohort of NAFLD patients (PBMC: n = 27, liver samples: n = 15) in comparison with healthy individuals (PBMC: n = 26, liver samples: n = 3) assessing the frequency and phenotype of 23 immune cell subtypes that were correlated with clinical data.

**Results:** PBMC of NAFLD patients showed decreased frequencies of total CD3+, CD8+ T cells, CD56dim NK cells and MAIT cells, but elevated frequencies of CD4+ T cells, Th2 cells compared to healthy controls. IHL of NAFLD patients showed decreased frequencies of total T cells, total CD8+ T cells, Vd2+ gamma delta T cells, and CD56bright NK cells, but elevated frequencies of Vdelta2- gamma delta T cells and CD56dim NK cells compared to healthy controls. The activating receptor NKG2D was significantly less frequently expressed among iNKT cells, total NK cells and CD56dim NK cells of PBMC of NAFLD patients compared to healthy controls. More strikingly, hepatic fibrosis as measured by fibroscan elastography negatively correlated with the intrahepatic frequency of total NK cells (r² = 0.3737, p = 0.02). Hepatic steatosis as measured by controlled attenuation parameter (CAP) value negatively correlated with circulating NKG2D+ iNKT frequency (r² = 0.3365, p = 0.0047).

**Conclusion:** Our data provide an overview of the circulating and intrahepatic immune cell composition of NAFLD patients, and point towards a potential role of NK cells and iNKT cells in the regulation of hepatic fibrosis and steatosis in NAFLD.

**SAT028**

**High-throughput sequencing identified MIR-193a as a potential biomarker of non-alcoholic fatty liver disease activity**

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**Background and Aims:** The current gold standard of non-alcoholic fatty liver disease (NAFLD) diagnosis is based on histological scoring of liver biopsies. Disease activity can be graded using the NAFLD activity score (NAS), calculated as the sum of Kleiner inflammation, ballooning and steatosis scores. Liver biopsies carry inherent risks and so circulating biomarkers are needed to circumvent the requirement for such invasive procedures. MicroRNAs (miRNAs) are small (~22 nt) non-coding RNA molecules that post-transcriptionally regulate gene expression and are known to be expressed in serum. Circulating miRNAs have been characterised as diagnostic biomarkers for a range of diseases. Accordingly, we sequenced over 2,000 serum miRNAs in a discovery cohort of patients across the NAFLD spectrum to establish a profile of circulating miRNAs from which novel disease biomarkers could be identified.

**Method:** miRNA libraries, using 15 μl serum for each of 183 NAFLD patients and ten healthy controls, were generated by HTG EdgeSeq and sequenced by Illumina NextSeq. Limma in the R software environment was used to perform analyses on the data. Data were normalised and corrected for batch effects. MiRNAs with a mean counts per million of >=100, a log2 fold-change (logFC) of >=0.3 and an adjusted p value of <=0.05 were classified as differentially expressed.

**Results:** Seven miRNAs were differentially expressed in severe disease activity (NAS=8) relative to mild (NAS=1–4), with miR-193a being the most significant (logFC = 0.68, p = 3.0 × 10^−07, AUROC = 0.71). Additionally, miR-193a was the most significant (logFC = 1.5, p = 3.5 × 10^−10, AUROC = 0.94) of 121 differentially expressed miRNAs above a more stringent logFC threshold of >=1 in NAFLD patients relative to controls. Distilling NAFLD into steatosis and non-alcoholic steatohepatitis (NASH) with fibrosis stage (F0-F4) confirmed a consistent and significant upregulation of miR-193a in each of the classifications relative to the controls.

**Conclusion:** We have identified a potentially clinically significant differentially expressed circulating miRNA, which appears to primarily reflect NAFLD grade and activity. Quantification of miR-193a may reflect NAFLD grade and activity. Distilling NAFLD into steatosis and non-alcoholic steatohepatitis (NASH) with fibrosis stage (F0-F4) confirmed a consistent and significant upregulation of miR-193a in each of the classifications relative to the controls.

**SAT029**

**Ageing causes lipid metabolism imbalance and exacerbates steatohepatitis in high-fat diet-fed mice**

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