### Background/Purpose

Acute-on-chronic liver failure (ACLF) is a defined clinical disease with high-mortality and mortality and characterized by acute severe hepatic failure (acute decompensation) with one, two or more extrahepatic dysfunctions (organ failure) on a background of liver cirrhosis. Therefore, it is important to better understand the development of ACLF. Effective treatments for ACLF are lacking and the mechanisms of ACLF development remain poorly understood. Therefore, it is necessary to develop an animal model with strict reference to the human outcome.

This study aims at generating animal models of acute decompensation (ACD) of cirrhosis developing ACLF, to identify those best reflecting the patient populations appearing as potential responders to a combinational therapy. Specifically, we create rat models of cholangitis or toxic liver injury. On top of CCl4 injections, we generate models of alcoholic (ASH) and non-alcoholic (NASH) steatohepatitis.

To reflect human ACLF new rat models of cirrhosis with different precipitating events are urgently needed. Thus, we mimicked excessive alcohol consumption by binge drinking, pneumonia by transversal inoculation and sterile inflammation by LPS injections.

### Methods

Liver cirrhosis was induced using bile duct ligation (BDL) or carbon tetrachloride (CCl4) injections in Sprague Dawley rats. Rats of the alcoholic steatohepatitis (ASH) group received ethanol (EtOH) in the drinking water ad libitum. Starting with 4% EtOH (week 1), followed by 8% EtOH for one week (week 2). Three precipitating events for the rest of the study were: 18% (week 3). Rats of the non-alcoholic steatohepatitis (NASH) group were fed with Western diet (containing 20% crude fat and 2.20% cholesterol) ad libitum. When sepsis was present (21 days of BDL, 12-14 weeks of CCl4, 5-6 weeks of CCl4+EtOH, 4-6 weeks of CCl4+LPS), as a clear sign of portal hypertension, ACLF was induced. To induce ACLF by sterile inflammation, rats were twice injected with LPS (1200 and 72h, 0.5µg/kg body weight) before the final experiment. Acute alcoholic hepatitis was induced by large drinking of EtOH (4g/kg body weight) 72h before the final experiment. As a model of pneumonia, we performed transversal inoculation (TNI) 72h before the final experiment. For this, 0.2µl of freshly collected cell suspension in 2µl sterile 0.9% NaCl-solution and inoculated.

For organ dysfunction characterization, invasive pressure measurements, blood chemistry analysis and quantitative polyclonal chain reactions (qPCR) (BM-1) were performed. For liver fibrosis assessment picrosirius red staining was performed in paraffin embedded liver sections. The neurological behavior test was performed according to Yarnell et al. (Curr. Proto. Neurosci., 2016). Brain water content was determined using the dry weight technique (Shah et al., Liver Transplant., 2013). Brain water content was determined using the dry weight technique (Shah et al., Liver Transplant., 2013).

### Results

#### Liver

Attributes developed a severe cirrhosis within 3 weeks after BDL characterized by liver, brain and circulation dysfunction. Only binge drinking as an ACLF precipitating event, led to further deterioration of liver function shown by increased levels of ALT, AST and GGT (Fig. 1a, 1b). In toxic liver disease induced by CCl4, rats developed a severe cirrhosis within 12-14 weeks characterized by liver, brain, circulation and respiratory dysfunction (Fig. 1c-1f). The injection of LPS and the TNI as precipitating events led to further deterioration in brain function, as shown in the neurological behavior tests and the increased brain water content (Fig. 2). Blood coagulation was also affected by LPS injections, as shown by the increased INR in the ASH group (Fig. 5).

In the NASH model, rats developed a severe cirrhosis within 4-6 weeks characterized by liver, kidney, brain, coagulation and circulation dysfunction (Fig. 1c-1f).

Additionally, LPS injections as a precipitating event in the NASH model, led to a deterioration in liver, brain and respiratory function (Fig. 1g).

In the ASH model, rats developed a severe cirrhosis within 6-8 weeks characterized by liver, brain, circulation and respiratory dysfunction (Fig. 1g-1n). The three precipitating events of binge drinking, LPS injections and TNI worsened the kidney function in all liver cirrhosis models (Fig. 4). Binge drinking, TNI and LPS injections impaired the brain function in all CCl4 groups, as assessed by the neurological behavior test and the brain water content (Fig. 2).

### Discussion/Outlook

ACLF is a frequent syndrome in patients with liver cirrhosis and therapeutic options are urgently needed. Therefore animal models which mimick this severe disease needs to be established. In the BDL model the precipitating events of binge drinking, LPS injections and TNI induced hepatic and renal failure, but no brain, coagulation or circulation dysfunction.

In toxic liver disease LPS injections and TNI induced mental as well as coagulation failure. In the NASH model LPS injections induced severe liver, brain and lung dysfunction and represents a suitable model for ACLF studies. However, in the ASH model all three precipitating events, alcohol, LPS and TNI induced severe renal, mental and coagulation dysfunction (Tab. 1). Taken together, the ASH model stands out as the most efficient cirrhosis model for ACLF induction. However the respiratory function was not affected by any precipitating event.

The bacterial transudation (Peritonitis) as a precipitating event of ACLF is still in progress in BDL rats to accomplish the task. The animal models generated in this WP will contribute to a better understanding of the pathogenesis of ACLF.