Gut liver axis in liver cirrhosis

Valorisation addendum

Valorisation addendum

Liver cirrhosis is the end result of a variety of chronic liver diseases, predominantly related to alcohol consumption, viral infections and metabolic disorders. It is characterized by distortion of normal liver architecture encompassing diffuse parenchymal fibrosis, nodules and vascular remodeling that lead to impaired liver function.[1] The liver is a central organ and is very important for whole body homeostasis. According to the National Center for Health Statistics, cirrhosis and chronic liver diseases are the twelfth leading cause of death in the United States.[2] In Europe, 1.8% of all deaths are caused by cirrhosis, accounting for approximately 170.000 deaths per year.[3] Worldwide, the number of patients progressing to liver cirrhosis is expected to rise, especially due to the obesity epidemic associated with the metabolic syndrome and non-alcoholic fatty liver disease (NAFLD), but also because of aging of patients with chronic viral liver diseases.

Liver cirrhosis is an asymptomatic disorder until complications, *i.e.* decompensation, because of portal hypertension occur. These complications, including variceal hemorrhage, spontaneous bacterial peritonitis (SBP) and hepatic encephalopathy are mainly responsible for the high morbidity and mortality, accounting for frequent hospital admissions and for 1.03 million deaths per year worldwide, 170.000 per year in Europe and 33.539 per year in the United States. Compared to patients with compensated cirrhosis, patients with decompensated cirrhosis report a poorer quality of life, which inevitably also leads to increased health-care utilization.
Despite recent advances in cause-specific treatments, liver transplantation is still the only effective and expensive curative option in cirrhotic patients with complications or hepatocellular carcinoma. Annually, 5500 liver transplants are performed in Europe with cirrhosis being the main indication.
As a result, liver cirrhosis is responsible for considerable health-care utilization and represents a large economic burden with high direct health-care costs as well as indirect costs related to work absenteeism and reduced work productivity. In 2004 in the United States, annual direct costs for chronic liver diseases and cirrhosis, excluding chronic hepatitis C, were estimated to be $2.5 billion with even higher estimates for indirect costs.

Considering these figures and the increasing prevalence of cirrhosis, further research to decrease the disease burden and health care costs of cirrhotic patients, is needed for patients as well as society. The results of this thesis will contribute in further insight into the pathophysiology of liver cirrhosis, thereby aiming to identify leads for preventing progression to end stage complicated liver disease. Furthermore, we aimed to identify non-invasive tools for diagnosing cirrhosis at an early stage and thereby prevent development of severe complications, *e.g.* by endoscopic screening of or

prophylactic treatment for gastrointestinal varices, ultimately aiming to increase health-related quality of life.

Liver biopsy is still the current standard for a definite diagnosis, but is difficult in the follow-up as repetitive biopsies are not accepted by patients and many health care workers. It is an invasive procedure with significant complications, such as pain and bleeding with a mortality rate of 1 in 10.000 and high costs.[4] Diagnostic tools to reduce the number of biopsies are therefore highly warranted.
Studies to find non-invasive alternatives for staging fibrosis are ongoing. As dynamic changes are currently considered to be involved in fibrogenesis, it is important to assess the functional metabolic capacity of the liver and search for markers that can measure ongoing pathophysiological processes and metabolic functions. Analysis of exhaled volatile organic compounds (VOCs) is already found to discriminate between cirrhosis and healthy controls, and was further studied in this thesis. VOCs have the advantage to reflect liver metabolic function and, therefore, may provide leads towards pathophysiological pathways. Furthermore, exhaled air collection is non-invasive, easy and requires only a minimal time investment. We found that VOCs can predict the presence of compensated cirrhosis among a heterogeneous group of patients with chronic liver diseases, with a higher accuracy than a panel of routine serological parameters. Therefore, VOCs may aid in reducing the number of liver biopsies in clinical practice, and will provide more insight into the pathophysiological processes and risk factors for disease progression. Furthermore, after validation of the current findings in other groups of patients, it would be of interest to further develop simple devices (*i.e*. sensors) to measure the VOCs, which can be implemented in daily clinical practice to monitor these patients over time. Thereby, these patients will not have to undergo (repetitive) invasive biopsies for diagnosing cirrhosis at an early stage to prevent development of complications, and results in an improved health-related quality of life.

Although chronic liver diseases, such as alcoholic liver disease and chronic infections by Hepatitis B and C, can lead to liver damage, fibrosis and ultimately cirrhosis, further insight is needed in (co-)factors increasing the risk of disease progression, *i.e.* to decompensated cirrhosis, and thereby aiming to decrease cirrhosis-related comorbidity and health care costs. In addition to the above-mentioned etiological factors, also the intestine may play a role, with its' barrier function aiming to prevent permeation of substances from the external (*i.e.* intestinal lumen) to the internal environment. However, studies are limited and often focused on the small intestine and/or one potential pathophysiological mechanism. This thesis has therefore focused on the role of the small and large intestine in patients with compensated cirrhosis. In particular, we demonstrated an impaired epithelial barrier function of the large intestine to be present in these patients. As the large intestine is colonized by a high number of (commensal) bacteria, the observed barrier dysfunction may enhance

bacterial translocation and contribute to further liver damage and the development of cirrhosis-related complications, such as SBP. Reinforcement of the intestinal epithelial barrier may therefore have therapeutic potential. For example, larazotide acetate, a novel tight junction (TJ) regulator peptide that prevents TJ opening, showed promising results in patients with celiac disease.[5] Furthermore, probiotics have shown to improve the epithelial integrity and reduce bacterial translocation. Results of a recent study showed that treatment with a probiotic reduced the risk of hospitalization and improved liver function and health-related quality of life in cirrhotic patients.[6] We therefore suggest that therapeutic modalities to restore epithelial barrier function, especially in cirrhosis, should be further investigated.

In this thesis, we also demonstrated an altered composition of the fecal microbiota in patients with compensated cirrhosis, which was even more pronounced with progression of liver cirrhosis. Based on these findings, the role of the fecal microbiota as marker for disease progression needs further study. Furthermore, it would be of interest to correlate this to findings on the VOCs in exhaled air, as a large part of them may result from the intestinal microbiota. Remarkably, we also found clear indications for involvement of the duodenal microbiota in cirrhosis and possible associations with small intestinal permeability. Bacteria have been shown to influence intestinal epithelial barrier directly by affecting the expression of TJ proteins or indirectly via elevated levels of lipopolysaccharide (LPS)/endotoxin and the subsequent inflammatory response.
Evidence indicates that the intestinal microbiota is an important player in the pathophysiology of several chronic liver diseases and development of cirrhosis-related complications. Modulating the intestinal microbiota by, for example, antibiotics or pre/probiotics may therefore influence the disease course and outcome of liver cirrhosis. In fact, these agents have proven successful in the prevention and treatment of several cirrhosis-related complications. Recently, interest is also growing in fecal microbiota transplantation for the treatment of several diseases associated with dysbiosis; the results look promising and may be an alternative treatment for patients with hepatic encephalopathy and SBP.
We think that better understanding of the alterations in the intestinal microbiota, especially with regard to their functional activity using state-of-the-art molecular techniques, will further elucidate the interaction between the microbiota and the intestine, leading to new insights in the pathogenesis, disease progression and may improve therapeutic strategies for cirrhosis. Future research will confirm the evidence for targeting the intestinal microbiota as a therapeutic strategy to restore dysbiosis and intestinal homeostasis and thereby prevent progression towards end stage complicated liver disease.

In this thesis, we showed that the intestine, *i.e.* its epithelial barrier and microbiota composition, is involved in compensated liver cirrhosis. It would be relevant to further

investigate whether the observed cirrhosis-related alterations in the intestine can be restored, for example by reinforcing the epithelial barrier and/or modulating the microbiota composition, and thereby prevent progression towards end stage complicated liver disease. This may decrease the high disease burden and health care costs of cirrhotic patients. Furthermore, we showed that non-invasive analysis of exhaled VOCs can predict the presence of compensated cirrhosis among a heterogeneous group of patients with chronic liver diseases, which is of relevance for both patients and society as early diagnosis of cirrhosis can prevent development of severe complications, and thereby improve health-related quality of life and decrease health care costs. In addition, analysis of exhaled VOCs may aid in reducing the number of invasive liver biopsies and could be implemented in daily clinical practice to monitor patients over time.

References

1. Anthony PP, Ishak KG, Nayak NC, Poulsen HE, Scheuer PJ, Sobin LH. The morphology of cirrhosis: definition, nomenclature, and classification. Bull World Health Organ 1977;55:521-40.
2. Hoyert DL, Xu J. Deaths: Preliminary Data for 2011. National vital statistics reports; vol 61 no 6. Hyattsville, MD: National Center for Health Statistics. 2011. Available from: http://www.cdc.gov/nchs/data/nvsr/nvsr61/nvsr61_06.pdf.
3. Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol 2013;58:593-608.
4. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. Hepatology 2009;49: 1017-44.
5. Khaleghi S, Ju JM, Lamba A, Murray JA. The potential utility of tight junction regulation in celiac disease: focus on larazotide acetate. Therap Adv Gastroenterol 2016;9:37-49.
6. Dhiman RK, Rana B, Agrawal S, et al. Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. Gastroenterology 2014;147:1327-37 e3.