Risk stratification in cirrhosis and acute-on-chronic liver failure : exploration of invasive and non-invasive prognostic markers

Kerbert, A.J.C.

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General introduction and scope of the thesis

GENERAL INTRODUCTION

Background and overall aim of this thesis

Liver cirrhosis is regarded as the end-stage of chronic liver disease. It results from different mechanisms of chronic liver injury that cause structural changes of the liver tissue leading to an increased resistance to portal blood flow and hepatic synthetic dysfunction.[1] Cirrhosis has traditionally been considered as an irreversible condition, but recent data show that regression and even reversal are possible.[2,3] In advanced stages of cirrhosis, however, liver transplantation is currently the only curative treatment option. This procedure is limited by the scarcity of donor organs and a significant number of patients is not able to benefit from transplantation due to disease progression, comorbidities or psychosocial issues.

Prognosis of cirrhotic patients varies widely and is mainly depending on the development of clinically decompensating events.[4] Information on prognosis is essential for any physician for providing information to the patient and forms the basis for the decision-making process for therapy. The available prognostic information is often inadequate, because individual patients frequently differ from the average patient population. Due to the wide range of disease severity in cirrhosis, exploration of prognostic markers is a challenging, but important, aspect of research in the field of chronic liver disease. Besides identifying patients who are at the highest or lowest risk of mortality, identification of robust prognostic markers may also help in understanding underlying pathophysiological processes and in improving treatment strategies. The overall aim of this thesis is to explore novel prognostic markers among different stages of chronic liver disease and in acute-on-chronic liver failure (ACLF).

Liver cirrhosis

Epidemiology

In developed countries, cirrhosis is an increasing cause of mortality. It is the 14th most common cause of death worldwide (>1 million deaths per year)[5] and the 4th in central Europe (170.000 deaths per year).[6] In Asia and sub-Saharan Africa, hepatitis B is the most common cause of cirrhosis, whereas hepatitis C, alcohol abuse and non-alcoholic fatty liver disease (NAFLD) are the main causes of liver disease in the more developed countries. Due to the increasing prevalence of overweight and diabetes over the past decades, NAFLD has become an endemic cause of liver disease in Europe.[6] It is associated with an increased mortality risk related to liver and cardiovascular disease and cancer.[7-9]

Pathogenesis and clinical presentation

Liver cirrhosis is the end-stage of liver fibrosis; a wound healing response that encapsulates injured regions by an extracellular matrix (ECM) or scar tissue. This occurs in patients with chronic liver injury and the rate is depending on the cause of liver disease, environmental and host factors. Inflammation and activation of hepatic stellate cells are key factors involved in the pathogenesis.[10-12] Stellate cell activation following chronic liver injury results in excessive production of ECM, angiogenesis and parenchymal extinction lesions.[13,14] These processes lead to sinusoidal remodelling, formation of intrahepatic shunts and endothelial dysfunction and may ultimately lead to the development of a hepatocellular carcinoma (HCC).[15] Another major clinical consequence is the resulting increased intrahepatic resistance to portal blood flow, which is the main factor contributing to an increase in portal pressure.

Portal hypertension plays a central role in the development of decompensating events and subsequent increased risk of mortality.[16] Splanchnic vasodilation, an adaptive response to the intrahepatic hemodynamic changes, contributes to a further increase in portal pressure by increasing portal inflow. In early stages of cirrhosis, splanchnic vasodilation is compensated by an increase in cardiac output, in order to maintain an adequate perfusion pressure.[16,17] However, in more advanced stages of cirrhosis, aggravation of portal pressure and peripheral vasodilation cannot be compensated anymore by a further increase in cardiac output. This leads to the development of a hyperdynamic circulation, which is characterized by a decreased effective arterial blood volume.[16] This again triggers the activation of counter regulatory systems, such as the renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system and the non-osmotic release of arginine vasopressin (AVP) from the posterior pituitary.[17] Activation of these vasoconstrictor systems helps to restore the effective arterial blood volume, but has negative effects on kidney function, particularly due to renal sodium and solute-free water retention, which are associated with the development of ascites, oedema and hyponatremia. Ultimately, intra-renal vasoconstriction and hypoperfusion may lead to the development of hepatorenal syndrome, which is associated with poor prognosis.[18,19]

According to the report of the Baveno VI consensus[20], determination of the severity of portal pressure provides relevant prognostic information. The hepatic venous pressure gradient (HVPG) is an estimate of the hepatic sinusoidal pressure, measured by transjugular catheterization of the hepatic vein, and is considered to be a good surrogate marker for portal hypertension (i.e., HVPG >5 mmHg).[21] For cirrhotic patients with portal hypertension who are at risk of developing decompensating events, the term 'compensated advanced chronic liver disease (cACLD)' has been proposed.[20] The threshold-HVPG at which complications of portal hypertension may develop is 10 mmHg. These patients are referred to as having 'clinically significant portal hypertension' (CSPH).[20] Due to differences in prognosis and subsequent

treatment goals, differentiation between patients with and without CSPH is important. HVPG measurement is currently the reference standard to assess the presence of CSPH. In patients with CSPH, formation of gastrointestinal varices may occur. Acute oesophageal variceal bleeding is associated with a 6 week mortality rate of 15-20%[22-24] and rebleeding rates of 60-70%.[25] Primary and secondary prophylaxis for variceal bleeding includes non-selective beta-blockers (NSBBs), endoscopic band ligation or sclerotherapy and transjugular intrahepatic portosystemic shunt (TIPS) placement.[20]

Spontaneous porto-systemic collateral formation and TIPS placement in cirrhosis may contribute to the development of hepatic encephalopathy (HE).[26] This neuropsychiatric syndrome describes a broad spectrum of neuropsychological abnormalities secondary to liver dysfunction and/or portosystemic shunting. Severity of HE is graded according to the West Haven criteria.[27] Patients with subclinical HE have subtle symptoms that may only be detectable using specialized tests. However, it is significantly associated with an increased risk of falling and car accidents.[28,29] Overt HE develops in 30-45% of patients with cirrhosis[30] and may present with symptoms ranging from disorientation and asterixis to coma. Symptoms are generally reversible and associated with a precipitating event, such as dehydration, infection, constipation or gastrointestinal bleeding. One-year mortality rates up to 64% have been reported.[1]

In cACLD, management of cirrhotic patients should focus on the prevention of any complication. Aetiological treatment may reduce portal hypertension and therefore prevent complications.[20] Furthermore, routinely screening for HCC and gastrointestinal varices is essential.[1] Patients with decompensated cirrhosis should be closely followed-up, as they might become liver transplant candidates.

Acute-on-chronic liver failure

Background

Acute decompensation of cirrhosis (AD) is characterised by the occurrence of one or more major complications of underlying liver disease (i.e., ascites, gastrointestinal bleeding, HE or bacterial infections) and is the main cause of hospitalization in cirrhotic patients. Acute-on-chronic liver failure (ACLF) is a distinct entity that occurs in patients with AD and is characterized by organ failures. Several working definitions have been developed for ACLF, which is not advantageous for executing and interpreting research to this complicated syndrome.[31-33] In order to define uniform diagnostic criteria for ACLF, the Acute-on-Chronic Liver Failure in Cirrhosis (CANONIC) study was performed by the European Association for the Study of the Liver- Chronic Liver Failure (EASL-CLIF) consortium.[34] In this study, a large cohort

of cirrhotic patients hospitalized for AD was prospectively followed and ACLF was found to be characterized by the presence of organ failure and a high short-term mortality rate (i.e., ≥15% within 28 days). For the diagnosis of organ failure, the sequential Organ Failure Assessment (SOFA) Scale was modified establishing a new scale specifically adapted for cirrhotic patients: the CLIF-SOFA score. Later, the CLIF Consortium Organ Failure score (CLIF-C OF score)[35] was designed in order to develop a more straightforward and evidence-based organ failure score (table 1). The prognostic significance of cut-off values for the diagnosis of organ failure as applied in the CLIF-SOFA score were confirmed in the CLIF-C OF score. ACLF is graded according to the number of organ failures. ACLF grade 1 consists of 3 groups: 1) single kidney failure, 2) single failure of the liver, coagulation, circulation or respiration with serum creatinine 1.5-1.9 mg/dL and/or grade I-II HE, 3) single cerebral failure with serum creatinine 1.5-1.9 mg/dL. ACLF grade 2 and 3 represent patients with 2 or 3 organ failures, respectively.[34]

Table 1. The CLIF-Organ Failure score system.

Organ/ system	Subscore = 1	Subscore = 2	Subscore = 3
Liver	Bilirubin <6 mg/dl	Bilirubin ≥6 mg/dl and < 12 mg/dl	Bilirubin ≥12 mg/dl
Kidney	Creatinine <2 mg/dl	Creatinine ≥2 mg/dl and <3.5 mg/dl	Creatinine ≥3.5 mg/dl or renal replacement
Brain (West-Haven grade for HE*)	Grade 0	Grade 1-2	Grade 3-4**
Coagulation	INR <2.0	INR ≥2.0 and <2.5	INR ≥2.5
Circulatory	MAP ≥70 mmHg	MAP <70 mmHg	Use of vasopressors
Respiratory PaO_2/FiO_2 or SpO_2/FiO_2	 >300 or >357	 ≤300 and >200 or >214 and ≤357	 ≤200*** or ≤214***

Adapted from Jalan R et al.[35] Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure.

The shaded area describes criteria for diagnosing organ failures.
*HE, hepatic encephalopathy; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; SpO_2 pulse oximetric saturation.
**Patients submitted to Mechanical Ventilation (MV) due to HE and not due to a respiratory failure were considered as presenting a cerebral failure (cerebral subscore=3)
***Other patients enrolled in the study with MV were considered as presenting a respiratory failure (respiratory subscore=3)

Clinical presentation

In the CANONIC study[34], patients with ACLF were found to be significantly younger than those without ACLF and main aetiologies of cirrhosis were alcohol abuse (60%) and hepatitis C (13%). The most common type of organ failure in ACLF was renal failure (56%), followed by

liver, coagulation, cerebral, circulatory and respiratory failure (44%, 28%, 24%, 17% and 9%, respectively). Patients without previous episodes of AD developed more severe grades of ACLF than patients with previous AD and had higher 28-day mortality rates (42% vs. 29%). In 43.6% of patients with ACLF, no precipitating event was identifiable. The most prevalent types of precipitating events for ACLF development were bacterial infections and active alcoholism (39% and 23%, respectively). However, mortality was independent of the presence and type of precipitating events. Interestingly, gastrointestinal bleeding appeared not to be a precipitating factor for ACLF.

Pathogenesis

The pathogenesis of ACLF is complex and is not yet completely understood. Traditionally, the 'peripheral arterial vasodilation hypothesis' was proposed to explain the pathogenesis of organ failure in ACLF.[36] This hypothesis is based on the activation of vasoconstrictor systems as an adaptive response to a decreased effective circulating blood volume in cirrhotic patients with portal hypertension and a hyperdynamic circulation, as outlined above. However, in the light of new knowledge, the peripheral arterial vasodilation hypothesis is thought not to completely cover the mechanism of ACLF development. Other concepts, especially systemic inflammation, are thought to be essential events preceding ACLF development.[36] This new course in approaching this complex syndrome, is referred to as the 'systemic inflammation hypothesis'. Recent studies revealed that markers reflecting systemic inflammation, such as white blood cell count C-reactive protein (CRP), pro-inflammatory cytokines and markers of macrophage activation are increased in cirrhosis and ACLF and the degree of systemic inflammation increases along with the severity of the underlying liver disease and ACLF grade.[34,37] The key concept of chronic systemic inflammation in advanced cirrhosis is bacterial translocation across the intestinal barrier that promotes the release of pathogen-associated molecular patterns (PAMPs).[38,39] PAMPS engagement to pattern recognition receptors may result in the release of pro-inflammatory cytokines leading to systemic inflammation. It is proposed that an acute increase in systemic inflammation following a precipitating event (i.e., active alcoholism or a bacterial infection) is the primary event in the onset of ACLF, and this may result in cardiovascular dysfunction, organ hypoperfusion and organ inflammation.[36]

Prognosis and prognostic biomarkers

Compensated and decompensated liver cirrhosis

Prognosis in liver cirrhosis is depending on the specific disease stage of the individual patient. Survival of patients with compensated cirrhosis is significantly better than that of patients in the decompensated stage (median survival time: >12 years and approximately 2 years, respectively).[4] Transition from a compensated to decompensated stage occurs at a

rate of 5-7% per year. Over the past decades, various studies have been performed in order to explore predictors of death in cirrhosis. Currently, the Child-Pugh (CP)[40] score and the Model of End-stage Liver Disease (MELD)[41] score are the most commonly used prognostic scores in clinical practice. The CP score includes the parameters albumin, bilirubin, ascites, HE and prothrombin time. This score is a significant predictor of death irrespective of the stage of cirrhosis. In the compensated stage, markers reflecting portal hypertension, such as the HVPG, presence of gastrointestinal varices, splenomegaly and platelet count, are particularly found to be significant predictors of death.[4] In the decompensated phase, factors as gastrointestinal bleeding and HCC become more predictive for survival. Also markers that reflect deterioration of renal function, such as creatinine, are powerful prognostic markers in the setting of decompensated cirrhosis. The MELD score was initially designed as a prognostic scoring system in patients undergoing a TIPS procedure and includes creatinine, bilirubin and the International Normalized Ratio (INR). This score is currently widely used as an organ allocation tool in the setting of liver transplantation. The MELD score is found to be a reliable short-term predictor of death in numerous studies, especially in more advanced stages of cirrhosis. The MELD and CP score characterize the severity of the underlying liver disease and kidney funcion, but fall short on assessing the severity of portal hypertension and associated circulatory dysfunction. Therefore, the MELD-sodium (MELD-Na) score has been proposed as a tool for organ allocation.[42] In this score, serum sodium is accounting for hemodynamic deregulations associated with end-stage cirrhosis. Incorporation of serum sodium in the MELD score has been shown to improve its prognostic accuracy. However, a limitation of the MELD-Na score is that marked changes in serum sodium may be influenced by numerous (exogeneous) factors, such as administration of diuretics and hypotonic fluids. Moreover, the CP and MELD(-Na) score do not account for events that are strongly involved in the onset of extra-hepatic organ failure, such as systemic inflammation and endothelial dysfunction. Therefore, several studies have explored potential biomarkers accounting for these events that may improve the prognostic accuracy of liver specific scoring systems. Factors such as vitamin D[43-45], C-reactive protein (CRP)[46], high-sensitivity troponin T, soluble urokinase-type plasminogen activator receptor[47], von Willebrand antigen[48-50] and serum-free cortisol[51] seem to provide important prognostic information.

Acute-on-chronic liver failure

Prognosis in ACLF is associated with the initial grade of ACLF. In the CANONIC study, 28-day and 90-day mortality rates were 22% and 41% respectively in ACLF grade 1, 32% and 55% in ACLF grade 2, and 73% and 78% in ALCF grade 3.[34] Although the grade at diagnosis of ACLF correlated with prognosis, the clinical course of ACLF during hospitalization (i.e., improvement, resolution or worsening) was shown to be the most powerful prognostic factor of short-term mortality. Patients achieving resolution of ACLF had similar 28-day survival

rates as patients with AD without ACLF. In addition, the disease course over time was found to importantly impact on prognosis. Assessment of the ACLF grade at day 3-7 after diagnosis was found to more accurately predict survival than assessment at diagnosis.[52]
Many previous studies investigating potential prognostic markers in cirrhotic patients with organ failure and ACLF have pointed towards the importance of the presence of organ failure in determining prognosis in cirrhosis. Factors accounting for systemic inflammation, such as macrophage activation marker[53], neutrophil-lymphocyte ratio[54] and neutrophil gelatinase-associated lipocalin[55], were in particular found to be of importance in assessment of prognosis in ACLF. Also markers reflecting hemodynamic dysfunction seem to contain important prognostic information: the HVPG correlated with markers of inflammatory response, norepinephrine levels, creatinine levels and severity of HE in alcohol-related ACLF.[56] Further research is needed in order to identify the interrelationship of the events involved in the pathogenesis of ACLF and to create prognostic scoring systems that are predictive for ACLF development which can be applied in clinical practice.

OUTLINE AND AIM OF THE STUDIES DESCRIBED IN THIS THESIS

Advanced liver cirrhosis is associated with systemic hemodynamic derangement leading to the development of severe complications associated with increased mortality. Research on arginine vasopressin (AVP), a key-regulator in hemodynamic homeostasis, in cirrhosis has been hampered by the difficulty of measuring AVP levels accurately. Copeptin is a surrogate marker of AVP and could have a role as a biomarker of prognosis in cirrhosis as it may reflect circulatory dysfunction. In **chapter 2**, we assessed the prognostic value of copeptin in cirrhotic and controls rats and in cirrhotic patients registered at the waiting list for liver transplantation. The animal model provided the opportunity to test the ability of copeptin as a biomarker in cirrhosis without interference of therapeutic interventions or the presence of renal dysfunction. Subsequently, we investigated copeptin as a prognostic biomarker in a larger and more heterogeneous population of cirrhotic patients derived from two European tertiary referral centres, as described in **chapter 3**. The results of a prospectively conducted study assessing copeptin as a prognostic biomarker of disease progression and acute decompensation of cirrhosis are presented in **chapter 4**. Finally, we investigated the prognostic potential of copeptin in cirrhotic patients with AD and ACLF (**chapter 5**).

Systemic hemodynamic dysfunction and the activation of endogenous vasoconstrictor systems are thought to be involved in the pathogenesis of ACLF. AVP plays a prominent role in the cardiovascular system and exerts its vasoconstrictive effects through the V1a receptor (V1aR).[57] Considering the important role of V1aR in regulating vascular tone, we hypothesized

that heterogeneity in V1aR may affect the risk of developing renal and circulatory failure in cirrhotic patients. Aim of the study described in **chapter 6** was to investigate whether genetic variation of V1aR is associated with the presence of circulatory failure, renal failure and outcome in cirrhotic patients with AD or ACLF.
Primary prophylaxis of variceal bleeding in cirrhotic patients currently consists of non-selective beta-blocker therapy to the maximum tolerated dose monitored by the reduction of the heart frequency.[20] However, reduction of the heart frequency and reduction in portal venous pressure do not correlate well.[58] A sufficient decrease in portal pressure to pharmacological prophylaxis is associated with a significant decreased bleeding risk. Clinical application of hemodynamic response monitoring using HVPG measurements is still under debate. In order to clarify some of the disputed aspects, a meta-analysis assessing the predictive value of hemodynamic monitoring using HVPG in primary prophylaxis for variceal bleeding in cirrhosis was performed. The results are presented in **chapter 7.**

Hepatic encephalopathy (HE), a complication of advanced liver cirrhosis, is associated with high recurrence and mortality rates.[59-61] There is increasing evidence that the MELD score underestimates the risk of mortality in patients with HE, who may therefore not receive liver transplantation in a timely manner in regions where the MELD score is used as an organ allocation system.[62-64] We assessed the impact of HE on mortality in cirrhotic patients awaiting liver transplantation using a propensity score analysis. Furthermore, two independent cohorts were used in order to validate our findings. The results of this study are outlined in **chapter 8**.

Chapter 9 summarizes the results of the studies described in this thesis and the findings of the studies are discussed in a broader perspective. Finally, a summary of the thesis in Dutch is given in **chapter 10**.

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