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

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General discussion and future perspectives

Hemodynamic derangement and prognosis in cirrhosis: the case of copeptin

Portal hypertension in cirrhosis is associated with the presence of abnormalities in the hepato-splanchnic circulation.[1] In advanced stages of cirrhosis, a marked reduction in peripheral vascular resistance leads to the activation of counter-regulatory systems, such as the non-osmotic release of arginine vasopressin (AVP). Activation of these vasoconstrictor systems helps to keep an adequate perfusion pressure, but has negative effects on peripheral organ perfusion and may lead to the development of ascites, hyponatremia and, ultimately, renal and multi-organ failure. Biomarkers reflecting the degree of hemodynamic dysfunction may therefore provide relevant prognostic information.

The AVP precursor peptide, pre-pro-vasopressin, consists of a signal peptide, vasopressin, neurophysin II and copeptin and is synthesized in the hypothalamus.[2] After processing to their active form, AVP, neurophysin II and copeptin are stored in the neurohypophysis and are simultaneously released into the bloodstream upon stimuli such as hypotension, hyperosmolarity and stress. AVP exerts its vasoconstrictive, adrenocorticotropic and antidiuretic effect via the V1a, V1b and V2 receptors, respectively. AVP may be interesting as a biomarker in various homeostasis-related conditions. However, AVP is difficult to measure as it is unstable in plasma and serum, has a short half-life (about 16-20 minutes) and is bound to platelets in the circulation. It is therefore not useful as a biomarker in clinical practice.[3] The 39-amino acid glycopeptide copeptin was first described by Holwerda et al. in 1972.[4] It has been suggested that copeptin plays an important role in the correct structural formation of the AVP precursor.[5] Once released into the bloodstream, however, the physiological function of copeptin remains unknown. Since the introduction of a new copeptin assay by Morgenthaler et al.[6], copeptin has been studied as a potential biomarker in various diseases and conditions. The advantages of measuring copeptin over AVP are that it is a more stable molecule (half-life of approximately 7-14 days) and that it is not bound to platelets. Moreover, only small amounts of plasma or serum are needed for measurement of copeptin concentration (50 µL), results can be obtained fast (<3 hours), no pre-analytical procedures are needed and the sensitivity is relatively high (detection limit of 1.7 pmol/L).[7] We explored the prognostic value of copeptin, as a surrogate marker of AVP, in several study populations with different stages of cirrhosis, as described in **chapters 2 till 4**.

A proof of principle study was performed by assessing serum copeptin levels in both cirrhotic rats and humans (**chapter 2**). We found that copeptin was significantly increased in cirrhotic rats as compared to control rats. The median serum copeptin concentration was also found to be significantly increased in cirrhotic humans as compared to that of healthy individuals (i.e., <5 pmol/L).[7,8] The animal study provided us with relevant information on serum copeptin levels, because the animals represented a homogenous cirrhotic population, with comparable

stages of cirrhosis and without presence of exogenous factors influencing AVP release or mean arterial blood pressure (MAP), such as age, body position, methods of measurement and use of medication. A significant negative correlation between serum copeptin and MAP was found in the animal study. Moreover, in the relatively small cohort of cirrhotic humans included in this first study, serum copeptin concentration was associated with outcome, independently of the Model for End-stage Liver Disease (MELD) and MELD-sodium (MELD-Na) score. Subsequently, we studied the prognostic value of serum copeptin on transplant-free survival in a larger and more heterogeneous population of hospitalized cirrhotic patients with either compensated or (acute) decompensated liver disease, as described in **chapter 3**. In consistence with the results of the human study in **chapter 2**, serum copeptin at time of hospital admission predicted mortality, independently of the MELD and MELD-Na score. In addition, copeptin was found to be an independent predictor of transplant-free survival in multivariate regression models adjusting for well-known prognostic factors, such as the Child-Pugh (CP) score, C-reactive protein (CRP), MAP and presence of ascites. Robustness of the predictive ability of serum copeptin for transplant-free mortality was shown by performing sensitivity survival analyses in subgroups of patients without renal failure, ascites and severe infections. The results of a prospectively conducted study are described in **chapter 4**, showing that plasma copeptin concentration independently predicts the development of cirrhosis-related complications and mortality within 3 months of hospitalization. This is a relevant finding, as it reveals the potential ability of plasma copeptin to identify cirrhotic patients who are at a higher risk of developing complications of chronic liver disease and mortality on a short term. These patients may require more intensive surveillance and treatment. Moreover, plasma copeptin concentration showed significant positive correlations with MELD score, AVP, markers of endogenous vasoconstrictor systems and renal function parameters. This finding supports our hypothesis that copeptin reflects hemodynamic dysfunction and may therefore be an interesting biomarker in cirrhosis. Altogether, **chapters 2-4** reveal that copeptin, as a biomarker of hemodynamic dysfunction, provides prognostic information independently of widely implemented prognostic scoring systems, such as the CP and MELD score.

Research on copeptin has been rapidly increasing over the past years. Copeptin is explored as a biomarker in a wide variety of diseases and conditions, associated with changes in fluid status[9-11], electrolyte (especially sodium) imbalance[12,13], physiological stress[14], as well as other acute and chronic diseases, including heart failure[15,16], myocardial infarction[17-19], diabetes mellitus[20-22], and sepsis[23-25]. The utility of copeptin in clinical practice has been thoroughly discussed for these conditions. Especially in diabetes insipidus, copeptin seems to have an established role in the differential diagnosis. In other conditions, promising data are available on the utility of copeptin as a biomarker, but further research is needed before it can be implemented into current clinical algorithms. In the setting of liver cirrhosis, some important

remaining issues need to be clarified in order to assess its utility as a biomarker. Firstly, it is currently not entirely elucidated how copeptin is cleared from the body. In a recent study, regional sampling of plasma copeptin revealed that copeptin is, at least partly, cleared by the kidneys in patients with left ventricular dysfunction undergoing elective cardiac catheterisation.[26] In consistence, Balanescu and colleagues detected copeptin degradation fragments in the urine.[27] As a consequence, elevated serum copeptin levels are likely to be causally related to renal function decline, which is supported by several studies reporting significant negative correlations between serum copeptin and estimated glomerular filtration rate (eGFR).[28-30] It is well known that renal dysfunction may occur in the course of cirrhosis, but the influence of renal dysfunction on serum copeptin concentration in this specific population and to what extent this influences the prognostic significance of copeptin concentration is yet unknown. Secondly, copeptin is used as a marker of the AVP system, but it should be noted that copeptin is a much larger molecule than AVP (42 vs. 9 amino acids). Therefore, their clearance rates are likely to be different. However, clearance rate of copeptin has not yet been studied. Although serum copeptin and vasopressin do strongly correlate, even in different osmolar states[27], relatively high serum copeptin levels with respect to AVP were found in patients with chronic kidney disease.[31] This finding suggests a more pronounced decrease in clearance rate of copeptin as compared to AVP when GFR decreases. Recently, high copeptin levels have been reported to be associated with the development of new-onset chronic kidney disease and a faster decline in eGFR over time in a general population.[29,30] These findings suggest that increased copeptin levels are not only explained by decreased clearance rates, but increased serum copeptin may also precede deterioration of renal function. Further studies are required to explore the relationship between renal function and serum copeptin levels in specific patient populations. In the setting of cirrhosis, it is particularly important to investigate the effect of diuretics and vasoactive therapy on serum copeptin levels. Finally, intrinsic biological activity of copeptin cannot be excluded.

In conclusion, copeptin shows potential as a robust biomarker in clinical practice for a wide variety of diseases and conditions. In the setting of liver cirrhosis, copeptin seems to be a promising prognostic biomarker, but additional research is needed to further explore its physiological function, metabolism and factors influencing the concentration and clearance of copeptin, before implementation in routine clinical practice could be justified.

Acute-on-chronic liver failure: a syndrome characterized by organ failure and high short-term mortality

Traditionally, the presence of extra-hepatic organ failure and a systemic inflammatory response, have been considered to be important factors influencing the clinical course and prognosis in acute-on-chronic liver failure (ACLF).[32] However, there was no uniform definition for this life-threatening syndrome and this hampered also the development of accurate prognostic scoring systems. Available prognostic scores for cirrhosis, such as the MELD and CP score, do not include variables concerning extra-hepatic organ failure and systemic inflammation and therefore do not adequately reflect prognosis in ACLF. One of the main goals of the recent prospective, multicentre Acute-on-Chronic Liver Failure in Cirrhosis (CANONIC) study[33], was to define clear diagnostic criteria for ACLF. This study revealed that ACLF is a syndrome distinct from acute decompensation of cirrhosis (AD), as it was associated with (multi-) organ failure and very high short-term mortality rates. ACLF was classified into 3 grades according to the presence and number of organ failures using a modified Sequential Organ Failure Assessment (SOFA) score: the CLIF-SOFA score. Prognosis was found to be highly dependent on the grade of ACLF. An important study performed in CANONIC population by Gustot and colleagues[34], revealed that ACLF is a very dynamic syndrome, which resolves or improves in up to 50% of patients. Prognosis was found to correlate better with clinical disease course than with the initial ACLF grade at time of hospital admission. They found that the final ACLF grade was obtained within 7 days of initial presentation in the vast majority of patients. They also showed that prognosis can therefore be more accurately assessed by the ACLF grade in the first week after ACLF development as compared to the ACLF grade at initial presentation. Reliable biomarkers possessing the ability to predict the early course of the disease are needed to discriminate between patients who need early specific treatment intervention, patients who may benefit from early liver transplantation and patients in whom treatment can be terminated due to dismal prognosis.

Another objective of the CANONIC study was to develop and validate novel prognostic scoring systems for patients with AD and ACLF. These prognostic models were developed using clinical and biochemical factors that were independently associated with mortality. For ACLF patients, the CLIF Consortium ACLF (CLIF-C ACLF) score was developed.[35] This score incorporates the CLIF-C Organ Failure (CLIF-C OF) score (a simplification of the CLIF-SOFA score), age and log-transformed white blood cell count. The CLIF Consortium AD (CLIF-C AD) score was designed to assess prognosis of hospitalized cirrhotic patients without ACLF.[36] Both scores were found to have a better prognostic accuracy than the CP and MELD score in these specific patient populations. The development of these CLIF-scores has significantly improved the ability in predicting 28- and 90-day mortality in critically ill cirrhotic patients and their accuracy extents to 79%. Still, there is a need for additional prognostic biomarkers,

preferably factors that are involved in the pathophysiology of the disease. Since publication of the CANONIC study, numerous studies have been performed in order to explore prognostic biomarkers in ACLF. These biomarkers can basically be classified into the following categories: oxidative stress (C-reactive protein[37], ischemia-modified albumin[38], nonmercaptalbumin 1 and 2[39], S100A12 and sRAGE[40], HMGB1[41]), immune dysfunction (MERTK+ cell expression[42] and neutrophil gelatinase-associated lipocalin[43]), cell death (M30/M65 antigen ratio[44,45]), dysbiosis[46] and hemodynamic dysfunction (HVPG[47], hyponatremia[48], von Willebrand factor[49] and copeptin[50]). These markers were shown to provide relevant prognostic information in the setting of ACLF. Further studies are needed for validation of these markers in large, heterogeneous ACLF populations and to assess their utility in prognostic scoring systems that best predict the risk of a severe early disease course and response to early intervention.

In **chapter 5** of this thesis, the results are described of a study assessing serum copeptin as a biomarker of circulatory dysfunction and outcome in patients admitted for AD. 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 is thought to play a role in the pathogenesis of ACLF.[32] Therefore, we hypothesized that biomarkers reflecting the degree of activation of vasoconstrictor systems would be interesting as prognostic biomarkers in AD and ACLF. Serum copeptin was found to be significantly elevated in patients with ACLF as compared to those with 'mere' AD. Remarkably, serum copeptin did not consistently increase through ACLF grade I-III. This finding suggests that although ACLF develops in the setting of hemodynamic dysfunction, it may not be associated with the severity of ACLF. High serum copeptin at hospital admission was found to be associated with increased mortality in cirrhotic patients admitted for AD, independently of the MELD, MELD-Na and CLIF-C OF score. In addition, copeptin was found to provide additional prognostic information to the CLIF-C OF score.

As previously described, AVP exerts its peripheral effects through three different receptors: V1a, V1b and V2. AVP induces vasoconstriction by binding to V1a receptor (V1aR).[2] We performed a study on the association between six single nucleotide polymorphisms (SNPs) of the V1aR gene and the development of organ failure in 826 patients admitted for AD of cirrhosis or ACLF, as described in **chapter 6**. As the activation of vasoconstrictor systems is thought to contribute to the pathogenesis of organ failure in ACLF, we hypothesized that heterogeneity in V1aR may affect the risk of developing renal and circulatory failure in cirrhosis. Genetic variation in the vasopressin 1a receptor was found not to be associated with circulatory or renal failure. Instead, two genetic variants of V1aR were found to be weakly, but significantly associated with coagulation failure (defined as an INR ≥2.5 according to the CLIF-C OF score). V1aR is involved in the coagulation cascade by stimulating platelet formation

and aggregation, but this does not explain the association found between the two SNPs and an INR ≥2.5 as the INR does not represent thrombocyte function. Other simple markers of coagulation function (such as prothrombin time, activated partial thromboplastin time and platelet count) did not significantly differ between patients with or without a mutation in V1aR. Based on the present study, it is uncertain whether the genetic V1aR association with coagulation failure in cirrhosis is a clinically relevant or an incidental finding.

Deterioration of systemic hemodynamic dysfunction in cirrhosis was thought to play an important role in the pathogenesis of ACLF.[32] However, in the light of new knowledge, it is now thought that the presence of systemic inflammation in cirrhosis is the key event in ACLF development.[51] This hypothesis proposes that ACLF develops as a result of aggravation of chronic systemic inflammation and associated systemic circulatory dysfunction already present in acute decompensation of cirrhosis. This successively results in cardiovascular dysfunction, organ hypoperfusion and inflammation. Increased bacterial translocation across the intestinal barrier in cirrhosis promotes the release of pathogen-associated molecular patterns (PAMPs), resulting in the release of pro-inflammatory cytokines leading to systemic inflammation.[52,53] Also the release of damage-associated molecular patterns (DAMPs)[54] by dying cells during acute liver injury may contribute to chronic systemic inflammation in cirrhosis. According to the hypothesis, ACLF would thus be the result of an acute increase in systemic inflammation precipitated by events such as active alcohol abuse or a bacterial infection. Next to an acute increase in systemic inflammation, direct tissue damage caused by bacterial products or processes leading to a decrease in tolerance to inflammation may also precipitate ACLF.[51]

This 'systemic inflammation hypothesis' was tested in a recently published study by Clària et al.[55] in a large population of patients with decompensated cirrhosis and ACLF. The presence of systemic inflammation was assessed by measuring 29 cytokines and oxidized albumin. The presence of systemic circulatory dysfunction was estimated by plasma renin and copeptin concentrations. In consistence with our data presented in **chapter 5**, plasma copeptin levels were found to be significantly higher in patients with ACLF as compared to those without. In addition, plasma copeptin levels were markedly higher in patients with ACLF and renal failure than in ACLF patients without renal failure. These results indicate that elevated plasma copeptin levels may not only reflect an increased release of AVP by the posterior pituitary, but also a decreased clearance rate of copeptin in patients with ACLF and renal failure. Copeptin may therefore be less suitable as a marker of hemodynamic dysfunction in this specific subgroup. In this context, plasma renin may be a more representative biomarker, as no difference in its plasma concentration was observed between ACLF patients with or without renal failure. This again underlines the need for better exploring the metabolism of copeptin. Furthermore,

it was confirmed that plasma copeptin concentration is not related to ACLF severity. In contrast, markers of systemic inflammation were found to be strongly associated with the severity of ACLF and were also more strongly associated with the clinical course of ACLF than markers of systemic hemodynamic dysfunction. It was shown that acute decompensation of cirrhosis is associated with very high plasma levels of cytokines and oxidized albumin and that ACLF develops when there is a further increase in these inflammatory markers. Based on these findings, Clària et al. conclude that their data supports the 'systemic inflammation hypothesis'. Nevertheless, it was stated that both non-hemodynamic and hemodynamic mechanisms are important in the pathogenesis of ACLF. This study provides us with very valuable information on the mechanisms of ACLF development. However, there are still a lot of questions that remain open. Due to the fact that approximately half of the patients already had ACLF at time of study enrolment, and the small number of patients who developed ACLF during follow-up, the critical time frame prior to ACLF development could not be studied. Therefore, the exact interrelationship between pathophysiological events that may precede the development of ACLF has not been fully discovered yet. This information is essential for developing preventive treatment strategies. Future prospective trials should aim at further exploration of the pathophysiological mechanisms that lead to the development of ACLF. This knowledge should be used to design prognostic scoring systems that can be used for risk assessment of ACLF development in hospitalized cirrhotic patients and to identify targets for causal treatment.

Risk stratification and treatment for portal hypertension

Diagnosis and surveillance

Assessment of the presence of clinically significant portal hypertension (CSPH; defined as a hepatic venous pressure gradient (HVPG) >10 mmHg) is essential in the management of cirrhotic patients. By definition, this condition is associated with a risk of gastro-oesophageal varices formation.[56] Acute bleeding from these varices is associated with a 6 week mortality rate of 15-20%.[57-59] Since 1986, a series of consensus meetings have been organised in order to develop definitions of key events in portal hypertension and variceal bleeding, to review the existing evidence on the pathophysiology, diagnosis and therapeutic options, and to formulate recommendations for management of treatment and conduct of clinical trials. According to the most recent consensus meeting ('Baveno VI')[56], HVPG measurement and endoscopic screening remain the reference "golden" standards to detect presence of CSPH and oesophageal varices. Patients with chronic liver disease who are at risk of developing CSPH are referred to as having so-called 'compensated advanced chronic liver disease' (cACLD). Several non-invasive methods have been proposed for detecting cACLD, such as elastographic techniques, duplex doppler sonography, magnetic resonance elastography and

combinations of laboratory tests.[59] In the Baveno VI consensus[56], it is stated that the risk of having varices requiring treatment in patients with a liver stiffness <20 kPa and platelet count of >150.000, is so low that endoscopic screening can safely be avoided. These patients can be followed up by yearly repetition of platelet count and transient elastography (TE), which is the method of choice to assess the degree of liver stiffness. In patients with cACLD and viral-related cirrhosis, TE is found to be useful in diagnosing CSPH. In other aetiologies, the diagnostic value of TE needs to be ascertained. The Baveno VI consensus states that there are no sufficient diagnostic non-invasive tools, besides TE, in the setting of portal hypertension, because of the lack of sufficient data. Further research is needed to improve non-invasive diagnosis of CSPH and varices. Availability of accurate non-invasive tools would be desirable instead of invasive methods, such as upper endoscopy and HVPG measurements.

Hemodynamic monitoring of treatment response to non-selective beta-blockers
In order to identify patients with an increased risk of variceal bleeding, we assessed the ability of monitoring the efficacy of primary prophylactic therapy with non-selective beta-blockers (NSBBs) by repeated HVPG measurements, as described in **chapter 7**. Although HVPG measurements are currently acknowledged to offer additional relevant information on treatment response and associated bleeding risk and prognosis, evidence for the routine use of HVPG measurements in clinical practice is low.[56] The meta-analysis performed included 6 prospective clinical trials showing the ability of repeated HVPG measurements to predict variceal bleeding risk by monitoring the efficacy of NSBB therapy. It was found that achieving a hemodynamic response to NSBB therapy (i.e., HVPG reduction <12 mmHg or >10-20% from baseline) is associated with a significant lower risk of a first variceal bleeding episode as compared to a non-response. However, it needs to be considered that there was heterogeneity in the included studies with respect to the time-interval between the HVPG measurements and follow-up time. More randomized controlled trials are needed in order to provide satisfactory evidence for recommendations on routinely monitoring pharmacological treatment response in future guidelines concerning primary prophylaxis for variceal bleeding. These studies should also take into account cost-effectiveness.

Before HVPG response-based treatment can be implemented in routine clinical practice, a few more remaining issues need to be considered. Firstly, it has not yet been demonstrated which alternative therapies would be most effective for hemodynamic non-responders. Several add-on therapies to improve NSBB-response rates have been studied so far, such as vasodilators, diuretics, statins and organic nitrates. However, currently available data do not seem to justify implementation of these additional therapies in standard clinical practice at this stage. There are data suggesting that carvedilol is more effective in decreasing HVPG than traditional NSBBs (i.e., propranolol and nadolol)[61] and switching to carvedilol in non-responders to propranolol

may result in achieving a hemodynamic response in some patients.[62] In addition to non-selective beta-blockade, carvedilol has mild anti-alpha 1 adrenergic activity, which results in decreased hepatic vascular tone and thus in a further reduction of portal pressure. This combined effect explains why carvedilol may be more effective in decreasing portal pressure than traditional NSBBs. However, it carries the potential risk of causing hypotension, which is undesirable in patients with decompensated cirrhosis. Long-term randomized controlled trials are needed in order to compare the occurrence of adverse events between carvedilol and traditional NSBBs and the effectiveness of both drugs in unselected patients.

It has recently been found that expression levels of vasoactive proteins in antrum mucosa (i.e., beta-arrestin 2, Ras homolog family member A and Rho-kinase 2) might reflect the hemodynamic response to NSBBs and their long-term protective effect.[63] This finding shows potential for a relatively simple approach using upper-endoscopic biopsies to facilitate the decision to treat with NSBB if varices are present. In this context, it needs to be considered that no trial thus far has demonstrated that selection of patients for NSBB treatment based on hemodynamic response leads to an overall better outcome as compared to unselected treatment of the whole group. Secondly, it has been questioned whether patients qualified as hemodynamic responders at short term will remain good responders on the long-term. Studies providing long-term follow-up with sequential HVPG measurements are limited.[64,65] These studies showed that not all patients remained good responders (i.e., 42-81%). Maintenance of a good hemodynamic response appeared to be associated with a decreased probability of developing cirrhosis-related complications, a decreased need for liver transplantation and significantly improved survival. Worsening HVPG in hemodynamic responders to medical treatment was related to deterioration of liver function.[65]

Summarizing, it has been clearly recognized that HVPG monitoring of hemodynamic response to primary pharmacological prophylaxis provides the clinician with relevant information on variceal bleeding risk, which cannot sufficiently be obtained otherwise. Nevertheless, HVPG measurement is not extensively used in routine clinical practice and data on cost-effectiveness are still lacking. Alternative therapeutic strategies aimed to reduce portal pressure are needed to provide sufficient protection against variceal bleeding in hemodynamic non-responders to NSBBs.

Hepatic encephalopathy in liver transplant candidates

Liver transplantation is currently the only curative treatment option in advanced cirrhosis. However, due to shortage of donors, there is still a significant number of patients that do not receive a liver transplantation in time and die at the waiting list (i.e., 18.4% in 2015 in Europe[66]). Therefore, optimization of organ allocation criteria is constantly under debate. The CP score has been used as an organ allocation tool for many years.[67] This score was initially designed to predict survival in cirrhotic patients undergoing surgery. In the setting of organ allocation, however, this score has several limitations including under- and overestimation of prognosis due to the subjective interpretation of the severity of ascites and hepatic encephalopathy (HE).[68] Currently, the MELD score is widely used in organ allocation. This score was initially designed as a prognostic scoring system in patients undergoing a trans-jugular intrahepatic portosystemic shunt (TIPS) procedure and incorporates laboratory markers of liver and renal function.[69] One of the limitations of the MELD score is that it may not adequately reflect the risks associated with other common manifestations of hepatic decompensation, such as ascites and HE, both of which are well-known for their association with poor survival.

HE is a complex, dynamic syndrome manifesting by a wide spectrum of neuropsychological symptoms ranging from subclinical cognitive alterations to coma. According to the recently published guideline[70], HE should be classified based on the underlying disease, the severity of the manifestations, its time course and the existence of precipitating factors. Patients with fully symptomatic HE are referred to as having overt HE (OHE), whereas subclinical abnormalities are defined as minimal HE (MHE) and covert HE (CHE). Diagnosing OHE is primarily based on clinical observation and examination. The difficulty in diagnosing OHE is to recognize neuropsychiatric symptoms as part of HE, as they may also result from other conditions in this population, such as the use of certain drugs, alcohol abuse, hypoglycaemia and psychiatric disorders. For this reason, patients can only be diagnosed with OHE after excluding other aetiologies. The use of specific quantitative tests is not recommended in clinical practice. In study settings, the West Haven criteria[71] is the golden standard for diagnosing and grading of (O)HE. This tool is, however, subjective and associated with inter-observer variability. Especially in CHE, minor symptoms, such as psychomotor slowing and shortened attention span, can easily be missed. In contrast, asterixis and disorientation have a good inter-observer reliability and are considered reliable diagnostic features of OHE. The presence of MHE and CHE can be tested using several psychometric and neurophysiological tests.[70] Testing for MHE and CHE is important, because these patients are at a higher risk of developing OHE and because it is associated with a poor quality of life. A limitation of the available testing strategies for MHE and CHE is the poor correlation between the different tests, because of the wide range of symptoms that can appear in HE.[72] Moreover, validity and specificity varies widely between the different available tests. Examination and interpretation

of the results should always be performed by a trained examiner, who is well informed about patient's current disease state, medical history and social context.

Several studies have been performed in order to explore the impact of the presence of HE on survival in cirrhosis. Previous studies have reported that the MELD score underestimates prognosis in cirrhotic patients with HE[73-75], who may therefore not undergo liver transplantation in a timely manner. Furthermore, the severity of HE appeared not to be correlated to the MELD score.[73, 76] In addition, the presence of severe HE at time of registration at the waiting list has been found to increase 90-day waitlist mortality, independently of the MELD-score.[77] These findings do suggest that it could be beneficial to incorporate the presence of HE to some extent in prioritizing liver transplant candidates. We performed a retrospective study exploring the impact of HE on mortality at the liver transplant waiting list in a Dutch cohort, as outlined in **chapter 8**. Aim was to assess the prognostic significance of HE, independently of the MELD score and presence of comorbidities related to HE development. In contrast to previous studies on this subject, we used a propensity score analysis to adjust for the presence of confounding factors that may impact on both the risk of HE development and mortality, such as infections, ascites, variceal bleeding or a TIPS procedure. The results of our study confirm that HE is indeed an independent risk factor for mortality in patients awaiting liver transplantation. This was validated in a representative and independent Dutch cohort. In a second validation cohort from Spain, with a different composition of the patient population accompanied by significantly higher transplantation rates due to higher donor availability and a shorter waiting list, no prognostic impact of HE was found. An important limitation of the present and previously published retrospective studies of the impact of HE on survival is the fact that diagnosing and grading of HE is observator-dependent, which may result in misclassification-bias.

Before it would be justifiable to consider implementing HE in the prioritizing criteria for organ allocation, widely applicable diagnostic tests with higher specificity and inter-observer reliability and acceptable costs are needed. So far, a number of (imaging) techniques have been investigated, such as electroencephalography (EEG), positron emission tomography (PET), computed tomography (CT) and magnetic resonance imaging (MRI). Montagnese et al.[78] performed a study in which they assessed the prognostic benefit of the addition of an EEG-based HE index to the MELD score. They found that the addition of an automatically obtained EEG-based index improves the prognostic accuracy of the MELD score. A recent study by Jackson et al.[79] aimed to optimise the diagnostic performance of the EEG in HE by defining new spectral thresholds and found that adoption of these thresholds significantly improves the utility of the EEG for diagnosis of HE. Especially in the setting of MHE, several studies investigated the ability of white matter imaging with MRI to identify MHE among

patients with cirrhosis.[80-82] White matter lesions are thought to be responsible for neurologic abnormalities and brain dysfunction.[83-86] Abnormalities of white matter in the brain, such as low-grade oedema and structural impairments, have been found to be present in patients with MHE and are associated with OHE development.[87-89] To date, MR techniques seem to be the most promising objective tools for diagnosing of HE in clinical practice.[90] To date, however, no specific recommendations on the use of MR techniques can be made, because larger scale studies are needed to validate currently available data. Nevertheless, these data suggest a potential future role for imaging techniques in diagnostic algorithms, particularly in patients with subclinical HE. In these patients, detection of abnormalities with brain imaging techniques may help identify patients who are at risk of developing OHE. Moreover, they may also play a role in grading of HE severity and monitoring of treatment response. In research settings, such objective techniques may help to better investigate the impact of the presence and severity of HE on survival and the need for implementation of HE in the prioritizing criteria for liver transplantation.

GENERAL CONCLUSION

In this thesis, the prognostic significance of several biological markers and clinical conditions in cirrhosis and ACLF has been assessed. A prognostic marker is defined as a biological characteristic that is objectively measured and evaluated to predict the course of a disease or a response to a therapeutic intervention. A biomarker can serve as a prognostic marker and is officially defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention'.[91] The ideal prognostic (bio-)marker should possess a number of qualities: easy to measure, cost-effective, consistent across gender, age and ethnic groups and able to monitor treatment effects and outcome. In addition, the ideal biomarker should have high sensitivity and specificity for a specific disease or condition.

As previously discussed in this summarizing discussion, further research should be performed in order to explore whether the markers assessed in this thesis would match all the above described criteria of an ideal prognostic (bio-)marker. Nevertheless, the studies described provide a basis for the direction of future studies in the field of risk-stratification in cirrhosis. We found a consistent, independent association of copeptin with outcome in various stages of cirrhosis, suggesting that this biomarker of the AVP system and systemic hemodynamic dysfunction may provide valuable prognostic information next to current prognostic scoring systems. In the setting of ACLF, recent studies in this field have found that markers reflecting systemic inflammation are most strongly associated with ACLF occurrence and severity.

Nevertheless, biomarkers assessing circulatory derangement, such as copeptin, could provide valuable information on the interrelationship between systemic inflammation and hemodynamic dysfunction in the period prior to ACLF development in future prospective studies.

Treatment response to primary prophylaxis with NSBB therapy monitored by repeated HVPG measurements was shown to be a strong predictor of a first variceal bleeding episode. At this stage, cost-effectiveness analysis in long-term follow-up studies is needed to prove its clinical utility. Furthermore, future research should focus on alternative and/or add-on therapies for non-responders to NSBB therapy.

Finally, we showed that the presence of HE contains important prognostic information for liver transplant candidates. Further research should focus on exploring and validating objective techniques to diagnose the presence and severity of HE and to confirm its independent negative impact on waitlist-mortality.

In conclusion, risk stratification in cirrhosis is challenging, as it is a very heterogeneous disease affecting multiple organ systems and physiological processes. The ideal prognostic marker does therefore not exist. As long as aetiological treatment and liver transplantation are not available for all patients, the most important challenge still is to identify the best combinations of prognostic markers for the specific stages of cirrhosis and ACLF in order to achieve the most accurate risk stratification and, ideally, to direct therapy.

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