Studies in Advanced Chronic Liver Disease

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Studies in Advanced Chronic Liver Disease

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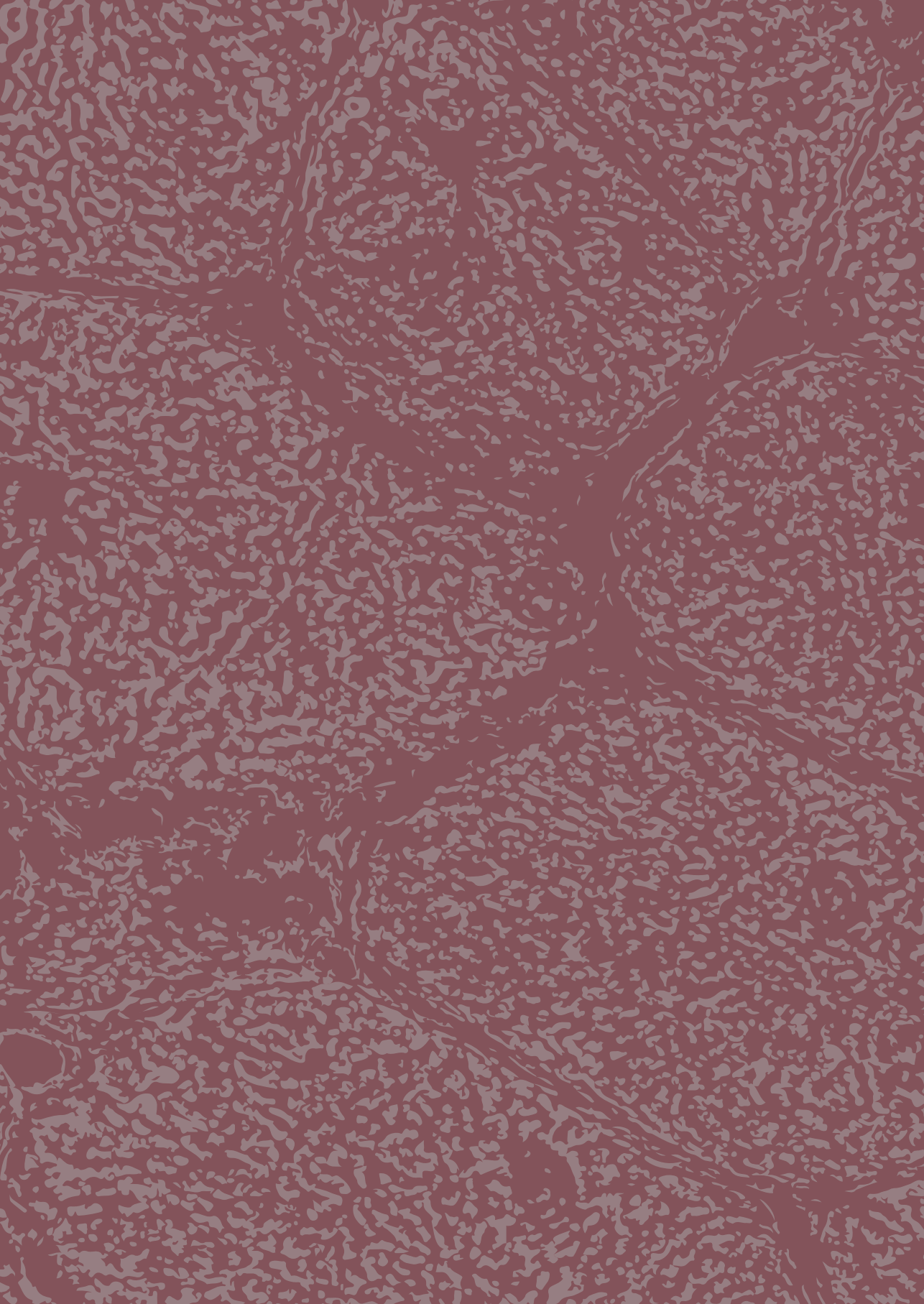
CONTENTS

Appendices



Part I
INTRODUCTION

CHAPTER 1
General introduction and aim

INTRODUCTION

The liver is a unique multifunctional organ, historically known for its exceptional regenerative capacity. The liver is strategically located regarding nutrient processing and detoxification, receiving blood from the portal venous system, which include veins from the intestinal tract, spleen, pancreas, and gall bladder. This allows the liver to function in the storage and metabolism of nutrients, the synthesis of plasma proteins, and the clearance of endogenous and exogenous molecules entering the human body.(1)

In advanced chronic liver disease, characterized by extensive nodular scarring (cirrhosis) and loss of functional cell mass, liver function and the regenerative capacity become progressively impaired. During the natural course several stages can be distinguished. In the first stage – compensated advanced liver disease – patients are clinically often asymptomatic and have an excellent prognosis with a 1-year mortality of 1.0 – 3.4%.(2) Transition into the second stage – decompensated advanced liver disease – occurs reportedly at an annual rate of 20 – 57%.(2) In this stage numerous complications involving all organ systems may develop, the most frequent being ascites, variceal bleeding, hepatic encephalopathy, infections, renal failure, pulmonary hypertension, hepato-pulmonary syndrome, and malnutrition. This thesis focuses on patients with this stage of disease, i.e. decompensated advanced chronic liver disease.

Aetiology

The most common causes of advanced liver disease include excess alcohol intake, viral hepatitis, primary sclerosing cholangitis, primary biliary cholangitis, autoimmune hepatitis, non alcoholic steatohepatitis, and exposure to toxins including medication.(1, 3, 4) Although liver disease may originate from a single cause, the disease is often caused by interacting causes and influenced by coexistent factors. The presence of potential cofactors, such as advanced age, male gender, obesity, high daily alcohol consumption, smoking, altered immunological status and genetic factors, could explain why certain individuals are more at risk to develop advanced chronic liver disease.(1, 5)

Pathophysiology

Many complications of advanced chronic liver disease are due to the development of portal hypertension.(6) Portal hypertension is most often caused by cirrhosis, but can have non-cirrhotic causes such as portal venous thrombosis, congenital liver fibrosis and nodular regenerative hyperplasia, however these aetiologies are outside the scope of this thesis.(7) Portal hypertension is defined by a sustained increase of the pressure in the portal venous system. According to the hemodynamic application of Ohm's law of fluid ($\Delta P = Q \times R$), the venous pressure gradient in the portal system (ΔP) is the result of blood flow volume (Q) multiplied by the resistance opposing this blood flow (R).(8) In advanced liver disease, intrahepatic mechanical and dynamic changes increase blood

flow resistance, resulting in an increased portal venous pressure. Examples of mechanical changes include anatomical distortion of the liver by fibrosis and nodule formation, and vascular occlusion by thrombosis; dynamic changes include an increased hepatic vascular tone and sinusoidal endothelial dysfunction. When portal pressure is increased above a hepatic venous pressure gradient of 10 mmHg, vascular pathways bypassing the liver may develop, a mechanism that can be regarded as a natural adaptation to increased portal resistance and pressure. Part of these portosystemic collateral vessels develop superficially in the gastrointestinal tract, in particular near the gastro-esophageal junction and in the distal rectum, where anatomic communications between the portal and systemic venous circulation are pre-existent or may easily develop, and are called varices.(1, 9, 10) As a response to a diminished blood flow to the liver due to the collateral circulation, endogenous vasodilators (such as nitric oxide and calcitonin gene-related peptide) induce vasodilatation of the splanchnic vasculature. Leakage of these vasodilators to the systemic circulation, due to portosystemic shunting or reduced degradation in hepatocytes, is considered to be the cause of a decrease in systemic vascular resistance, characterized by a reduced effective arterial blood pressure and volume. In response the renin-angiotensin-aldosterone system is activated, resulting initially in an increase in cardiac output, heart rate, and plasma volume, resulting in sodium and water retention. This phenomenon is named 'hyperkinetic or hyperdynamic circulation' and results in an increased splanchnic blood flow contributing and further aggravating portal hypertension in a vicious circle.(9)

Complications of advanced chronic liver disease

Patients with advanced chronic liver disease are susceptible to develop any of the many potential complications, such as ascites, variceal bleeding, hepatic encephalopathy, bacterial infections, jaundice, hepatorenal syndrome, hyponatriaemia, hepatopulmonary syndrome, hepatic hydrothorax, malnutrition and cardiac failure.(1, 11) In most patients multiple complications occur that may manifest simultaneously or subsequently.(12) Further, complications often develop secondary to other complications, e,g, spontaneous bacterial peritonitis, hepatic hydrothorax and umbilical hernias may complicate ascites and variceal hemorrhage may precipitate encephalopathy and renal failure.

Ascites and spontaneous bacterial peritonitis

Ascites, an accumulation of free fluid in the peritoneal cavity, is the most frequent first decompensating event in patients with advanced liver disease. Sinusoidal portal hypertension leads to fluid effusion from the vessels into the peritoneum. Due to the hyperkinetic circulation, splanchnic dilatation and renal sodium and water retention, the intravascular volume is expanded allowing the maintenance of ascites formation.(13) The prognosis of ascites formation is co-dependent on the development of ascites infection, denoted as spontaneous bacterial peritonitis (SBP), by definition an infection developing

in the absence of an intra-abdominal source such as appendicitis or visceral perforation.(14) The most common theory implicates that bacterial colonization of ascites is caused due to gut bacteria migration through the intestinal wall into the lymph system/systemic circulation or secondary translocation from a concomitant infection from extra-intestinal sites (e.g. urogenital or respiratory tract). In addition, in cirrhosis several abnormalities in the systemic immune system have been described, decreasing the opsonisation and killing of translocated bacteria.(14) The prognosis of SBP is poor with a mortality as high as 29% during the first month after hospital admission.(15)

Bacterascites is a different clinical entity than SBP, characterized by the presence of bacteria without a neutrophil reaction.(16) Its clinical significance seems to vary according to the mode of acquirement and the presence of clinical symptoms.(17, 18) Ascites is also associated with other complications, in particular hepatic hydrothorax and inguinal, umbilical and cicatricial hernias.(19, 20)

Development of varices and variceal bleeding

Collateral/variceal formation starts due to increased portal pressure at sites with pre-existing communicative vessels between the portal and systemic circulation.(10) These small calibre vessels are functionally of no or minor importance in healthy conditions, but open up in portal hypertension. Varices are superficially located porto-systemic collaterals that develop mainly in the distal oesophagus, at the gastro-esophageal junction and in the distal rectum. Varices may rupture and cause life-threatening bleeding. Approximately 5% of bleedings originate from varices outside the gastro-oesophageal junction area and these varices are referred to as ectopic.(21) Ectopic varices may develop anywhere along the gastrointestinal tract and around enterocutaneous stomas, but can also be present in the biliary system, pelvic organs, peritoneum and skin. Abdominal and pelvic surgery is a main risk factor for the development of ectopic varices in patients with portal hypertension because postoperative adhesions and the creation of entero- and ureterostomies facilitates the formation of portosystemic collaterals.(21) The risk for rupture of varices is dependent on the size of varices, the severity of liver disease and local abnormalities of the variceal wall known as ''red spots''.(22) The bleeding risk has been reported to range from 5 – 76% per year.(23) Variceal bleeding is associated with an increased mortality up to 20% within 6 weeks.(24)

Hepatic encephalopathy

Hepatic encephalopathy refers to a complex of neuropsychiatric changes in personality, intellectual capacity, cognitive function, and consciousness, due to advanced liver disease.(25) The clinical spectrum varies from non-manifest or very subtle psychological changes that may only become apparent during specific testing, to marked changes in behaviour, lethargy and coma. Patients become more susceptible for hepatic encephalopathy with increasing age. The most accepted theory of pathogenesis implies that impaired

hepatocellular metabolism and porto-systemic shunting cause an increased systemic level of gut-derived toxins effecting brain function.(26) Ammonia has been identified as the most important toxin causing cerebral edema. Hepatic encephalopathy is frequently provoked by causes such as infections, gastrointestinal bleeding, constipation and use of diuretics or psychoactive drugs, and correction of the underlying cause may be curative.(27) However, this complication may also occur spontaneously, i.e. without clear precipitating factors and constitute a chronic condition. Hepatic encephalopathy heralds a poor prognosis, in particular when symptoms occur spontaneously or have a chronic character.(1)

The medical management of hepatic encephalopathy primarily involves the treatment of underlying conditions and correction of precipitating factors, such as gastrointestinal bleeding, dehydration, electrolyte disturbances, infections, obstipation, use of diuretics and sedative drugs. Since many decades lactulose and lactitol are the main drug treatment options. More recently rifaximin is also shown to have important therapeutic potential.(28) This drug is currently used as a second line treatment, in particular when symptoms recur or persist despite lactulose treatment.

Malnutrition

Malnutrition is common in advanced chronic liver disease, affecting more than 60% of patients, and refers in this context to undernutrition.(29, 30) The condition is multifactorial and the main pathophysiologic mechanisms include: inadequate dietary intake (due to loss of appetite or early satiety), impaired digestion and absorption (related to portal hypertension or bacterial overgrowth), metabolic alterations (impaired glucose storage as glycogen, increased lipid turnover), and hypermetabolism due to chronic inflammation.(31)

Malnutrition is diagnosed based on the presence of sarcopenia, characterized by a loss of muscle mass, decreased muscle strength, and reduced physical performance. Although various methodologies exist to assess sarcopenia, detection of muscle mass depletion on CT is currently considered to be the gold standard.(1, 30, 32)

Although malnutrition is a consequence of liver disease and the severity of malnutrition is correlated to the stage of liver disease, malnutrition itself can affect the natural course of liver disease and independently worsen prognosis.(33) Studies have shown that malnutrition is associated with increased waiting list mortality and reduced post-transplant survival and overall survival.(32) Malnutrition is also associated with a higher risk for developing complications such as hepatic encephalopathy and bacterial infections.(29)

Medical treatment

Treatment in advanced liver disease aims to prevent further decompensation and death and is primarily based on treatment of the underlying liver disease. Examples

include alcohol abstention in alcoholic liver cirrhosis, antiviral therapy in viral hepatitis, ursodeoxycholic acid in primary biliary cholangitis, immunosuppression in autoimmune hepatitis, weight loss in non alcoholic steatohepatitis, and ceasing chemical or medication exposure in toxic liver disease. Avoidance of (further) decompensation is often based on treatment of the pathophysiological mechanism: increased portal sinusoidal pressure (transjugular intrahepatic port-systemic shunt placement (TIPS); liver transplantation), collateral formation/bleeding (non-selective beta-blockers; endoscopic variceal band ligation; variceal obliteration with tissue adhesives; balloon-occluded retrograde transvenous obliteration), hyperkinetic circulation (sodium restricted diet; diuretics; terlipressin; somatostatin; albumin), hyperammonemia (lactulose; rifaximin), and bacterial translocation/infection (antibiotic therapy).(1)

However, the abovementioned medical interventions are also accompanied by complications, for example in patients using long-term antibiotic treatment for the secondary prevention of spontaneous bacterial peritonitis an antibiotic-resistant bacterial flora may emerge, and diuretic therapy and TIPS are significant risk factors for hepatic encephalopathy. In this population with patients being in precarious equilibrium, the '*primum non nocere*'-principle (translated from Latin to 'First, do no harm') should be conscientiously kept in mind. Thus, the indication of medical interventions should be carefully weighed against the potential complications of treatment.

Liver transplantation

Liver transplantation is a life-saving, highly invasive procedure for patients with progressive irreversible liver injury.(1, 34, 35) However, liver transplantation is limited by the scarcity of suitable donor organs resulting in a waiting list mortality of approximately 20%.(36)

Patients undergo an extensive liver transplantation screening including evaluation of the liver disease, surgical suitability, anaesthetic suitability, presence of infectious diseases, presence of malignant diseases, mental health condition, and nutritional condition. To this end, the indication and suitability for liver transplantation can be determined, contra-indications for transplantation can be excluded (e.g. uncorrectable cardiopulmonary disease, ongoing extra-hepatic infection, metastatic malignancy, and severe brain damage) and management of the liver disease and complications can be optimized.(34)

Overall 1 and 5 year survival rates after liver transplantation are approximately 90% and 70%, respectively, and are mainly influenced by graft dysfunction, rejection, infection, and co-morbidities.(4)

AIM OF THE STUDIES IN THIS THESIS

In patients with severely advanced liver disease, we studied clinical, diagnostic and therapeutic aspects of frequent complications, including spontaneous bacterial peritonitis, bacterascites, other infections, (ectopic) variceal bleeding, hepatic encephalopathy and

malnutrition, with the general aim to evaluate current and new diagnostic and therapeutic strategies and with the ultimate aim to optimize patient management.

More specifically the highly prevalent problem of infections in advanced liver disease was addressed by studying diagnostic methods, microbiological characteristics and therapeutic aspects of spontaneous bacterial peritonitis and bacterascites, and the impact of infections in patients with most severely advanced disease, i.e. patients listed for liver transplantation.

In addition to studying infectious problems in candidate patients for transplantation, we performed an in depth study of the value of screening colonoscopy, a standard but invasive procedure potentially associated with an increased risk for complications, such as infections, in this vulnerable population. In this population we also studied diagnostic and prognostic aspects of malnutrition, a frequent and important feature of advanced liver disease, and evaluated the validity of a recent international guideline in a well-characterized Rotterdam cohort.

While the efficacy of TIPS has been widely studied in gastro-esophageal variceal bleeding, relatively few and only small studies have been performed in patients with bleeding form ectopic varices. We studied the efficacy and safety of TIPS in ectopic variceal bleeding in a multicentre cohort, in particular to assess potential differences between subtypes of ectopic varices.

Finally, aspects related to the recent introduction of rifaximin to prevent recurrent overt hepatic encephalopathy were studied in a population in our hospital.

In Part II, clinical, diagnostic, microbiological and therapeutic aspects of ascites, ascitic infections, and other common infections complicating end-stage liver disease are considered. **Chapter 2** discusses the current recommended diagnostic approach in patients presenting with ascites and summarizes potential future diagnostic targets. **Chapter 3** reports a prospective study of the diagnostic accuracy of leucocyte esterase reagent strips for detection of spontaneous bacterial peritonitis. In **Chapter 4**, the causative microorganisms of spontaneous bacterial peritonitis over a decade were studied in order to detect potential changes in the causative pathogens and antibiotic sensitivity. **Chapter 5** contains a study on bacterascites, a not infrequent but relatively little studied clinical entity, in particular to assess clinical and microbiological characteristics and put our findings in perspective with current general guidelines. In **Chapter 6**, the results are described of a study examining the frequency and clinical impact of infections in patients listed for liver transplantation.

In Part III of this thesis, findings and implications of diagnostic assessments during liver transplantation screening are discussed. In **Chapters 7 and 8** the yield and safety

of screening colonoscopy in patients evaluated for liver transplantation are reported. The studies report the prevalence of advanced colorectal neoplasms and compare the incidence of clinical events after colonoscopy with the standard risk in patients with advanced chronic liver disease in an effort to make a recommendation to optimize colorectal cancer screening in this population. **Chapter 9** include a study describing the frequency of malnutrition in patients screened for liver transplantation, evaluated by different tools, and show the impact on clinical outcome.

Part IV includes studies evaluating treatment of ectopic variceal bleeding and hepatic encephalopathy. In **Chapter 10** we report the results of a study evaluating the long-term control of bleeding and clinical course in subgroups of patients with ectopic variceal bleeding treated with TIPS. In **Chapter 11**, the addition of rifaximin to lactulose treatment in the secondary prevention of hepatic encephalopathy was evaluated by studying the effect on hospital resource use and safety.

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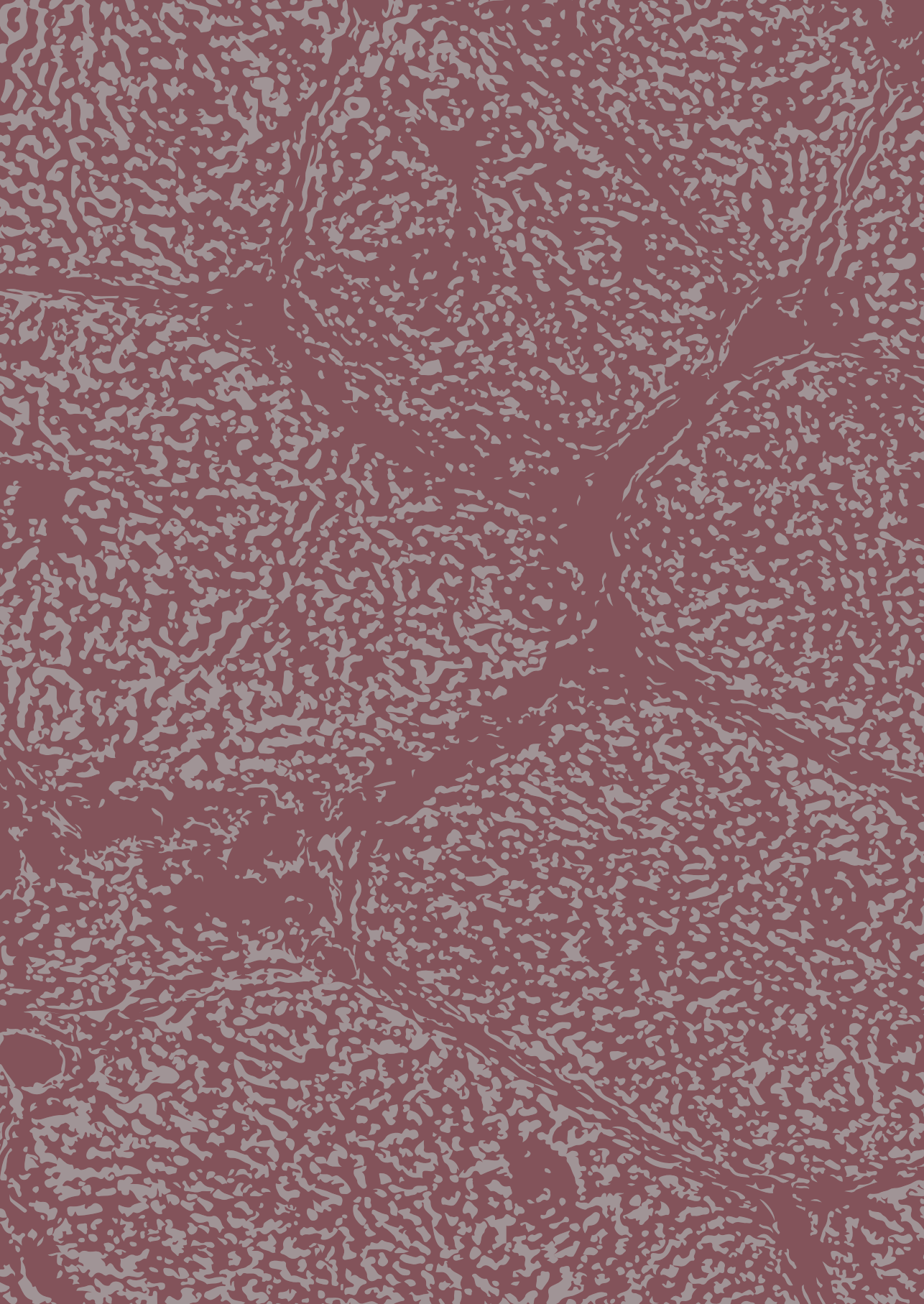

Part II
ASCITES AND INFECTIONS

CHAPTER 2

The diagnostic work-up in patients with ascites: current guidelines and future prospects

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ABSTRACT

Accumulation of fluid in the peritoneal cavity - ascites - is commonly encountered in clinical practice. Ascites can originate from hepatic, malignant, cardiac, renal, and infectious diseases. This review discusses the current recommended diagnostic approach towards the patient with ascites and summarizes future diagnostic targets.

INTRODUCTION

Ascites is a pathologic accumulation of fluid in the peritoneal cavity. It is a symptom of numerous medical conditions and has a broad differential diagnosis.(Table 1) Ascites can be classified by the underlying pathophysiologic mechanism: portal hypertension, peritoneal disease, hypoalbuminaemia and miscellaneous disorders. Liver cirrhosis (75%) is the most common cause in adults in the Western world, followed by malignancy (10%), heart failure (3%), tuberculosis (2%), and pancreatitis (1%).(1) An adequate diagnosis is necessary for successful treatment.

Ascites can be classified as: mild ascites only detectable by ultrasound (grade 1), moderate ascites evident by moderate symmetrical distension of the abdomen (grade 2), and large or gross ascites with marked abdominal distension (grade 3).

Ascites is a common problem and patients present to a broad range of medical specialties. This review aims to provide a comprehensive overview of the current diagnostic approach of ascites and also discusses recent developments in ascites research.

Table 1. Differential diagnosis of ascites.

Portal hypertension
Cirrhosis
Alcoholic hepatitis
Hepatic congestion
Congestive cardiac failure
Constrictive pericarditis
Hepatic venous outflow obstruction (hepatic vein thrombosis, sinusoidal obstruction syndrome)
Portal vein thrombosis
Non-cirrhotic portal hypertension
Malignancy
Peritoneal carcinomatosis
Hepatocellular carcinoma
Mesothelioma
Metastatic liver disease
Other intra-abdominal malignancies
Infectious
Spontaneous bacterial peritonitis
Secondary bacterial peritonitis
Tuberculous peritonitis
Chlamydia

Table 1. Differential diagnosis of ascites. *(continued)*

Miscellaneous
Pancreatitis
Hypoalbuminaemia
Nephrotic syndrome
Lymphatic leakage
Myxedema
Urinary leakage

Diagnosis

History

Patients with ascites should be questioned about a pattern of body weight gain, change in abdominal girth, and ankle oedema. Information about the medical history, medication use, lifestyle, risk factors for liver disease, and infectious disease risk (e.g. migration) are relevant to discover the underlying aetiology.

Physical examination

A screening physical exam should be carried out in every patient, with awareness on signs of liver disease (erythema palmare, spider naevi, splenomegaly), heart failure (peripheral oedema, jugular venous distension, third heart sound, pulmonary rales) and malignancy (lymphadenopathy).(2)

The abdomen should be inspected for the presence of bulging flanks and percussion can reveal flank dullness. Flank dullness is present when approximately 1500 mL of ascites is present. These combined findings have a sensitivity of 75% and a specificity of 57%.(3) Shifting dullness, determined by a 3 cm flank dullness shift when the patient changes between a supine to a lateral decubitus position, has a sensitivity of 69% and a specificity of 69%. Detection of a fluid wave or puddle sign is less reliable.(3,4) Complications accompanying ascites such as umbilical, inguinal and other hernias and pleural fluid (hepatic hydrothorax) are particularly common in cirrhotic patients.

Blood tests

It is recommended to assess serum levels of creatinine, urea, electrolytes, prothrombin time and liver function tests and to order a complete blood cell count.(5)

Abdominal ultrasound

Abdominal ultrasound is the first-line imaging method to confirm the presence and quantity of ascites.(5-7) Additionally ultrasound can provide crucial information about the cause of ascites, detect signs of portal hypertension (splenomegaly and portosystemic collaterals), and offer guidance during paracentesis.

Abdominal paracentesis

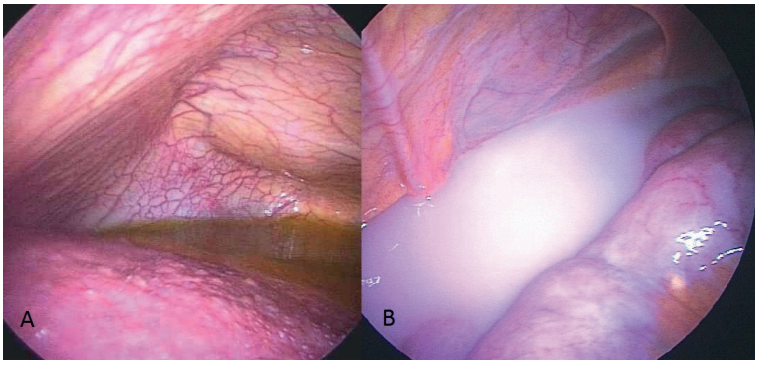
Abdominal paracentesis is the most important step in the diagnostic work-up. It is indicated in every patient with new-onset ascites, patients with known ascites and clinical deterioration or a new presentation at an emergency department. Paracentesis is usually performed in the left lower quadrant, 3 cm cranial and 3 cm medial from the anterior superior iliac spine. Other sites include the right lower quadrant and the midline linea alba between the umbilicus and the pubic bone.(7) Paracentesis should be performed under sterile conditions. Complications occur infrequently and include abdominal wall hematoma (1%), hemoperitoneum (<0.1%), bowel perforation (<0.1%), and infection (<0.1%).(7, 8)

Ascitic fluid analysis

Visual inspection

Visual inspection of the ascitic fluid can show a milky, cloudy, bloody, straw coloured or clear appearance.(Figure 1) Milky ascites suggests the presence of chylomicrons, containing predominantly triglycerides, and is therefore called chylous ascites. Chylous ascites can be caused by malignancy, (iatrogenic) trauma, liver cirrhosis, infection, pancreatitis, congenital disease and more uncommon causes.(9) Cloudy ascites, also known as pseudochylous ascites, may indicate peritonitis, pancreatitis or a perforated bowel. Bloody ascites is often associated with malignancies or result from traumatic paracentesis, whereas straw coloured or clear ascites is common in liver cirrhosis.(10) The first impression of the appearance of ascites is non-specific, but can steer the direction of diagnosis.

Figure 1. Appearance of ascitic fluid.



A: straw coloured ascites in a patient with micronodular liver cirrhosis. B: chylous ascites in a patient with lymph vessel obstruction caused by a small bowel neuroendocrine tumour.

Biochemical testing

Serum-ascites albumin gradient

The serum-ascites albumin gradient (SAAG) is the most sensitive marker to distinguish between ascites due to portal hypertension/hepatic congestion and other causes, with an accuracy of 97%.(11) The SAAG is obtained by subtracting the ascitic fluid albumin level from the serum albumin level, both measured at the same time. A value equal or greater than 1.1 g/dL (or 11 g/L) indicates underlying portal hypertension or hepatic congestion; a value smaller than 1.1 g/dL indicates aetiologies not due to portal hypertension, such as malignancy, pancreatitis or infection.(6, 11)

Total protein

Current international guidelines still recommend measuring the total protein concentration in ascites.(5-7) Traditionally, this was thought to indicate the aetiology of ascites according to the transudate-exudate concept, but this approach is now generally considered inferior. The total protein concentration does have prognostic value as concentrations smaller than 15 g/L are associated with an increased risk for spontaneous bacterial peritonitis (SBP) in cirrhotic patients.

Amylase

The amylase concentration in ascitic fluid should be measured in particular when pancreatic disease is considered. Pancreatic ascites can be caused by leakage from pancreatic pseudocysts or due to pancreatic duct rupture. An amylase ascitic fluid/blood serum concentration ratio of 6.0 is indicative for pancreatic disease, considering that a ratio of 0.4 is normal in non-pancreatic ascites.(12) However, high-levels of amylase have also been detected in patients with malignancy and other conditions making it a rather non-specific finding. Still it can be of significant value in patients with comorbidities such as alcoholic cirrhosis and pancreatitis.(13)

Triglycerides

A concentration of triglycerides in the ascitic fluid that exceeds the blood serum level (2.2 mmol/L) indicates chylous ascites. Previous abdominal surgery, pancreatitis, trauma and (retro-peritoneal) lymphoma are among the main causes.(9) Malignancy is diagnosed in 80% of patients with chylous ascites, however, it must be noted that ascites in up to 6% of cirrhotic patients has a chylous character.(14)

Adenosine deaminase activity

Adenosine deaminase activity (ADA), an enzyme of purine metabolism, is a reliable marker to differentiate tuberculous ascites from other aetiologies. An ADA cut-off value between 36 to 40 IU/L has a high sensitivity (100%) and specificity (97%) for diagnosing

abdominal tuberculosis.(15) In the Netherlands, the ADA assay is available in a limited number of centers.

Glucose and lactate dehydrogenase

Traditionally, determining glucose and lactate dehydrogenase concentrations in ascites constituted part of the diagnostic work-up. A lower glucose concentration in ascites than in blood serum can indicate the presence of bacteria, white blood cells or cancer cells. (16, 17) A low level of lactate dehydrogenase is associated with non-malignant ascites, high levels suggest a malignant aetiology.(18) Unfortunately both measurements are influenced by the SAAG, are non-specific and are no longer recommended.(19)

Urea and creatinine

A very uncommon cause of ascites is urinary leakage into the peritoneal cavity. Urinary ascites is associated with pathological bladder changes and outlet obstruction.(20, 21) Normally the ascites/plasma creatinine ratio is approximately one, whereas a ratio of five is reported in case of urinary ascites. Importantly, urinary ascites can be accompanied by pseudo-renal failure due to peritoneal absorption of urea.(20)

Non-biochemical testing

Polymorphonuclear leukocytes counts

An ascites polymorphonuclear neutrophil (PMN) count should be performed in the ascitic fluid of all patients with ascites being admitted to the hospital or showing clinical signs suggestive of SBP. A PMN count equal or greater than 250 cells/mm^3 (0.25 x 10^9 cells/L) confirms the diagnosis of SBP in the absence of an evident intra-abdominal source of infection.(22) A PMN count repeated after 48 hours of antibiotic administration can distinguish between SBP and secondary bacterial peritonitis, a decrease suggests SBP and a sustained increase secondary bacterial peritonitis. A repeated PMN count after 48 hours after starting antibiotic therapy is recommended to document the efficacy of antibiotic therapy for SBP.(7, 16) Although SBP is mainly a complication of ascites due to portal hypertension, it may also develop in patients with ascites of other aetiologies.

Bacterial cultures

Ascitic fluid should be cultured if SBP is clinically suspected. Bedside inoculation of 10 mL under sterile conditions using blood culture bottles, containing aerobic and anaerobic media, leads to identification of an organism in ~80% of patients with SBP.(7, 23, 24) Ascitic fluid cultures should be carried out before antibiotic treatment is initiated.

PCR bacterial DNA Mycobacterium tuberculosis

Bacterial DNA of Mycobacterium tuberculosis in ascitic fluid can be detected using

polymerase chain reaction (PCR) and can be performed when tuberculous ascites is suspected. This method has a high sensitivity (94%) compared to microscopic acid-fast bacilli smears (~0%) and mycobacterial culture (~50%).(25, 26) Alongside a higher diagnostic accuracy, PCR offers a timesaving method in contrast to current Mycobacterium culture techniques. PCR is a widely available biomolecular technique, however, PCR specific for the genus of Mycobacterium may not be available in all centers. Furthermore, culturing Mycobacterium from ascitic fluid or peritoneal biopsy remains the gold standard test according to international guidelines, also allowing antibiotic susceptibility testing.(7)

Cytology

Ascitic fluid cytology should be performed in case of suspicion of malignant ascites or in doubt of the underlying aetiology (e.g. no decrease in PMN count after 48 hours of antibiotic treatment). Clearly, positive cytology is highly indicative for peritoneal carcinomatosis. The sensitivity of cytology is 83%, but can be as high as 97% if three samples from separate paracenteses are analysed.(27) Crucial factors are avoiding any time delay between obtaining the ascitic fluid and cytology processing as well as obtaining at least 50 mL ascitic fluid, or even 1000 mL if the first test was negative.(27) The sensitivity of cytology in patients with hepatocellular carcinoma and ascites is low (~27%).(28)

Diagnostic laparoscopy

If the conventional work-up fails to disclose the cause of ascites laparoscopy should be considered. Laparoscopy offers the advantages of visual inspection of the peritoneal cavity in combination with the ability to obtain targeted biopsies for histological and microbiological studies. The procedure may be particularly helpful to diagnose peritoneal carcinomatosis, tuberculous peritonitis and other peritoneal or omental diseases such as mesothelioma and sclerosing peritonitis.(29, 30) Figure 2 shows schematically the diagnostic approach to the patient with ascites.

Diagnostic developments

Novel markers in ascitic fluid analysis have been proposed for the initial differential diagnosis as well as for predicting prognosis in specific diseases. Most discoveries either target on simplifying, accelerating or reducing the costs of the diagnostic process or they result from advancing biochemical laboratory techniques.

Leucocyte esterase reagent strips

Leukocyte esterase reagent strips are widely used for urinary analysis with the advantages of a simple, inexpensive and rapid bedside method. Several studies have examined the usefulness of this method for diagnosing SBP and found a sensitivity and specificity of this test ranging from 80 – 93% and 93 – 98%, respectively.(31) The negative predictive

value is remarkable high ranging from 97 – 99%, which makes it an ideal tool to rule out SBP.(31) Together with the other advantages, the reagent strip could gain a place in routine practice. Recently, an ascitic-specific reagent strip with a cut-off value of 250 cells/mm^3 was introduced, which could further improve the diagnostic accuracy.(32)

Viscosity

A few studies have reported the potential usefulness of viscosity measurement of ascitic fluid. Measuring viscosity was found to be able to discriminate between portal hypertension and non-portal hypertension related aetiology and showed a high correlation with the SAAG.(33) These preliminary results await confirmation by additional studies.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a protein, fundamental in the process of vasculogenesis and angiogenesis. High concentrations of vascular endothelial growth have been associated with malignant ascites.(34) Additional research is necessary to define the diagnostic value of this test.

Bacterial DNA, cytokines and other proteins

Bacterial DNA was studied in two series of 30 patients with ascites due to liver cirrhosis. The presence of bacterial DNA in ascites was regularly found documenting bacterial translocation, which could indicate a worse clinical prognosis in this patient group, without implicating a diagnosis of SBP. Markers, such as endotoxin and peptidoglycan/β-glucan, could predict a poor clinical outcome.(35, 36) Another study, including 52 patients with SBP and 27 control patients with cirrhotic ascites, found that blood serum concentrations of procalcitonin and an ascitic fluid concentration of calprotectin were significantly higher in SBP patients. Both serum and ascitic levels of TNF-α and IL-6 were significantly higher in SBP patients than in non-SBP patients.(37) These findings need to be confirmed in larger series of patients.

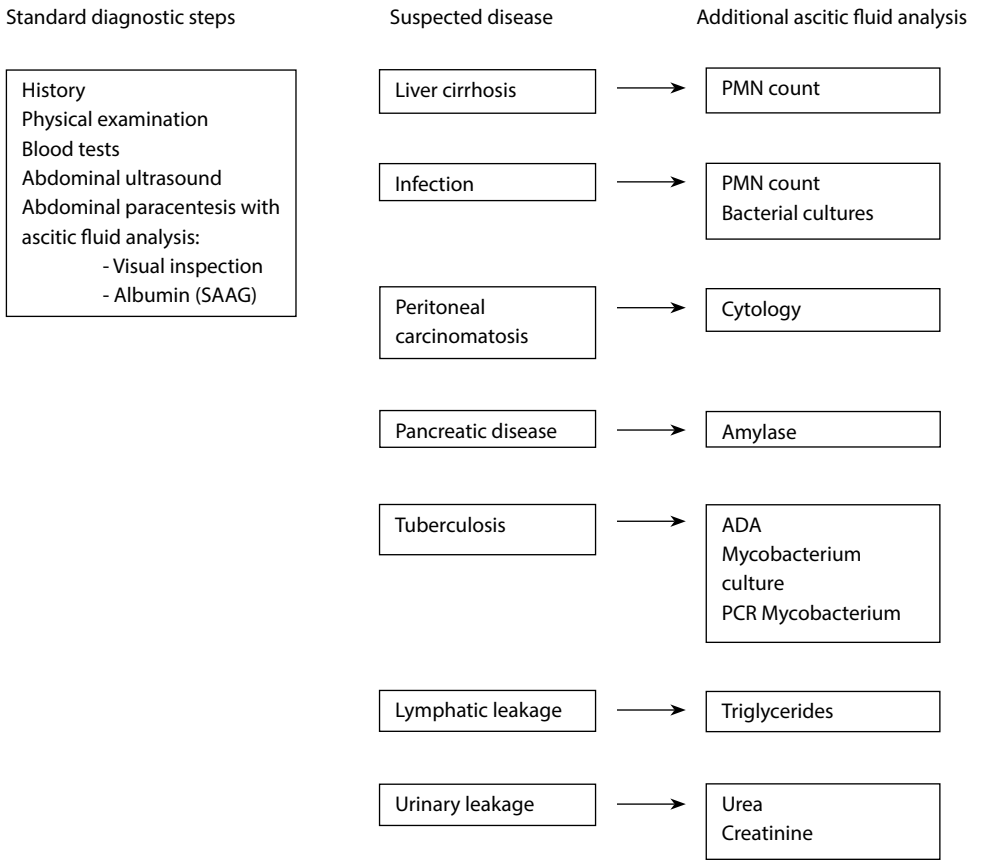
Platelet indices

Increased platelet indices, e.g. mean platelet volume and platelet distribution width, have been reported in blood of cirrhotic patients with SBP. The diagnostic accuracy was not sufficient, however, to consider these indices as a potential diagnostic tool.(38)

Tumour markers

Several studies have addressed the diagnostic value of tumour markers in ascitic fluid including α-fetoprotein (AFP), des-gamma-carboxy prothrombin, carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA19-9) and cancer antigen 125 (CA-125). Increased concentrations have been associated with underlying malignancies but are also found in medical conditions such as gastritis, diverticulitis, cirrhosis and pancreatitis.(33)

Figure 2. Diagnostic approach to the patient with ascites.



When the cause of ascites remains unknown after performing the tests stated above, diagnostic laparoscopy should be considered.

ADA, adenosine deaminase activity; PMN, polymorphonuclear neutrophil; SAAG, serum-ascites albumin gradient.

SYNOPSIS

The differential diagnosis of ascites is broad and includes a large number of benign and malignant causes. A structured diagnostic approach will likely reveal the aetiology in the large majority of cases and is based on the following elements: history, physical examination, blood tests, abdominal ultrasound and diagnostic paracentesis. Standard ascitic fluid analysis includes visual inspection and determination of the serum-ascites albumin gradient. In patients with suspected infection or underlying liver disease a PMN count and bacterial cultures are standard. According to clinical circumstances other established diagnostic studies are ascites cytology and determination of levels of amylase and triglycerides. In exceptional cases measuring urea and creatinine levels may be crucial. Adenosine deaminase activity measurements, Mycobacterium cultures and PCR for Mycobacterial DNA are indicated when tuberculosis is considered. Leucocyte esterase reagent strips are useful, in particular to rule out SBP in patients with a low a priori risk. New diagnostic markers such as viscosity, VEGF, bacterial DNA, cytokines and platelet indices have been proposed, but further research is needed to validate the value of these markers.

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CHAPTER 3

Reagent strips are efficient to rule out spontaneous bacterial peritonitis in cirrhotics

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ABSTRACT

Background: The gold standard to diagnose spontaneous bacterial peritonitis (SBP) is a polymorphonuclear neutrophil count ≥ 250 cells/μL in ascitic fluid. This test is laborious and expensive. Urine reagent strips measuring leukocyte esterase activity have been proposed as a rapid and inexpensive alternative. The aim of this study was to assess the diagnostic accuracy of the Combur reagent strip for diagnosing SBP. Furthermore the possible advantage of photospectrometer reading over visual reading of the strip was investigated.

Methods: This prospective study includes all ascitic fluid samples of cirrhotic patients undergoing diagnostic or therapeutic paracentesis over a 12-month period. The samples were collected for standard diagnostic work-up and in addition tested with a bedside Combur reagent strip. The strip was read visually and with an automated spectrometer.

Results: A total of 157 samples were obtained from 53 patients, and spontaneous bacterial peritonitis was diagnosed in 12 patients based on ascitic PMN count. The sensitivity, specificity, positive predictive value and negative predictive value of the reagent strip according to the photospectrometer were 100%, 93%, 55% and 100% respectively, and 75%, 99%, 82% and 98%, respectively, for visual interpretation. The diagnostic accuracy of the photospectrometer was found to be higher than visual interpretation ($p = 0.007$).

Conclusion: The diagnostic accuracy of leucocyte esterase reagent strips read out by a photospectrometer was comparable to the gold standard test and was excellent to exclude SBP. Our results support implementation of reagent strips in the diagnostic work-up of ascitic fluid.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a life-threatening complication in cirrhotic patients with ascites.(1) Late or misdiagnosed SBP can lead to increased mortality due to consequences such as gastrointestinal bleeding, development of hepatorenal syndrome and progressive liver failure. Therefore, the threshold for performing diagnostic paracentesis and ascitic analysis should be low.(2)

The reported prevalence of SBP in cirrhotic patients differs from 0-2,8% in outpatients to 10-30% in hospitalised patients.(3-9) The gold standard test to diagnose SBP is a polymorphonuclear neutrophil (PMN) count of equal or greater than 250/µL in ascites using a manual counting chamber, regardless of the outcome of the culture of ascitic fluid. (2) This analysis is laborious, time-consuming and expensive. Automated cell counting has been proposed to be a reasonable alternative with a high diagnostic accuracy.(10)

In the past two decades several studies have examined the use of leukocyte esterase reagent strips for the bedside diagnosis of SBP.(8, 11-25) These strips are widely used for rapid urinary analysis and the principle is based on the detection of leukocyte esterase activity of granulocytes.

Varying levels of diagnostic accuracy to diagnose SBP with reagent strips have been reported, with a sensitivity ranging from 45-100%, a specificity from 90-100%, a positive predictive value from 42-100% and a negative predictive value from 93-100%.(8, 9, 11-31) These inconsistent results could be related to variability in reagent strips, patient populations, different cut-off values and the subjective interpretation of the reagent strip result. However, the consistent high negative predictive value could make the reagent strips a very useful rule-out tool.

This study was performed to (1) assess the diagnostic accuracy of reagent strips in comparison with the current gold standard test for diagnosing SBP in a mixed population of low-risk and high-risk patients, and to (2) investigate the possible advantage of automated analysis of the reagent strips over visual non-automated reading.

MATERIALS AND METHODS

Study design

This prospective cohort study was carried out at the department of Gastroenterology and Hepatology in a referral centre for liver disease in the Netherlands. The study was designed and carried out in accordance with the principles of the Helsinki Declaration and approval was given by the local medical ethical committee of the hospital.

Patients

Consecutive patients with cirrhosis undergoing diagnostic or therapeutic paracentesis were prospectively enrolled from July 2006 up to and including July 2007. The total study

population was subdivided into a low- and high-risk population for the development of SBP. The low-risk population was defined as patients undergoing therapeutic, large volume paracentesis or outpatients undergoing diagnostic paracentesis.(4, 5, 9) The high-risk population was defined as hospitalized patients undergoing a standard diagnostic paracentesis at admission or because of clinical deterioration.(2) Patients with ascites secondary to causes other than liver disease were excluded.

Methods

Paracentesis was performed under strict sterile conditions. Ascitic fluid was routinely analyzed in the central clinical laboratory with automated determination of the white blood cell count with differential. Ten millilitres of fluid was inoculated at bedside in aerobic and anaerobic blood culture bottles (Bactec®). Fluid was collected in a sterile tube and assessed by two leukocyte esterase reagent strips (Combur^{10} strips, Roche Diagnostics). Both strips were read out after 60 seconds, one strip visually and one with a photospectrometer (Urisys 1100®, Roche Diagnostics). The observer was unaware of the results of the spectrometer. The observer could differentiate between 4 different colour shades corresponding to 0, 25, 100 or 500 leukocytes/µL.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp.). A mean and standard deviation was computed for continuous variables and compared with the Student's t-tests if normally distributed. A two-sided *p*-value <0.05 was considered significant. Sensitivity, specificity, positive and negative predictive values with confidence intervals of 95% were calculated. ROC-curves were computed and the optimal categorical cut-off point was analysed. Diagnostic performance between photospectometer reading and visual interpretation was statistically compared using a McNemar test.(32)

RESULTS

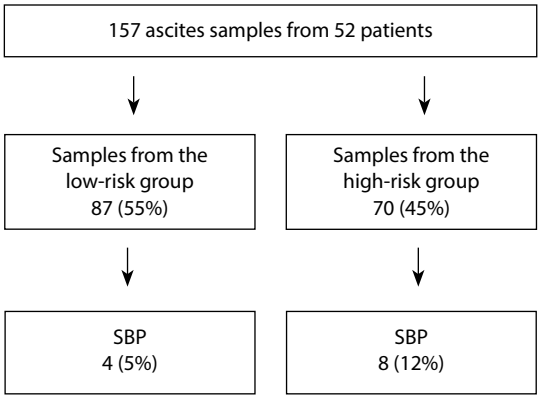
A total of 157 ascitic fluid samples were collected from 52 patients (range 1–14 samples per patient); 87 samples (55%) were obtained in the low-risk population and 70 (45%) in the high-risk population.(Table 1) The prevalence of SBP according to polymorphonuclear count was 4 (4,5%) in the low-risk group and 8 (11,6%) in the high-risk group.(Figure 1) In the low-risk population, one culture (25%) was positive, identifying an Enterococcus faecium, whereas three cultures (37,5%) were positive in the high risk population, identifying Enterococcus coli, Haemophilus parainfluenzae and Pseudomonas aeruginosa in one case each.

Table 1. Baseline characteristics in 52 patients in the low- and high-risk group.

	All patients (n=52)	**Low-risk group (n=20)**	**High-risk group (n=32)**	***p*-value**
Male, *n (%)*	35 (67%)	17 (85%)	18 (56%)	0.038
Age*, *years*	51 ± 10	51 ± 8	51 ± 11	0.581
Child-Pugh score*	10 ± 1.5	9 ± 1.4	10 ±1.6	0.962
Etiology of liver cirrhosis, *n (%)*				0.325
Alcohol	18 (35%)	11 (55%)	7 (22%)	
Cryptogenic	10 (19%)	3 (15%)	7 (22%)	
Viral	7 (13%)	2 (10%)	5 (16%)	
Viral + alcohol	3 (6%)	0 (0%)	3 (9%)	
Other	14 (27%)	4 (20%)	10 (31%)	

*Mean ±standard deviation.

Figure 1. Flowchart study participants and sample collection.



Photospectrometer versus visual reading

Of the total of 12 (25%) cases of SBP, three were not detected by optical reading of the strip but correctly diagnosed with the photospectrometer. With visual reading, the sensitivity for diagnosing SBP was 75% (95% CI 43-93), the specificity 99% (95% CI 95-100), the positive predictive value 82% (95% CI 48-97) and the negative predictive value 98% (95% CI 94-100). The diagnostic accuracy for automated reading was slightly superior ($p = 0.007$ McNemar test): sensitivity 100% (95% CI 70-100), specificity 93% (95% CI 87-97), positive predictive value 55% (95% CI 33-75) and negative predictive value 100% (95% CI 97-100).(Table 2) ROC curve analysis indicated that the diagnostic accuracy of the strips was optimal at a cut-off of 100 leukocytes/µl.

Table 2. Diagnostic accuracy of visual and automated reading of the leukocyte esterase reagent strip compared to gold standard.

	Visual reading	Photospectrometer reading
Sensitivity	75% (95% CI 43 - 93%)	100% (95% CI 70 - 100%)
Specificity	99% (95% CI 95 - 100%)	93% (95% CI 87 - 96%)
Positive predictive value	82% (95% CI 48 - 97%)	55% (95% CI 33 - 75%)
Negative predictive value	98% (95% CI 94 - 99%)	100% (95% CI 97 - 100%)

Low- and high-risk group analysis

The diagnostic performance of the strip with automated reading in the low- and high-risk populations was similar: the negative predictive value was 100% (95% CI 92 -100%) and the specificity was 93% (95% CI 83 -98%).

Table 3. Overview of studies assessing the diagnostic value of Combur leukocyte esterase reagent strips for diagnosing SBP.

Author, year [corresponding number in reference list]	Samples	Prevalence SBP (%)	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Thevenot, 2004 [13]	100	9 (9%)	89	100	100	99
Sarwar, 2005 [26]	214	38 (18%)	83	83	42	97
Braga, 2006 [27]	100	9 (9%)	100	98.9	92.3	100
Campillo, 2006 [18]	443	33 (7%)	63	99.2	91	92.9
Rerknimitr, 2006 [28]	200	42 (21%)	88	81	55	96
Rerknimitr, 2010 [30]	250	30 (12%)	90	93.2	64.3	98.6
Present study, 2015	157	12 (8%)	100	93	55	100

DISCUSSION

The results of the present study support the diagnosing value of leukocyte esterase reagent strips in ascitic fluid analysis in patients with cirrhosis. In particular, this simple, quick and inexpensive method could reliably rule out SBP, with a 100% negative predictive value in populations low- and high-risk for SBP. Automated reading of the reagent strip was superior to visual interpreting and prevented false-negative results.

The diagnostic accuracy of the Combur[10] strip in ascitic fluid analysis has been studied previously by several groups.(13, 18, 26-28, 30) The results of these studies were

comparable with our results in terms of a high negative predictive value of reagent strip testing.(Table 3) The cumulative data suggest that the sensitivity of strips for diagnosing SBP is variable and may not be optimal. A negative test result, however, strongly predicts absence of SBP. Thus, in patients undergoing diagnostic paracentesis, a negative reagent strip result may imply that further diagnostic studies - polymorphonuclear neutrophil count and bacterial cultures - are not useful and can be omitted. Obviously, preventing unnecessary diagnostic studies in a substantial proportion of patients presenting with ascites may lead to a marked reduction in costs.

Although an automated reader has been used in previous studies, this study is, to our knowledge, the first to compare visual and automated reading of reagent strips in ascitic fluid analysis.(7,15) Our results suggest that automated reading is superior and may be the preferred method in clinical practice. Additional studies would be useful to confirm this finding.

One of the limitations of our study may be that the reagent strips we used are not specifically designed for ascitic fluid analysis. The cut-off levels are not based on the PMN-count of 250 leukocytes/μL, the gold standard for SBP. It has been suggested that protein could interfere with the test and has a negative effect on the accuracy. One study found significantly higher mean ascitic protein content in patients with false-negative results than in patients with true-positive results.(29) Furthermore little is known regarding the effects of the different composition of ascites as compared to urine, for example with respect to bilirubin or pH level, on reagent strip diagnostic accuracy. Remarkable results - a 100% sensitivity and negative predictive value - have been reported with the Periscreen strip, a strip with specific characteristics for ascitic fluid analysis.(31) These results await confirmation in a large cohort, which is currently investigated in the Per-DRISLA study.(33)

In conclusion, this study adds to already available data suggesting that Combur reagent strips are useful for ascitic fluid analysis in cirrhotic patients. Cumulative evidence clearly indicates that a negative test result reliably rules out SBP. We found reagent strips an inexpensive, time- and money-saving tool, which is available both during and after regular working hours. Reading the strips with a photospectometer may be superior to visual reading.

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CHAPTER 4

Microbiology and antibiotic susceptibility patterns in spontaneous bacterial peritonitis: a study of two Dutch cohorts at a 10-year interval

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ABSTRACT

Background: Recent investigations suggest an increasing prevalence of Gram-positive and antibiotic-resistant bacteria causing spontaneous bacterial peritonitis (SBP), probably related to changes in antibiotic prescription patterns, in particular more widespread and long-term use of antibiotic prophylaxis with quinolones.

Objective: The primary objective of this study was to assess potential changes in the microbiology of SBP in two patient cohorts studied at a 10-year interval. Further aims were to study prognostic factors and outcome of SBP.

Methods: A retrospective double-cohort study, including all ascitic cultures from patients with cirrhosis obtained 2003–2005 and 2013–2014, was conducted.

Results: In total 312 patients were included, 125 patients in the first and 187 patients in the second cohort. SBP was diagnosed in 132 of 840 analyzed ascitic fluid samples; 62 samples were culture positive. An increase of Gram-positive bacterial isolates was noted from 26% to 46% between cohorts (p=0.122). The prevalence of multidrug-antibiotic–resistant pathogens increased from 25% to 32% (p=0.350). Survival after SBP among the two cohorts was comparable.

Conclusion: This single-center study in the Netherlands found a modest but non-significant increase in the proportion of patients with SBP caused by Gram-positive bacteria and multidrug-antibiotic–resistant bacteria over a 10-year period. Our findings differ from reported data in other countries and suggest empiric antibiotic prophylaxis and treatment of SBP should be based on national and regional microbiological findings and resistance patterns.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a common infection in patients with cirrhosis and ascites. Reportedly, this infection can be diagnosed in up to 30% of cirrhotic patients with ascites who are admitted to the hospital. SBP is associated with significant morbidity and mortality.(1-4) Intestinal bacterial translocation, altered immunity and the presence of ascites are key in the development of SBP.(5-7)

SBP is diagnosed by a polymorphonuclear neutrophil (PMN) count in ascitic fluid equal to or greater than 250/µl. Approximately 40% of SBP episodes are culture positive.(3, 8, 9) Numerous, in particular older, studies reported that Gram-negative enteric bacteria were involved in the majority of SBP episodes. International guidelines recommend third-generation cephalosporin as empirical treatment for SBP and quinolones for secondary prophylaxis.(10, 11) However, in the last decade Gram-positive bacteria and antibiotic-resistant bacteria have been increasingly found to cause SBP.(3, 8, 9, 12-14) This change in microbiology has been attributed to long-term and widespread quinolone use and increased prevalence of hospital and intensive care unit admissions. These findings have raised doubts about the currently recommended antibiotic strategy in SBP.

The prevalence of antibiotic-resistant pathogens substantially differs geographically.(15) Antibiotic consumption has been identified as the main cause for increasing rates of antibiotic resistance. The Netherlands is known for a restrictive antibiotic policy and has had the lowest antibiotic use in Europe for years.(15-17) Consequently, microbiological study results in SBP in our country could differ from those observed in other countries - i.e. Spain, Greece, Germany and the United States - over time. This would mean that international guidelines for prophylaxis and treatment of SBP would need to differentiate between countries based on antibiotic resistance rates. Therefore, we investigated causative microorganisms in two patient cohorts who were hospitalized with a 10-year interval in a tertiary referral hospital in the Netherlands. In addition, we aimed to identify the patients most at risk for SBP and to evaluate the associated short- and long-term survival.

MATERIALS AND METHODS

All consecutive ascitic cultures performed in patients with cirrhosis between January 2003 and December 2005 (first cohort) and between January 2013 and December 2014 (second cohort) at Erasmus MC, University Medical Center, Rotterdam, were included. Demographic, clinical, biochemical and survival data from patient hospital records were retrospectively studied to evaluate the prevalence, risk factors, microbiology, and mortality of SBP. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in an approval by the ethical review board of the Erasmus MC in February 2017.

The diagnosis of cirrhosis was based on liver histology or a combination of clinical, biochemical, and radiologic findings.(18) SBP was defined as a PMN count equal to or

greater than 250/μL in ascites without evidence of an intra-abdominal source of infection.(10, 11) All ascites samples obtained during the two study periods were studied, implying that, if applicable, multiple samples per patient were taken into account. However, only the first positive culture per SBP episode, and thus one culture per SBP episode, was included in the analysis. Multidrug-antibiotic–resistant organisms (MDR) were defined, according to international guidelines, as an acquired resistance to at least three antibiotic classes.(19) Nosocomial acquisition was defined as SBP diagnosed at least 48 hours after hospital admission. During the study periods, the standard antibiotic prophylaxis for SBP was norfloxacin 400mg daily.(10, 11, 20) The primary choice for empirical antibiotic treatment for SBP was ceftriaxone 2000mg daily for five to seven days and the secondary choice was amoxicillin/clavulanic acid 1000/200mg every eight hours for five to seven days, according to international guidelines.(10, 11, 20)

All ascitic fluid samples were routinely analyzed in the central clinical laboratory with automated determination of the white blood cell count with differential. In addition, at least 10 ml of ascitic fluid was inoculated at bedside under sterile conditions in aerobic and anaerobic blood culture bottles (Bactec®) for culture in the central medical microbiology laboratory. For identification of positive cultures, the ascitic fluid was plated on agar and current identification methods were used. Susceptibility was determined with the VITEK® 2 system (VITEK AMS; bioMerieux Vitek Systems Inc, Hazelwood, MO, USA). Cultures collected until 2013 were called resistant using Clinical and Laboratory Standards Institute criteria; later cultures were called resistant using European Committee on Antimicrobial Susceptibility Testing criteria 2013.

Statistical analysis

A mean and standard deviation (SD) was computed for approximately normally distributed variables and compared using the Student's *T*-test. Non-normally distributed continuous variables were summarized by their median and interquartile range (IQR), and compared using the Mann-Whitney ranks sum test. Categorical variables were expressed with percentages and compared using the Chi-square test. A two-sided p value < 0.05 was considered significant. Patients were followed up to a maximum of one year. This time frame was chosen based on the severity of decompensated advanced chronic liver disease. Survival was analyzed using the Kaplan-Meier method. The actual 30-day mortality and one-year mortality was calculated after the first ascitic fluid analysis; both liver transplantation and death were considered as events. The survival rates were compared using log-rank test. Univariable and multivariable Cox's proportional hazard analyses were carried out to identify independent predictors for 30-day mortality and one-year mortality after SBP. The variables selected for univariable analysis were based on previous studies: gender, age, etiology of liver disease, community- or nosocomial-acquired SBP, positive microbial ascites culture, causative microorganism, antibiotic susceptibility, use of

antibiotic prophylaxis, use of immunosuppressant drug, hepatocellular carcinoma (HCC), model for end-stage liver disease (MELD) score, albumin in serum, platelets in serum, and protein in ascites at time of ascites analyses. Variables with a *p* value of < 0.10 in univariate analysis were included in the multivariate Cox's proportional hazards model.

RESULTS

In the first (2003–2005) cohort of 125 patients, 343 ascitic fluid samples were obtained for analysis. In the second (2013–2014) cohort of 187 patients, 497 samples were obtained. The diagnosis of SBP was established in 132 of the total 840 (16%) ascitic fluid samples in 95 patients.(Figure 1)

The total study population included 197 men and 115 women with a mean age of 56 years (±12) and a mean MELD score of 19 (±8). Norfloxacin was used by 12% and 10% of patients at the time of paracentesis in the first and second cohort, respectively (*p*=0.638).

The clinical characteristics of patients with and without SBP are shown in Table 1. Patients with SBP had more frequent liver disease of autoimmune origin, more frequently used immunosuppressive drugs (*p*=0.012) and had higher baseline MELD scores (*p*=0.020).

Figure 1. Flow chart of study population.

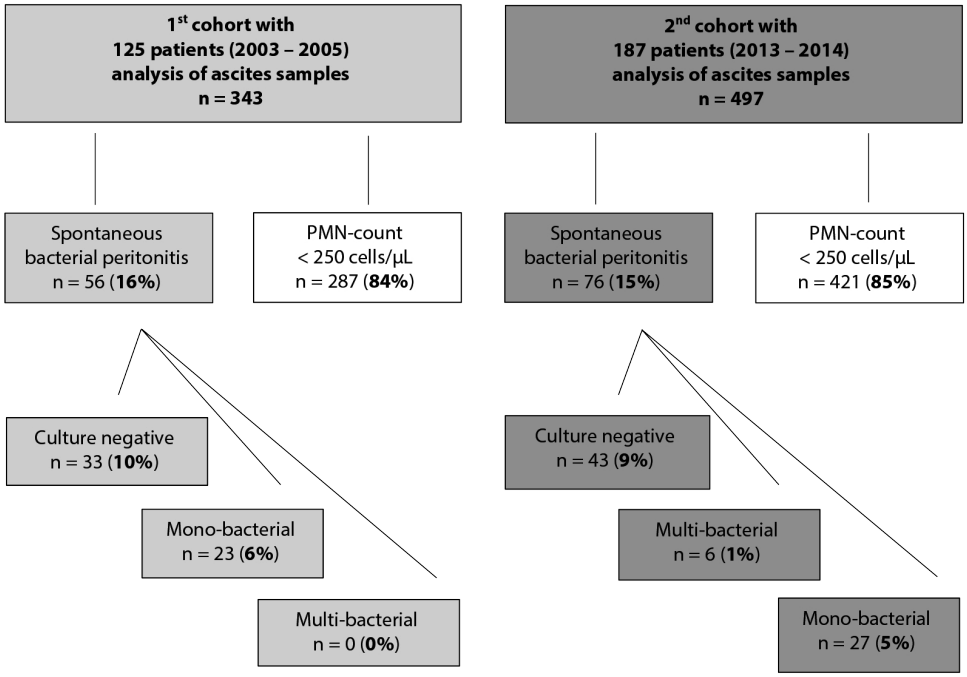


Table 1. Clinical characteristics of the study population with and without SBP.

	SBP negative (n=217)	**SBP positive (n=95)**	***p*-value**
Male, *n (%)*	138 (64%)	59 (62%)	0.802
Age in years	57 (±12)	54 (±13)	**0.036**
Etiology of cirrhosis, *n (%)*			
Alcohol	92 (42%)	31 (33%)	
Viral	51 (24%)	23 (24%)	**0.045**
Auto-immune	28 (13%)	24 (25%)	
Other	46 (21%)	17 (18%)	
Child-Pugh class, *n (%)*			
A	25 (12%)	5 (5%)	0.215
B	74 (34%)	33 (35%)	
C	118 (54%)	57 (60%)	
MELD score	19 (±8)	21 (±8)	**0.020**
Creatinine (mmol/L)	96.8 (±1.7)	110.1 (±1.8)	0.052
Albumin (g/L)	29 (±6)	29 (±6)	0.912
INR	1.5 (±1.0)	2.0 (±1.5)	**0.006**
Bilirubin (mmol/L)	62 (±3)	76 (±4)	0.260
Thrombocytes (109/L)	107 (±2)	97 (±2)	0.304
Ascites protein (g/L)	10.6 (±2.0)	16.3 (±2.6)	**0.009**
Hepatocellular carcinoma, *n (%)*	30 (14%)	11 (12%)	0.589
Diabetes mellitus, *n (%)*	37 (17%)	11 (12%)	0.218
Use of immunosuppressant drug, *n (%)*	21 (10%)	19 (20%)	**0.012**
Use of norfloxacin, *n (%)*	26 (12%)	8 (8%)	0.353

Microbiology

In the two cohorts a culture-positive SBP was found in 23/56 (41%) and 33/76 (43%) episodes with SBP, respectively.(Figure 1) The microbiological culture results are shown in Table 2. In the first cohort, 61% of culture-positive SBP was due to Gram-negative bacteria vs. 51% in the second cohort. Candida albicans was isolated in four cultures. Although the percentage SBP with Gram-positive organisms increased over time, the differences were not statistically significant (p=0.122).(Table 2)

Table 2. Microbiological findings in two cohorts of patients; 62 organisms were identified in 56 episodes of culture-positive SBP.

	cohort 2003 – 2005 (n=23)	**cohort 2013 – 2014 (n=39)***
Gram-negative bacteria	14 (61%)	20 (51%)
Escherichia coli	9	13
Enterobacter aerogenes	-	2
Enterobacter cloacae	-	2
Pseudomonas aeruginosa	2	-
Klebsiella pneumoniae	1	3
Morganella morganii	1	-
Aeromonas spp.	1	-
Gram-positive bacteria	6 (26%)	18 (46%)
Staphylococcus aureus	1	3
Staphylococcus haemolyticus	-	1
Staphylococcus (coagulase negative)	3	3
Enterococcus faecium	-	5
Streptococcus oralis	1	3
Streptococcus anginosus	-	1
Streptococcus salivarius	-	1
Streptococcus viridans	-	1
Streptococcus pneumoniae	1	-
Yeast	3 (13%)	1 (3%)
Candida albicans	3 (13%)	1 (3%)

* Including 6 cultures showing two microorganisms.

Antibiotic susceptibility patterns

In the first cohort 5/20 (25%) of the isolated bacteria were MDR versus 12/38 (32%) in the second cohort.(Table 3) There was no significant change in prevalence of MDR organisms over time (p=0.350). In the MDR organisms, the most frequently detected resistance mechanism was due to extended-spectrum beta-lactamase (ESBL) production (Escherichia coli n=9, Klebsiella n=1). Furthermore, methicillin-resistant Staphylococcus aureus (MRSA) (n=4), intrinsically cephalosporin-resistant Enterobacter (n=2), and vancomycin-resistant Enterococci (VRE) (n=1) were found. There was no evidence that the risk of SBP caused by MDR organisms was related to a Gram-negative or Gram-positive microbiologic isolate (p=0.192), a nosocomial acquisition of the infection (p=0.677), a previous history of SBP (p=0.245), or the use of antibiotic prophylaxis (p=0.316).

Three of the 20 (15%) and six of the 38 (16%) isolated organisms were norfloxacin resistant, in the first and second cohort, respectively (p=0.274).

Analysis with respect to ceftriaxone showed that in the first cohort 3/20 (15%) of bacterial isolates were resistant to this agent as compared to 5/38 (13%) in the second cohort. Amoxicillin/clavulanic acid-resistance was slightly more prevalent. In the first cohort 6/20 (30%) organisms were found to be resistant versus 8/38 (21%) in the second cohort. The frequency of ceftriaxone and amoxicillin/clavulanic acid-resistant organisms did not differ significantly between the cohorts (p=0.952 and p=0.254, respectively).(Table 3)

Table 3. Antimicrobial susceptibility patterns of bacteria from culture-positive SBP.

	Bacterial isolates in SBP (n=58)		***p*-value**
	cohort 2003 – 2005 (n=20)	cohort 2013 – 2014 (n=38)	
Multidrug resistant	5 (25%)	12 (32%)	0.350
Norfloxacin resistant*	3 (15%)	9 (24%)	0.274
Ceftriaxon resistant*	3 (15%)	5 (13%)	0.952
Amoxicillin/clavulanic acid resistant*	8 (40%)	8 (21%)	0.254

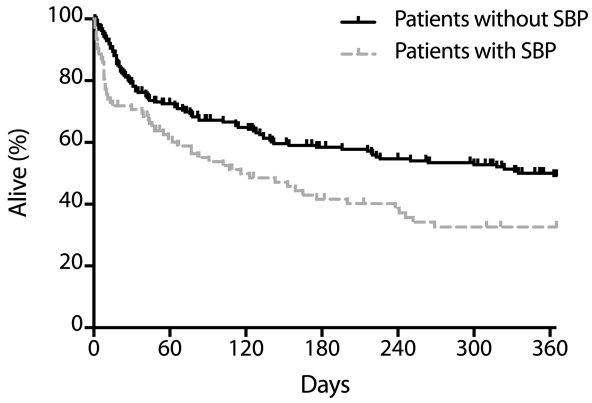
* Intrinsically and/or acquired antimicrobial resistance.

Survival

At one year, 153 patients had died (49%) and 48 patients had received a liver transplant (15%). Furthermore, 83 patients (27%) were alive after one year, while 28 patients (9%) were lost to follow-up. The median follow-up time of the patients not reaching the endpoint of death or liver transplantation was 365 days (IQR 12 days). The survival of patients with SBP did not differ significantly between the cohorts (log-rank p=0.442), nor did survival differ significantly between the cohorts for patients without SBP (log-rank p=0.216).

The median survival after the first ascites analyses was 168 days for SBP-negative patients and 77 days for SBP-positive patients (log-rank p=0.001).(Figure 2) The 30-day mortality rate was 33% (32/95) for patients with SBP compared to 25% (55/217) for patients without SBP.(Figure 2) Univariable and multivariable Cox-regression analyses were carried out to identify risk factors for 30-day mortality and one-year mortality after SBP. MELD score was the only independent predictive factor for 30-day mortality (hazard ratio (HR) 1.106 per point, 95% CI 1.061–1.154, p<0.001) and one-year mortality (HR 1.060 per point, 95% CI 1.030–1.091, p<0.001).(Table 4)

Figure 2. One-year mortality after first ascites analysis. SBP-negative patients (black solid line) have a median survival of 168 days and SBP-positive patients (grey dotted line) of 77 days (log-rank p=0.001).



*Liver transplantation and death are considered as event.

Table 4. Demographic and clinical factors after SBP in 95 patients predicting 1-year mortality using Cox-regression analysis.

	Univariate			**Multivariate**		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Gender						
Female (ref.)	**1**		**<0.001**			
Male	**0.424**	0.263-0.682				
Age (per year)	0.999	0.981-1.017	0.911			
Etiology						
Alcohol (ref.)	1					
Viral	0.841	0.444-1.596	0.206			
Auto-immune	1.545	0.836-2.858				
Other	0.837	0.431-1.625				
Acquisition SBP						
Community	**1**		**0.078**			
Nosocomial	**1.761**	0.938-3.303				
Positive microbial ascites culture	1.406	0.886-2.231	0.148			
Causative microorganism type						
Gram-negative bacteria (ref.)	1		0.561			
Gram- positive bacteria	0.764	0.359-1.625				
Yeast	1.511	0.444-5.146				
Multi-drug resistant microorganism	**1.989**	0.972-4.073	**0.060**			
Antibiotic prophylaxis	0.662	0.286-1.531	0.335			
Immunosuppressant use	**0.508**	0.260-0.992	**0.047**			
HCC	0.971	0.465-2.026	0.937			
MELD score (per point)	**1.060**	1.030-1.091	**<0.001**	**1.060**	1.030-1.091	**<0.001**
Albumin in serum (per point)	0.967	0.927-1.008	0.109			
Platelets in serum (< 150 x 10^9/L)	**2.179**	1.226-3.870	**0.008**			
Low protein in ascites (<15 g/L)	1.287	0.530-3.124	0.578			

DISCUSSION

In this single-center study in the Netherlands, the microbiological characteristics and antibiotic susceptibility patterns of organisms causing SBP in liver cirrhosis patients were compared between the periods 2003–2005 and 2013–2014. No significant increase in Gram-positive bacteria was observed and Gram-negative bacteria remained the primary cause. Bacteria resistant to empirical treatment with third-generation cephalosporin accounted for 13%–15% of all causative pathogens.

There was no evidence that mortality was influenced by causative microorganisms, antibiotic susceptibility, use of prophylactic antibiotics, intensive care admission or a nosocomial acquisition of SBP. The main predictors for mortality were age, MELD score, and platelet count.

Our most important finding is that in the era of quinolone prophylaxis for SBP, we cannot confirm observations made elsewhere regarding a significant increase in Gram-positive and MDR organisms causing SBP.(3, 8, 9, 12, 13) Although in the Netherlands antibiotics are used prudently, a rise in quinolone use in the last decades has been described.(21, 22)

Third-generation cephalosporin may poorly cover the causative pathogens in SBP, with reported antibiotic resistance rates ranging from 57% to 69%.(9, 23, 24) However, our results show a susceptibility rate of 85–87%. We hypothesize this difference can be most likely attributed to different national antibiotic policies. High consumption of antibiotics has been related to higher rates of antibiotic resistance.(15) The Netherlands has always had a restrictive national policy regarding antibiotic prescription and a conservative approach toward the prescription of new broadspectrum antibiotic agents.(15, 16, 25)

The microbiology and susceptibility patterns' differences can be hospital and region dependent, implying difficulties with recommending antibiotic treatment and prophylaxis in international guidelines. The results of this study in the Netherlands do not support the need to revise guidelines as previously proposed.(3, 23) Empirical antibiotic treatment should be based on known regional and national differences of antibiotic resistance patterns.

A previous study from our institution on the microbiology of SBP in the period 1987–1991, before the implementation of long-term quinolone prophylaxis in the relevant patient population, reported that causative pathogens were isolated in 25 of 31 SBP episodes.(26) Gram-negative bacteria were detected in 60% of the episodes and Gram-positive bacteria in 40% of the episodes. Despite the small sample size, the proportions of causative pathogens seem comparable to those identified in the cohorts reported here.

Although optimizations in bacterial culture techniques have been implemented, an organism was isolated in a minority of all SBP episodes (40%). This is a stable percentage over the last decade and similar proportions have been documented in other studies.(3, 8, 9) It may be expected that, with technologies arising from bacterial DNA detection and microbiome studies, more causative pathogens can be identified rapidly in the future for targeted antibiotic therapy.

There are a few limitations of the study regarding methodology. This study was designed as a retrospective, double-cohort study, which implied some laboratory and clinical data were missing. For instance, we have no information about short-term antibiotic use prescribed by general practitioners and clinicians outside the hospital, but it is not to be expected that this would differ significantly between the cohorts.

Furthermore, multiple tests have been performed that may lead to an increased risk of finding spurious significant results and results should be interpreted while keeping this in mind. Prospective cohort studies comparing multiple regions during a large time frame could provide more insight as to the microbiology of SBP in diverse regions over time.

In this study we show that the microbiology of pathogens causing SBP did not change significantly in our center over the last decade. These findings suggest that guidelines with respect to antibiotic prophylaxis and treatment of SBP should carefully take into account potential national and regional differences in the microorganisms causing SBP and antibiotic resistance patterns.

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CHAPTER 5

Bacterascites: a study of clinical features, microbiological findings and clinical significance

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ABSTRACT

Background: Knowledge about bacterascites is limited and management guidelines are based on small patient series. The purpose of this study was to add further insight into the clinical characteristics, microbiological findings and prognosis of patients diagnosed with bacterascites.

Methods: Retrospective analysis of patients with advanced chronic liver disease diagnosed with bacterascites and SBP between January 2003 and August 2016.

Results: In this study, 123 patients were included with 142 episodes of bacterascites. The median MELD score was 20 and clinical symptoms of infection were present in 78%. Empiric antibiotic treatment was initiated in 68%. In 26 untreated patients undergoing repeated paracentesis, 42% were diagnosed with either ongoing bacterascites or SBP. The presence of signs or symptoms of infection was not an independent predictor for mortality or spontaneous resolution of infection. The 1-month and 1-year mortality rates of the 123 patients studied, were 32% and 60%, respectively; these results were in line with data pertaining to the prognosis of SBP.

Conclusions: Patients with bacterascites and SBP are highly comparable with respect to severity of liver disease and overall prognosis. If left untreated, bacterascites is likely to persist or to evolve to SBP in a significant proportion of patients. The results of this study support current guidelines regarding the treatment of ascitic fluid infection, but could not confirm the prognostic relevance of symptomatic disease at the time of diagnosis. We suggest that the threshold to initiate antibiotic treatment, in particular in cases with severely advanced liver disease, should be low.

INTRODUCTION

Bacterascites is defined by an ascitic fluid polymorphonuclear neutrophil (PMN) count below 250/μL and a positive ascitic fluid culture result in the absence of an evident intra-abdominal, surgically treatable source of infection.(1) It is a different clinical entity than spontaneous bacterial peritonitis (SBP), which is characterized by a neutrophil reaction in ascites regardless of the bacterial culture result. Bacterascites is prevalent in 8-11% of all patients with cirrhosis and ascites, and the clinical significance seems to vary according to how the infection was acquired.(2-7)

Several hypotheses have been proposed to explain the potential underlying pathophysiological mechanisms. The most common theory implicates that the bacterial colonization of ascites is caused by bacterial translocation from the intestinal lumen or by secondary translocation from a concomitant infection from extra-intestinal sites (e.g. urogenital or respiratory tract). The absence of an inflammatory response could be interpreted as an early phase of SBP, in which the neutrophil response has not commenced yet, or a spontaneously resolving infection, determined by good host defenses or less virulent pathogens.(1, 4) In this context, the term 'symptomatic bacterascites' has been introduced for patients with bacterascites and clinical symptoms of infection, in order to identify those patients who may require treatment. Furthermore, bacterascites caused by commensal skin bacteria has been attributed to exogenous contamination of the ascitic fluid sample and bacterascites with multiple pathogens may be caused by traumatic paracentesis.(2, 3) The indication for antibiotic treatment of bacterascites is generally regarded to be dependent on the supposed pathophysiologic mechanism and the clinical situation.

The AASLD practice guideline regarding the management of ascites states that patients with ascites and convincing signs or symptoms of infection should receive empiric antibiotic treatment.(8) This recommendation is based on one study with 36 cases of bacterascites receiving a follow-up paracentesis, in which 62% of the cases spontaneously resolved and 38% progressed to SBP.(4) The EASL clinical practice guideline endorses this recommendation and further states asymptomatic patients should undergo a second paracentesis when culture results come back positive. Patients in whom the repeated ascitic PMN count is greater as or equal to 250/μL should be treated for SBP, and the remaining patients (i.e., PMN count below 250/μL) should be followed up.(9) This guideline is based on a consensus document of the International Ascites Club in 2000.(1)

Although bacterascites is not an uncommon condition, relatively few studies on prognostic factors and outcome of this ascitic fluid infection have been reported. (2-7) Therefore, the purpose of this study was to assess the clinical characteristics, microbiological findings and clinical course in consecutive patients diagnosed with bacterascites. We further attempted to study the prognostic impact of bacterascites in comparison to SBP, and to define the most logical therapeutic approach.

PATIENTS AND METHODS

Study design and data collection

All consecutive ascites cultures performed in patients with advanced chronic liver disease between January 2003 and August 2016 at Erasmus MC, University Medical Center, were retrospectively reviewed to identify patients fulfilling the diagnostic criteria for bacterascites. The Medical Ethics Committee Erasmus MC, approved the study protocol on February 27th, 2017 and stated that written informed patient consent was not necessary considering the design of the study.

All ascites samples obtained during the study period were reviewed, implying that, if applicable, multiple bacterascites episodes per patient were taken into account. The lower-case letter n was used to indicate the number of patients and the capital letter N for the number of bacterascites episodes.

Paracentesis was performed in patients with new-onset ascites, clinical deterioration, and large-volume removal in refractory ascites.(9) White blood cell (WBC) and PMN count in ascites were automatically determined and aerobic and anaerobic blood culture bottles (Bactec®) were used for bacterial cultures. Blood cultures taken within two hours before or after ascites cultures were considered to be concomitant.

Demographic, clinical, biochemical and survival data from patient hospital records were collected.

To determine the prognostic impact of bacterascites in comparison with reported outcomes of SBP, a control cohort was established with patients from our center with SBP, performed as described in a previous publication.(10) In order to create homogenous groups for survival analyses, patients with both episodes of bacterascites and SBP were categorized as SBP when the first ascites infection was SBP or bacterascites developed within 48 hours to SBP. Patients with bacterascites developing SBP after 48 hours, but within 30 days were excluded from survival analysis. In addition, the MELD score-dependent relation of the prognosis of bacterascites patients was studied and compared with SBP patients.(11, 12)

Furthermore, a PubMed search was performed on December 1st, 2017 with the following search terms: spontaneous bacterial peritonitis (ALL) AND (outcome (ALL) OR mortality (ALL)) AND prognos* (ALL). The studies were reviewed and included when the following criteria were met: (1) observational studies, (2) study population consisted of patients with SBP defined as a PMN count of 250/μL or greater in ascites, (3) minimum study population of 50 adult patients, (4) reporting survival analysis and 1-month or in-hospital mortality rate, and (5) written in English. Interventional studies (e.g. randomized controlled trials), studies with a selected population (e.g. HIV patients), and abstracts were excluded. Study and clinical characteristics were collected from the included studies.

Definitions

Bacterascites was defined as an ascitic fluid sample with a PMN count below 250/μL and a positive bacterial culture, in the absence of evidence for an intra-abdominal source of infection.(1) Infection acquisition was categorized as nosocomial (infection was detected after 48 hours after hospital admission), health-care associated (<48 hours after hospital admission in patients with any 90-day prior health-care contact), or community acquired (within 48 hours after hospital admission in patients without any 90-day prior health-care contact).(13) Ascites was graded as diuretic-responsive or diuretic-refractory, and hepatic encephalopathy (HE) as none, or West Haven grade 1-4. Patients were categorized as symptomatic, when one or more of the following symptoms, shown to be frequently present in patients with SBP, were recorded: abdominal discomfort (feeling of fullness), abdominal pain/tenderness, fever, and a change in mental status (recorded as HE grade).(14) Recent gastrointestinal (GI) bleeding was defined as a diagnosed upper GI bleeding in the 72 hours prior to index paracentesis. During the study period, the standard primary antibiotic treatment in patients with variceal bleeding was oral norfloxacin 400 mg twice daily or intravenous ceftriaxone 1 g daily during five days. The secondary prophylaxis for SBP was norfloxacin 400 mg once daily.(8, 9)

Statistical analysis

Continuous variables were reported as mean with standard deviation (SD), after visual confirmation of approximate normality, and compared using the Student's *T*-test. Categorical variables were reported as count with proportion and compared using the Chi-square test. A two-sided *p*-value <0.05 was considered significant.

Transplantation-free survival was analyzed using Kaplan-Meier survival analysis. Follow-up started at the time of the first ascitic fluid analysis. A multivariable logistic regression analysis was carried out to identify predictors for treatment of bacterascites, a multivariable logistic regression analysis in the untreated patient group to identify risk factors for worse outcome (i.e. liver-related death before culture results were known, SBP development, and persisting bacterascites), and a multivariable Cox's proportional hazard analysis to identify independent predictors for 3-month mortality. These analyses were performed using the candidate predictor variables: age, gender, etiology of liver disease, MELD score, hepatocellular carcinoma, gastrointestinal bleeding, HE, grade of ascites, symptoms of infections, immunosuppressive medication use, antibiotic prophylaxis use, Staphylococci cultured, and PMN count in ascites, with the addition of initiation of antibiotic treatment for bacterascites in the Cox's regression. The regression models were employed using the backward stepwise selection method with removal testing based on the probability of the likelihood-ratio statistic. Statistical analyses were performed using IBM® SPSS® Statistics for Windows, version 21.0.

RESULTS

Patients

Between January 2003 and August 2016, 142 episodes of bacterascites were diagnosed in 123 patients. The demographic, clinical and laboratory data are summarized in Table 1. Patients with bacterascites were mainly male with a mean age of 63 years (±14) and a median MELD score of 20 (IQR 14–25).

Table 1. Baseline demographic and clinical patient characteristics.

	Patients with bacterascites (n=123)
Male, *n (%)*	76 (62%)
Age in years, *mean (SD)*	63 (± 14)
Etiology of cirrhosis, *n (%)*	
Alcohol	35 (29%)
Viral	26 (21%)
Auto-immune related	19 (15%)
Alcohol + viral	10 (8%)
NASH	9 (7%)
Other	24 (20%)
MELD score, *median (IQR)*	20 (14 – 25)
Child-Pugh score, *median (IQR)*	8 (7 – 10)
Child-Pugh class, *n (%)*	
class A	30 (24%)
class B	61 (50%)
class C	32 (26%)
HCC, *n (%)*	21 (17%)
Sodium (mmol/L), *mean (SD)*	136 (± 8)
Creatinin (µmol/L), *median (IQR)*	109 (73 – 168)
Albumin (g/L), *mean (SD)*	29 (± 6)
Total bilirubin (µmol/L), *median (IQR)*	51 (26 – 135)
INR, *mean (SD)*	1.7 (± 0.7)
Ascites, *n (%)*	
Diuretic-responsive	33 (27%)
Diuretic-refractory	90 (73%)
Hepatic encephalopathy, *n (%)*	
None	73 (59%)
West Haven grade 1 - 2	32 (26%)
West Haven grade 3 - 4	18 (15%)
PMN count in ascites (cells/µL), *mean (SD)*	48 (± 61)
Protein level in ascites (g/L), *mean (SD)*	16 (± 10)

Table 1. Baseline demographic and clinical patient characteristics. *(continued)*

Recent GI bleed, *n (%)*	35 (28%)
Use of norfloxacin, *n (%)*	
Primary prophylaxis	27 (22%)
Secondary prophylaxis	-
Admission status during paracentesis, *n (%)*	
Inpatient	103 (84%)
Outpatient	20 (16%)

Abbreviations: GI, gastrointestinal; HCC, hepatocellular carcinoma; INR, international normalized ratio; IQR, interquartile range; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; PMN, polymorphonuclear neutrophil

Bacterascites

The infection was in 11% of the bacterascites episodes community acquired, in 55% health-care related, and in 34% nosocomial acquired.

One or more clinical symptoms of infection were present in the majority (78%) of patients with bacterascites. Sole abdominal discomfort was reported by 18%, HE by 16%, abdominal pain by 9%, and fevers or chills by 3%. A combination of these symptoms were present in 32%: 13% had HE and abdominal pain, 11% HE, fever and abdominal pain, 5% fever and abdominal pain, and 3% HE and fever.

Symptomatic patients had a similar age (mean 64 vs 63 years; p=0.907), MELD score (median 20 vs 19 points; p=0.313), serum creatinine (median 106 vs 104 mmol/L; p=0.606) and PMN count in ascites (median 23 vs 19 cells/μL; p=0.576) compared to asymptomatic patients. Table 2 shows additional characteristics in relation to the presence of symptoms. Monomicrobial bacterascites was just as likely to be symptomatic as polymicrobial bacterascites (p=0.660). Seventeen percent of patients with bacterascites were diagnosed with concomitant infections of the respiratory tract (6%), urinary tract (5%) or skin (6%). Concomitant blood cultures were obtained in 42% and 17% of symptomatic and asymptomatic cases, respectively. The proportion of positive blood cultures did not statistically differ between the groups.

Fourteen patients were diagnosed with more than one episode of bacterascites. In 5 patients, the second episode was diagnosed within 5 days, and in 9 patients after a median time of 31 days. Of these 14 patients, 9 patients died, 4 patients received a liver transplant, and 1 patient was lost to-follow-up. The median time till one of the endpoints was reached was 74 days. When patients with a single episode were compared with patients with multiple episodes, there were no statistical differences in age, gender, MELD score, PMN count in ascites, presence of symptoms, antibiotic prophylaxis use, or presence of a GI bleed.

In 36 of 142 bacterascites episodes (25.4%), an admission to the intensive care unit (ICU) was necessary; 14 cases (9.9%) of bacterascites were diagnosed on the ICU and in 22 cases (15.5%) patients were admitted to the ICU after organ failure development following bacterascites diagnosis. ICU admission was less than 7 days in 21 cases, between 7 and 14 days in 6 cases, between 14 and 30 days in 4 cases, longer than 30 days in 5 cases.

Table 2. Clinical and infection characteristics according to symptoms associated with bacterascites (N=142 episodes).

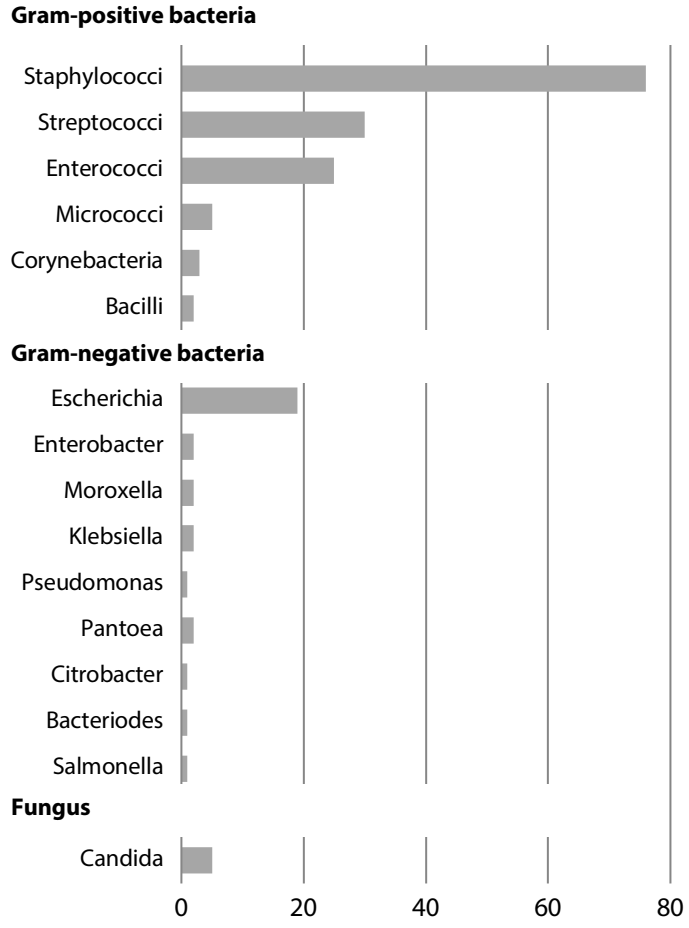
	No symptoms (N=31)	Any symptoms (N=111)	Abdominal discomfort (N=26)*	Abdominal pain (N=52)*	New-onset/ worsening HE (N=56)*	Fever (N = 32)*
Clinical characteristics						
MELD score, *median (IQR)*	19 (10)	20 (11)	17 (7)	21 (12)	23 (11)	25 (10)
PMN count in ascites (cells/μL), *median (IQR)*	19 (44)	23 (50)	28 (41)	30 (62)	21 (48)	40 (79)
Infection characteristics						
Acquisition infection, *N (%)*						
Community acquired	4 (13%)	11 (10%)	3 (11%)	6 (12%)	3 (5%)	3 (9%)
Health-care acquired	15 (48%)	63 (57%)	14 (54%)	35 (67%)	30 (54%)	18 (56%)
Nosocomial	12 (39%)	37 (33%)	9 (35%)	11 (21%)	23 (41%)	11 (35%)
Repeated paracenteses after 48 hours, *N (%)*	15 (48%)	70 (63%)	17 (65%)	30 (58%)	32 (57%)	18 (56%)
Ascites culture monomicrobial, *N (%)*	26 (84%)	89 (80%)	21 (81%)	39 (75%)	46 (82%)	24 (75%)
Concomitant infection, *N (%)*						
Respiratory tract	1 (3%)	8 (7%)	0	3 (6%)	7 (12%)	6 (19%)
Skin	1 (3%)	8 (7%)	1 (4%)	6 (12%)	4 (7%)	2 (6%)
Urinary tract	0	7 (6%)	0	3 (6%)	3 (5%)	4 (12%)
Concomitant blood culture, *N (%)*						
Non taken	25 (80%)	65 (59%)	23 (88%)	24 (46%)	27 (48%)	10 (31%)
Negative	3 (10%)	21 (19%)	1 (4%)	13 (25%)	15 (27%)	8 (25%)
Positive	3 (10%)	25 (22%)	2 (8%)	15 (29%)	14 (25%)	14 (44%)

*Patients could have multiple symptoms per episode. More details are described in the Results section in the paragraph Bacterascites.

Microbiology

Monomicrobial bacterascites was found in 81% of all episodes, consequently multiple pathogens were cultured in 19% of all episodes. In polymicrobial bacterascites two or three different organisms were cultured. Gram-positive bacteria were predominantly cultured in monomicrobial bacterascites. The 177 species cultured in monomicrobial and polymicrobial bacterascites are listed in Figure 1. Staphylococci were most often isolated (43%), followed by Streptococci (17%), Enterococci (14%) and Escherichia (11%). The cultured species of these four most common found genera are subtyped in Supplementary Figure 1.

Figure 1. Type of pathogens cultured in 142 bacterascites episodes classified by genus in absolute numbers.



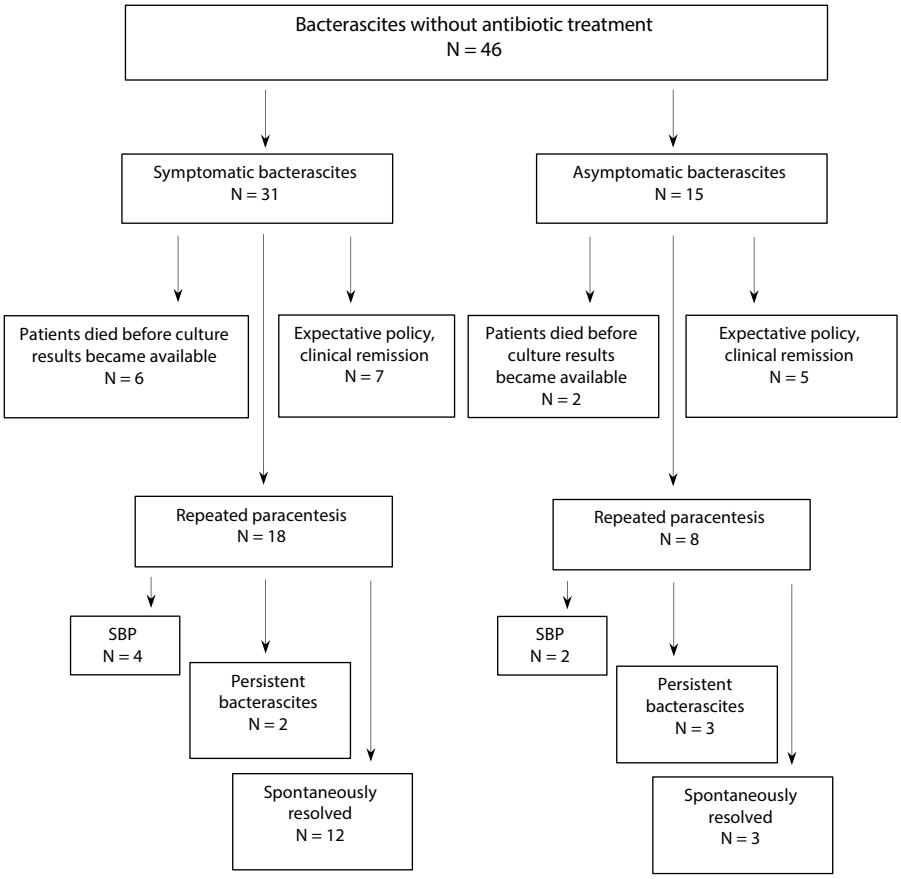
Multidrug-antibiotic resistant (MDR) organisms were isolated in 25% of all episodes. Methicillin-resistant Staphylococci were the most frequently found MDR organism (N=26), followed by extended-spectrum beta-lactamase (ESBL) producing bacteria (N=8). A similar proportion of patients using primary antibiotic prophylaxis were infected with a MDR organism (29.0%) compared to patients without prophylaxis (23.1%) (p=0.506).

Antibiotic therapy

In 96 (68%) of the total 142 episodes of bacterascites antibiotic treatment was initiated: in 49 episodes after paracentesis and before culture results became available, in 47 episodes after culture results were known. In 16 cases the antibiotic treatment was modified based on culture results. Amoxicillin-clavulanic acid combination was most often prescribed (30%), followed by cephalosporin (14%) and vancomycin (10%). Symptomatic patients more often received treatment compared to asymptomatic patients (72% vs 52%; p=0.031). Patients with higher MELD score (HR 1.156 per point, 95%CI 1.060–1.260, p=0.001), higher PMN count in ascites (HR 1.017 per point, 95%CI 1.005–1.030, p=0.007), an infection with another bacterial genus than Staphylococci (HR 3.512, 95%CI 1.333–9.253, p=0.011), and a female gender (HR 2.837, 95%CI 1.066–7.547, p=0.037) were more likely to receive antibiotic treatment for bacterascites.

In 46 episodes of bacterascites, antibiotic treatment was not initiated. In 31/46 episodes (67%), patients had signs or symptoms of infection. A total of 111 episodes of bacterascites was symptomatic. The ascitic PMN count in 80 patients who were treated with antibiotics was significantly higher (median 31, range 0–235) than the count (median 13, range 0-71) in those patients (n=31) who did not receive antibiotic treatment (p=0.002). Figure 2 shows a flow chart of the clinical course of the untreated episodes of bacterascites. Of these 46 bacterascites episodes, the patient died before culture results were known in 8 cases, in all cases the patients died of decompensating liver disease. These 8 patients had a median MELD score of 29 (IQR 18–30), median serum creatinine of 147 µmol/L (IQR 100–250), and 5/8 patients were admitted at the ICU; either before paracentesis (2 patients), or after paracentesis (3 patients). SBP developed in 6 cases, and bacterascites persisted in 5 cases. The latter group and those diagnosed with SBP were immediately treated with antibiotics. A logistic regression analysis was performed in the untreated patients to identify risk factors for liver-related death before culture results were known, SBP development, and persisting bacterascites (19/46) compared to clinical remission (27/46). We found that MELD score (HR 1.286 per point, 95%CI 1.071–1.546, p=0.007) and age (HR 1.113 per year, 95%CI 1.027–1.205, p=0.009) were independent risk factors.

Figure 2. The clinical course of patients with bacterascites without antibiotic treatment.



Clinical course and outcome

The survival analyses included 114 patients with bacterascites and 88 patients with SBP, after the exclusion of patients with both SBP and bacterascites. The median follow-up time in 114 patients was 38 days (IQR 15–272 days). In this study cohort, 27 patients were alive or lost to follow-up, 16 patients received a liver transplant, and 71 patients died. The causes of death were in 36 patients (50.7%) liver disease-related, in 29 patients (40.9%) unknown, and in 6 patients (8.4%) a combination of liver disease-related and non-liver disease-related.

The cumulative mortality rates in bacterascites patients (1-month: 36%; 3-month: 56%; 6-month: 62%; 1-year: 66%) are statistically comparable to that reported for SBP patients (1-month: 34%; 3-month: 54%; 6-month: 67%; 1-year: 77%) (log-rank test p=0.397).(Figure 3)

The most important predictive factors for 3-month mortality after bacterascites diagnosis were: MELD score and the presence of hepatic encephalopathy.(Table 3) Figure 4 shows the MELD score-dependent relation of survival in 114 patients with bacterascites and 88 patients with SBP.

Table 3. Independent predictive factors of 114 bacterascites patients predicting 3-month mortality (58 events) identified by multivariable Cox-regression analysis.

	HR	**95% CI**	***p*-value**
MELD score (per point)	1.099	1.082 – 1.156	<0.001
Hepatic encephalopathy			0.002
None (reference)	1		
West Haven grade 1 – 2	1.411	0.697 – 2.856	
West Haven grade 3 – 4	3.209	1.614 – 6.381	

Abbreviations: MELD, model for end-stage liver disease.

Figure 3. Comparable cumulative survival curves shown for 114 patients with bacterascites (grey solid line) and 88 patients with SBP (black solid line) (log-rank test p=0.3973). The dashed lines with corresponding colors display the 95% confidence interval.

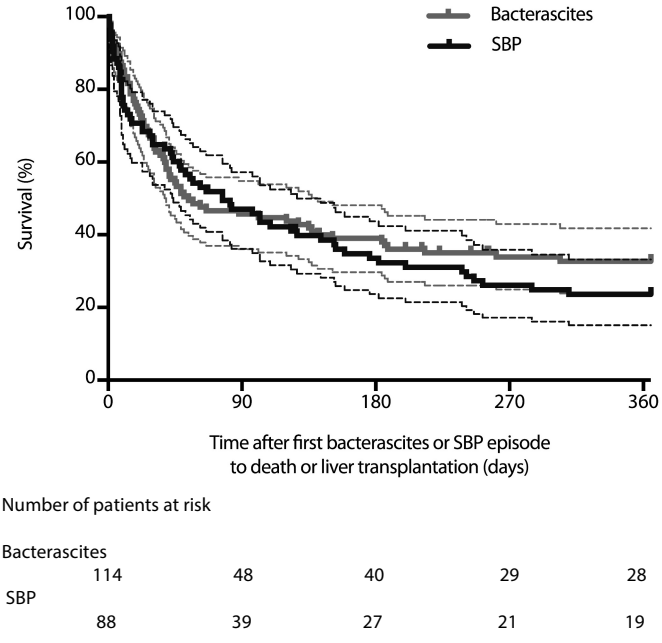
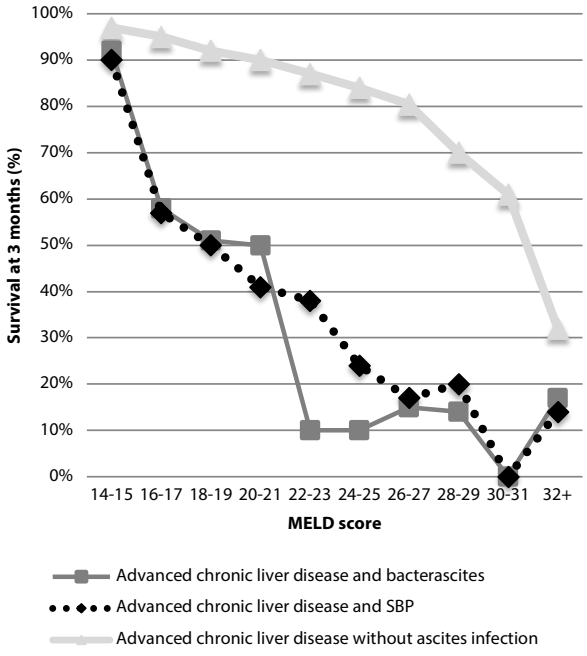


Figure 4. The figure shows a MELD score-dependent relation for the three-month survival after bacterascites diagnosis of 114 patients (dark grey solid line with squares). The survival of 88 patients with SBP (black broken line with diamonds) and advanced chronic liver disease (light grey solid line with triangles) are plotted for comparison.



Bacterascites in comparison with SBP in the literature

Our literature search for relevant studies of SBP identified 17 publications.(Table 4)(10, 15-30) The reported baseline clinical characteristics including age, gender distribution, and liver disease severity scores of SBP were comparable to those identified in our patient population with bacterascites. In addition, the cumulative mortality rate in our bacterascites cohort (1-month: 36%; 6-month: 62%; 1-year: 66%) also appears comparable to that reported for SBP (1-month: 13-49%; 6-month: 52-59%: 1-year: 49-70%).

Table 4. Overview of observational studies assessing the clinical characteristics and mortality rates of patients with SBP in comparison with our present study with bacterascites patients.

Author, year [corresponding number in reference list]	Number of patients	Male gender (%)	Mean age	CP class A/B/C (%) or mean CP score	Mean MELD	1-month or in-hospital mortality	6-month mortality	1-year mortality
Toledo, 1993 (15)	185	64%	56	1/22/74	-	44%	-	-
Follo, 1994 (16)	197	63%	55	-	-	24%	-	-
Navasa, 1998 (17)	52	63%	64	10.5	-	23%	-	-
Thuluvath, 2001 (18)	348	57%	58	-	-	33%	-	-
Soylu, 2005 (19)	87	71%	53	0/10/90	-	26%	-	-
Song, 2006 (20)	106	79%	55	0/28/72	-	33%	59%	-
Nobre, 2008 (21)	73	77%	62	0/23/77	23	37%	-	-
Cheong, 2009 (22)	236	70%	57	10.6	-	49%	-	-
Terg, 2009 (23)	127	-	-	-	18	17%	-	-
Kim, 2010 (24)	130	68%	52	10.7	-	13%	52%	70%
Tsung, 2013 (25)	95	74%	59	2/31/67	-	39%	55%	63%
Tandon, 2013 (26)	184	66%	55	-	20	27%	-	-
Cho, 2014 (27)	336	77%	61	10.9	22	38%	-	
Lim, 2014 (28)	75	88%	59	11.0	19	25%	-	-
Hassan, 2015 (29)	100	68%	57	0/15/85	18	22%	-	-
Balaraju, 2017 (30)	150	86%	48	5/21/74	22	31%	59%	-
Oey, 2017 (10)	95	62%	54	5/35/60	21	33%	-	49%
Present study	114	62%	63	24/50/26	20	36%	62%	66%

Abbreviations: CP, Child-Pugh; MELD, model for end-stage liver disease.

DISCUSSION

Bacterascites is an infectious complication occurring in patients with advanced or end-stage liver disease, and is associated with a high short-term mortality risk. Bacterascites tends to persist, or to evolve to SBP, in a significant proportion of cases. Further, our findings indicate that bacterascites diagnosed in in-hospital patients has great similarities to SBP. In particular, patients with bacterascites and SBP present with a similar degree of liver insufficiency and have a comparable poor prognosis.

In our cohort, 78% of patients showed clinical symptoms of infection, which is reasonably similar to the rates of 66-71% in previously published studies.(4, 6) Although it is generally accepted that SBP is frequently accompanied by clinical symptoms, a proportion of 13-32% patients with SBP has been reported to be asymptomatic.(6)

With the results of this study, it is difficult to clearly elucidate the underlying pathogenesis of bacterascites. Gram-positive bacteria were frequently isolated in bacterascites, which is in line with findings from other bacterascites series.(2, 5-7) However, only a minority of episodes of bacterascites was considered to be due to exogenous contamination. In many cases, patients showed evident symptoms of infection, had a concomitant positive blood culture, or there were evidence of a porte d'entrée from the skin through an inserted line or catheter. It could be hypothesized that Gram-positive bacteria are less virulent or less likely to induce an inflammatory host reaction, which has been previously postulated in studies analyzing patients with bacteremia.(31, 32) Furthermore, traumatic paracentesis were highly unlikely to explain any cases of bacterascites in our cohort. In all polymicrobial bacteracites a maximum of three different organisms were cultured and not the variety of bowel flora expected after perforation.(33) Thus, it is more likely bacterascites is an actual colonization of ascites, either behaving as a different form of peritonitis or with a large probability of evolving into a classic infection.

Earlier reports have suggested bacterascites patients might have higher levels of bactericidal and opsonic activity, reflected by higher protein concentrations in ascites, preventing a full-blown inflammatory response.(4, 34) Despite the fact that protein concentrations in ascites were measured on an irregular basis in our cohort, the mean protein level of 16 g/L does not indicate patients are deviant from SBP patients.(35, 36)
Unexpectedly, female gender was one of the factors increasing the odds to receive antibiotic treatment for bacterascites. We found a correlation between female gender with auto-immune-related liver disease etiology and immunosuppressant use. Female patients were more likely to have auto-immune hepatitis, primary biliary cirrhosis, or non-alcoholic steatohepatitis and more often used immunosuppressive medication. Possibly, the threshold to prescribe antibiotic treatment for bacterascites is lower in patients using immunosuppressive medication.

One of the goals of this study was to determine the clinical course of bacterascites. In two-thirds of the cases, the treating physician decided to initiate antibiotic treatment.

However, in the 46 episodes not treated with antibiotics, 17% died before culture results were known. In the 26 untreated patients undergoing repeated paracentesis, 42% were diagnosed with either ongoing bacterascites or SBP.

The results of this study do not support the importance to distinguish clinically between symptomatic and asymptomatic bacterascites. The proportion of untreated bacterascites, which spontaneously resolved, was equal in symptomatic and asymptomatic patients. Furthermore, the presence of signs or symptoms of infection was not an independent predictor for mortality.

The rate of 25% MDR bacteria found in all bacterascites episodes was relatively high for the Netherlands, but it is in line with current international microbiologic SBP studies reporting MDR bacteria rates of 27–67%.(37-39) The involvement of MDR bacteria in bacterascites was not associated independently with mortality in our study. Whether there is an independent association between MDR bacteria and a worse prognosis is still unclear with contrasting findings in studies regarding SBP.(10, 20, 22, 40, 41)

As shown in Figure 4, the prognosis after bacterascites is worse than the reported prognosis based on the MELD score, as developed in a large cohort of patients with advanced chronic liver disease.(11, 12) The relatively high rate of short-term mortality suggests bacterascites is either directly endangering the patient or a symptom of a critical condition. Therefore, this data suggests that these patients should be medically supported by all available means including antibiotic treatment. Timely and appropriate antibiotic treatment, as has been proven effective in SBP, seems appropriate in bacterascites.(9) Taking in consideration that 27 of the 46 untreated cases of bacterascites in our cohort spontaneously resolved bacterascites, this clinical measure might induce a significant over-treatment. This study evidently does not proof that treating bacterascites in patients with spontaneously resolving infection will improve prognosis. Although we found age and MELD score important predictors for patients with a worse clinical course, it is clinically difficult to accurately distinguish these patients from patients resolving the infection spontaneously.

To the best of our knowledge, this is one of the first studies concentrating solely on bacterascites by analyzing a large cohort of consecutive patients. Our cohort with 123 patients is substantially larger than previously reported cohorts including 18-48 patients. (2-7) One of the limitations of this study is that, due to the retrospective design, the natural course of bacterascites could not be optimally studied. For instance, it may well be that patients received antibiotic treatment while the bacterascites would have resolved spontaneously. It should also be pointed out that 14 patients had multiple bacterascites episodes, which could have led to a possible statistical bias, since a correlation between episodes of the same patient was ignored. Prospective studies would be necessary to further define the natural history of bacterascites and the optimal diagnostic and therapeutic strategy. Such studies could also confirm our finding that bacterascites carries a mortality risk comparable to that of SBP.

In conclusion, bacterascites is a complication of cirrhosis comparable to SBP with respect to clinical background and prognosis. Also considering that bacterascites seems to persist or to evolve into SBP in a substantial proportion of cases, with no clear differences in the course of symptomatic versus asymptomatic patients, our results may suggest that the (antibiotic) treatment strategy in bacterascites and SBP should be the same.

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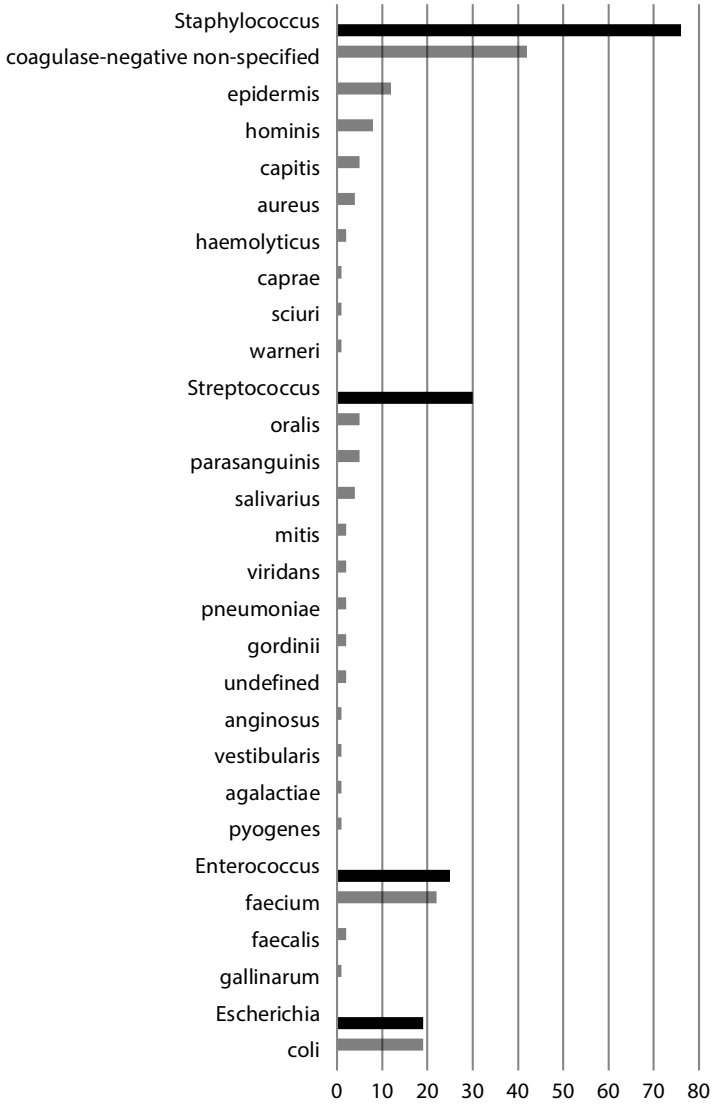
Supplementary Figure 1. Four most common found bacteria genera cultured in bacterascites subtyped by species in absolute numbers.



CHAPTER 6

The impact of infections on delisting patients from the liver transplantation waiting list

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ABSTRACT

Background: Approximately 20% of the patients listed for liver transplantation die before transplantation can be accomplished. Understanding risk factors for waiting list mortality may help to improve survival and organ allocation. Infections are very common in patients with cirrhosis and are associated with significant morbidity and mortality. This study analysed the frequency and characteristics of infections in patients awaiting liver transplantation, identified risk factors for withdrawal from the waiting list and evaluated the impact of infections on the clinical outcome.

Methods: Retrospective analysis of consecutive patients listed for liver transplantation in Erasmus MC, University Medical Center, Rotterdam, the Netherlands from 2007 to 2014.

Results: Infections occurred in 144 of 327 studied patients (44%). In this cohort, 23.4% of the patients on the liver transplantation waiting list were delisted or died before transplantation. Patients with an infection were 5.2 times more likely to become delisted than non-infected patients. In the 30 days after the first infection patients were 33.8 times more likely to become delisted compared to non-infected patients. High age, high MELD score, refractory ascites and inappropriate antibiotic therapy were independent predictors for delisting due to infection.

Conclusions: Infections occur frequently in patients on the liver transplantation waiting list and rapidly worsen patient's prognosis.

INTRODUCTION

Liver transplantation (LT) is a life-saving procedure for patients with sustained irreversible liver injury.(1, 2) However, LT from deceased donors is limited by the scarcity of suitable donor organs. The accumulating incidence of liver disease worldwide increases donor organ shortage and leads to a prolonged time for patients on the liver transplantation waiting list.(3) The median pretransplant waiting time among active wait-listed adults was 9 months in 2015 in the United States and approximately 10 months in the Eurotransplant region.(4, 5) In the United States 19.8% of the listed patients died in 2015 before transplantation could be accomplished, which was comparable to the 18.4% mortality of listed patients in the Eurotransplant region.(4, 5)

During the time awaiting transplantation, patients are at risk for progressive liver failure.(6) Infections are an important precipitating factor for acute decompensation and acute-on-chronic liver failure.(7, 8)

Infections are present at admission or develop during hospitalization in 20 – 60 % of patients with liver cirrhosis and are associated with 4-fold increased mortality; up to 30% of patients has been reported to die within 1 month and another 30% within 1 year. (9-11) Intestinal bacterial overgrowth, increased bacterial translocation, and an altered inflammatory response are considered major etiological factors.(12, 13)

Knowledge about risk factors for waiting list mortality may help improve organ allocation and reduce waiting list mortality. A recent study found that hospitalized cirrhotic patients with infections complicated by extrahepatic organ failure are at higher risk for delisting and death before LT.(14) However, the frequency of infections in wait-listed patients and the subsequent risk of delisting and death after infection have not been clearly established.

This study aimed to (1) analyse the frequency and epidemiology of infections in patients awaiting LT, (2) identify risk factors for infection-related removal from the waiting list, and (3) evaluate the impact of having an infection on the clinical outcome of listed patients.

PATIENTS AND METHODS

Patients

All consecutive patients on the liver transplantation waiting list from 2007 – 2014 at Erasmus MC, University Medical Center, Rotterdam, were studied retrospectively. Patients with acute liver failure or listed for a non-primary liver graft were excluded. Patients delisted because of clinical improvement, intercurrent psychiatric disorders (mostly substance-related disorders), non-liver-related mortality, or patients declining an offered organ were excluded.

Data collection

Demographic and clinical data, and information on the clinical course, including details of infectious complications, were retrieved from hospital medical records. Diagnosis of infection and the type of infection were made according to definitions formulated by the Centers for Disease Control (CDC).(15-18) Episodes, clinically interpreted and treated as infection, without satisfying CDC-criteria were reviewed by two clinicians (infectious disease specialist and research physician). Statistical sensitivity analyses were performed to assess whether this subgroup was comparable to the group meeting CDC criteria for infection. All infections of patients were evaluated; hospitalized and non-hospitalized infections in both our center and in other centers. Infections taking place in other centres were communicated to physicians of our transplant center. Additional information was requested if information regarding the infection in other centres was not sufficient. Multidrug-resistant (MDR) bacteria were defined as bacteria with non-susceptibility to at least one agent in three or more antimicrobial categories, extended-spectrum β-lactamase (ESBL), or carbapenemase-producing Enterobacteriaceae.(19) Inappropriate antibiotic therapy was defined as: use of antimicrobial agents to which a pathogen was resistant in vitro or administration of antibiotic therapy with a delay of at least 24 hours after diagnosis of infection. Multidrug resistance and inappropriate antibiotic therapy were determined in a subgroup of patients with available antimicrobial susceptibility patterns and sufficient information about the timing of antibiotic therapy. Renal failure was defined as increase in serum creatinine of >50% from baseline, or a rise in serum creatinine of ≥ 26.4 μmol/L (≥ 0.3 mg/dL) within 48 hours.(20) Refractory ascites was defined as ascites that did not recede or that reoccurred shortly after therapeutic paracentesis, despite sodium restriction and diuretic treatment.(21) Data were collected from the time of waiting list placement until the follow-up was completed. The follow-up was complete when a clinical endpoint was reached: 1) liver transplantation, 2) delisting or death due to infection, 3) delisting or death for other reasons (e.g. unmet Milan criteria), or 4) still registered on the waiting list on 1st May 2016. Delisting or death due to infection was defined as definite withdrawal from the list within 30 days after an infection was diagnosed due to clinical deterioration with suspicion of infection outside the liver. The endpoint 'becoming delisted from the liver transplantation waiting list due to infection' will be systematically used and will include: an inactive waiting list status without reactivation, delisting with infaust prognosis and death due to infection.

Statistical methods

A mean and standard deviation (SD) was computed for numerical variables, if normally distributed, and compared using the Student's *T*-test. Non-normal distributed continuous variables were summarized with a median and interquartile range (IQR), and compared using the Mann–Whitney Ranks Sum test. Categorical variables were expressed with

percentages and compared using the χ2 test. A two-sided *p*-value < 0.05 was considered significant. The probability for the occurrence of infection for the length of time after waiting list placement was presented using Kaplan-Meier. Patients were censored when a clinical endpoint was reached. Logistic regression modelling was employed to determine possible predictors for withdrawal from the waiting list due to infection and each determinant was reported with an odds ratio (OR). The analysed variables were age, gender, aetiology, MELD score, medication use, type of infection, microorganism of infection, MDR bacteria, inappropriate antibiotic therapy, events of acute decompensation, intensive care unit (ICU) admission, or an invasive procedure 30 days prior to infection. A time-dependent Cox proportional hazard model was used to study the non-proportional hazards effect of the first infection on the competing endpoints: liver transplantation, delisting or death due to infection, delisting or death with other reasons, and waiting on the list. The hazard for delisting is presumably highest during and right after the infection, while the hazard for liver transplantation commences to increase after the recovery of the infection. Thus, infection could have a non-proportional hazard on the competing endpoints compared to non-infected patients. The landmark analysis method was used to study time intervals after infection and the landmarks 30 days and 180 days after infection were chosen. The model was adjusted for covariates age, gender, aetiology, and MELD score at listing. The effect of the first infection on the various endpoints was assessed for the interval of 30 days following infection, the interval between 30 to 180 days following infection and after 180 days. Furthermore, the likelihood on becoming delisted in relation to the number of infections was analyzed using a multivariate Cox regression adjusted for age, gender, aetiology, and MELD score at listing. The odds on clinical endpoints are reported as hazard ratio (HR) on liver transplantation and delisting. In the logistic regression model, as well as the Cox proportional hazard models, variables with a *p*-value of < 0.20 in univariate analysis were included in a multivariate analysis, and maintained in the multivariate model with a *p*-value < 0.10. Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 21.0 (Armonk, NY: IBM Corp.).

RESULTS

Patients

Four hundred forty-five patients were registered on the national liver transplant waiting list between January 2007 and January 2014. Three hundred twenty-seven patients were eligible for the present analysis.(Figure 1) The mean follow-up time was 208 days (IQR 56 – 406). The study cohort included 217 men and 110 women. At time of waiting list placement, patients were aged 54 (IQR 46 - 60) years and had a median MELD score of 16 (IQR 11 – 19). The baseline demographics and clinical characteristics of infected and non-infected listed patients at waiting list placement are shown in Table 1. Patients with infections had more frequent viral hepatitis or primary sclerosing cholangitis (PSC) as

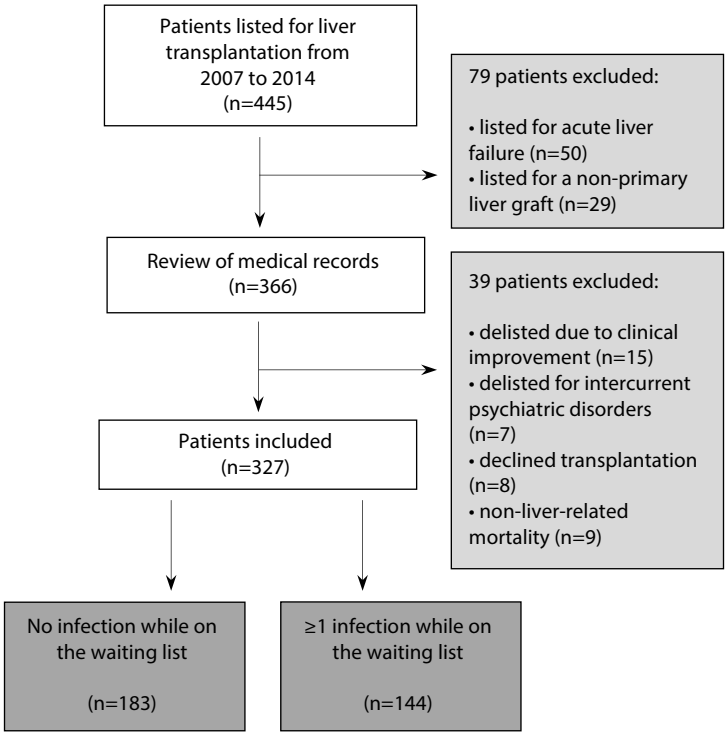
aetiology, and less frequent hepatocellular carcinoma (HCC) (p<0.001). Furthermore, patients with infections had higher baseline MELD scores (p=0.003), and more often used antibiotic prophylaxis (p=0.005), diuretics (p=0.005), and laxatives (p=0.026).

Table 1. Demographic and clinical characteristics of patients at the time of listing for liver transplantation with respect to development of infections.

Characteristics	**All patients n = 327**	**Patients without infection n = 183**	**Patients with infection(s) n = 144**	***p*-value**
Age (years)*	54 (46 - 60)	54 (48 - 61)	52 (43 – 59)	0.239**
Male gender, *n (%)*	217 (66%)	129 (70%)	88 (61%)	0.075
Blood group, *n (%)*				
0	146 (45%)	79 (43%)	67 (47%)	
A	117 (36%)	69 (38%)	48 (33%)	0.136
B	44 (13%)	20 (11%)	24 (17%)	
AB	20 (6%)	15 (8%)	5 (3%)	
Aetiology of liver disease, *n (%)*				
Alcohol	49 (15%)	28 (15%)	21 (15%)	
Viral	32 (10%)	7 (4%)	25 (17%)	
PSC	72 (22%)	28 (15%)	44 (31%)	**<0.001**
HCC	90 (27%)	76 (42%)	14 (10%)	
Auto-immune & PBC	23 (7%)	7 (4%)	16 (11%)	
Other	61 (19%)	37 (20%)	24 (17%)	
MELD score*	16 (11 - 19)	15 (10 - 18)	17 (14 – 20)	**0.003****
Child-Pugh score	8 (6 - 10)	8 (5-10)	9 (8-10)	**<0.001****
Medication use, *n (%)*				
Antibiotic prophylaxis	86 (26%)	37 (20%)	49 (34%)	**0.005**
Diuretics	196 (60%)	97 (53%)	99 (69%)	**0.005**
PPI	160 (49%)	82 (45%)	78 (54%)	0.102
Corticosteroids	31 (10%)	11 (6%)	20 (14%)	**0.016**
Non-corticosteroid immunosuppressives	19 (6%)	5 (3%)	14 (10%)	**0.008**
NSBB	112 (34%)	66 (36%)	46 (32%)	0.436
Laxatives	110 (34%)	52 (29%)	58 (40%)	**0.026**

* Data are displayed as median with interquartile range. ** Mann-Whitney Rank Sum test.
(HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; NSBB, non-selective beta-blocker; PBC, primary biliary cirrhosis; PPI, proton-pump inhibitor, PSC, primary sclerosing cholangitis)

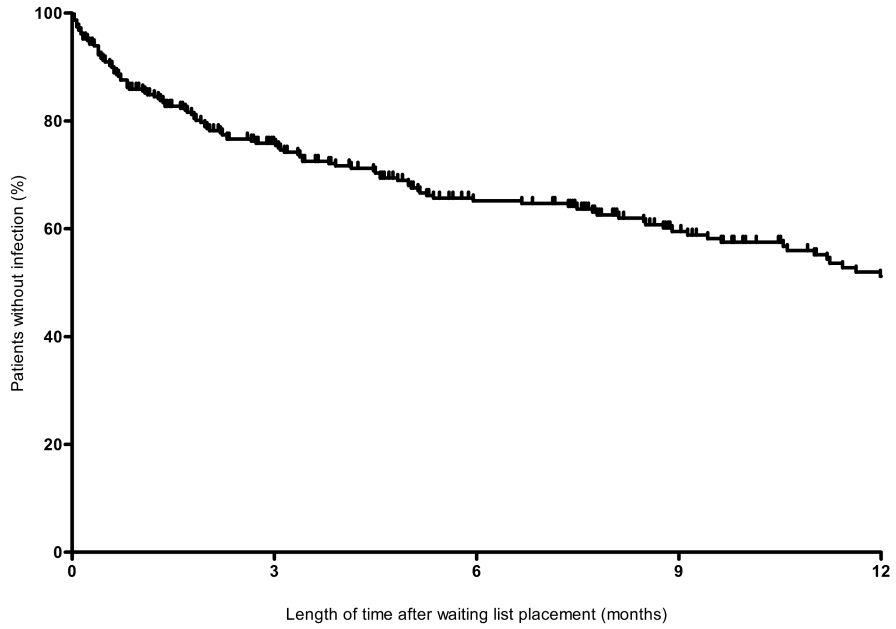
Figure 1. Flow diagram of study population.



Infections

In 144/327 (44%) of the listed patients at least one infection occurred; the number of infections in these patients ranged from one to eleven. The actuarial percentage of patients with an infection was 23% at 3 months, 29% at 6 months, 33% at 9 months and 37% after 12 months.(Figure 2) The median duration on the waiting list for patients with an infection was 381 days (IQR 137 - 753) compared to 163 days (IQR 43 - 320) for patients without infection (p<0.001). In total 318 infections occurred. Sixty-five patients experienced a single infection, 39 patients two infections, 40 patients three or more infections. Cholangitis (24%) was the most common infection, followed by spontaneous bacterial peritonitis (SBP) (18%), urinary tract infection (12%), respiratory infection (9%), bloodstream infection (7%), and gastro-intestinal infection (6%). The majority (83%) of infections were met by CDC criteria.

Figure 2. The actuarial percentage of patients without an infection in the first year after waiting list placement.



In 78/318 (25%) of all infections microbiological studies were negative. Gram-negative bacteria were cultured in 73 infections (22%) and Gram-positive bacteria in 58 infections (18%).(Table 2) The antimicrobial susceptibility patterns and sufficient information about the timing of antibiotic therapy were available in 190 infections. Of these infections, 25% were caused by multidrug-resistant organisms. The majority of multidrug-resistant organisms were Enterococci spp. (48%), followed by Enterobacteria spp. (32%), and Staphylococci spp. (13%). The initial antibiotic therapy was considered inappropriate in 34% of the infections. The reasons for inappropriate therapy were: microorganism not expected (Enterococcus n=20, Candida n=11, virus n=3, Pseudomonas n=1, Staphylococcus n=1, Streptococcus n=1), organism with acquired antibiotic resistance (n=14), negative cultures and clinical improvement after antibiotic switch (n=6), and administration of antibiotic therapy, according to guidelines, with a delay of at least 24 hours after diagnosis of infection (n=7).

Table 2. Results of microbiological studies for the most common types of infection.

Type of infection	Gram-negative bacteria	Gram-positive bacteria	Fungus	Multiple organisms	Negative or no culture performed
SBP (n=58)	21 (36%)	16 (28%)	0	2 (3%)	19 (33%)
Cholangitis (n=75)	11 (15%)	10 (13%)	0	3 (4%)	51 (68%)
Urinary tract (n=39)	19 (49%)	11 (28%)	0	2 (5%)	7 (18%)
Respiratory (n=29)	2 (7%)	1 (3%)	0	5 (17%)	21 (73%)
Bloodstream (n=22)	9 (41%)	8 (36%)	1 (5%)	4 (18%)	0
Gastro-intestinal (n=19)	4 (21%)	2 (11%)	2 (11%)	0	11 (57%)

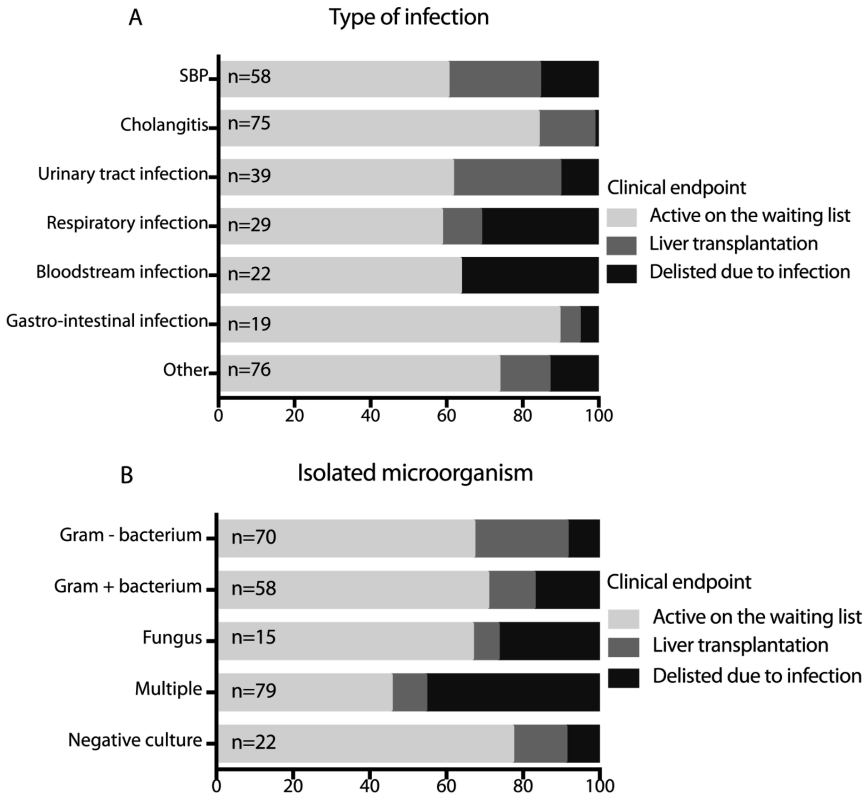
(SBP, spontaneous bacterial peritonitis)

Risk factors for delisting

In our study cohort, 245 (74.9%) patients underwent liver transplantation, 42 (12.8%) were delisted due to infection, 34 (10.4%) were delisted for other reasons, and 6 (1.8%) were still on the waiting list at the end of follow-up in the context of this study. The proportion of patients receiving a liver graft was higher in patients without infections (80.9%) as compared to patients with infections (67.4%) (p=0.012).

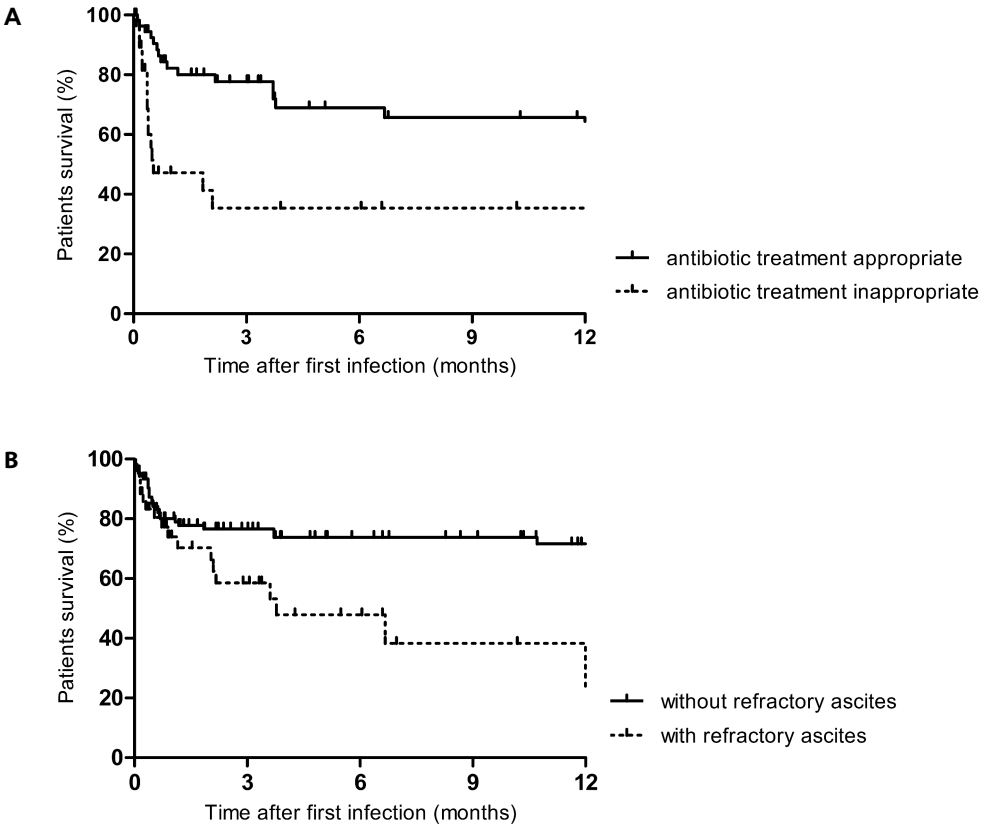
In 13.2% (42/318) of all infections, patients were delisted in the 30 days following infection. In this time interval no patients were delisted due to other reasons than infection. Risk factors associated with delisting due to infection were identified and univariate analysis indicated 15 possible predictors.(Supplement 1) Bloodstream infection, respiratory infection and SBP more often led to delisting compared to cholangitis, urinary tract infection and gastro-intestinal infection.(Figure 3a) In addition, delisting occurred more often after infections caused by multiple organisms or fungus in comparison with infection caused by single bacteria or when no microorganisms could be identified.(Figure 3b) Furthermore, an initial inappropriate antibiotic therapy and the presence of refractory ascites were significant predictors for delisting or death. (Figure 4)

Figure 3. Clinical endpoint 30 days after infection shown in boxplots for group variables A) type of infection (p=0.003), and B) isolated microorganism (p=0.001).



Multivariate logistic regression analysis revealed four independent predictors for delisting after adjusting for gender: age (OR 1.1 per year; 95% CI 1.0 – 1.2; p=0.001), MELD score (OR 1.3 per point; 95% CI 1.2 – 1.4; p<0.001), inappropriate antibiotic therapy (OR 3.7; 95% CI 1.1 – 12.4; p=0.035), and refractory ascites present within 30 days prior to infection (OR 3.3, 95% CI 0.9 – 12.0).(Table 3)

Figure 4. The survival of patients in the first year after the first infection. (A) Patients with an appropriate and inappropriate antibiotic treatment are shown in different curves. The solid line shows values for patients with an appropriate antibiotic treatment and the dotted line for patients with an inappropriate antibiotic treatment. (B) Patients with and without refractory ascites are shown in different curves. The solid line shows values for patients without refractory ascites and the dotted line for patients with refractory ascites.



Sensitivity analyses were performed on the subgroups of CDC-validated infections and non-CDC-validated infections. The multivariate logistic regression model identified the same predictors for delisting within the 30 days following infection in both groups. There were no statistical significant differences between the subgroups and the complete study cohort.

Table 3. Multivariate regression analysis of risk factors for infection-related withdrawal from the waiting list.

Risk factors	Odds ratio	95% CI	*p*-value
Multivariate model (adjusted for gender)			
Age at time of infection (per year)	1.133	1.049 – 1.223	**0.001**
MELD score (per point) at time of infection	1.295	1.169 – 1.435	**<0.001**
Refractory ascites 30 days prior to infection (n=83)	3.348	0.932 – 12.024	**0.064**
Inappropriate antibiotic therapy (n=58)	3.683	1.096 – 12.376	**0.035**

(MELD, model for end-stage liver disease)

The risk for delisting in the first month, half year, and afterwards

The Cox proportional hazard model showed that patients with one or more infections were more at risk of becoming delisted than patients without infections (HR 5.2; 95% CI 3.0 – 8.8; $p<0.001$). There is a time-dependent hazard for becoming delisted or receiving a liver transplant following the first infection compared to wait-listed patients without infection. The hazard for delisting is highly increased in the first 30 days after infection (HR 33.8; 95% CI 7.2 – 157.9; $p<0.001$), declines between 30 to 180 days (HR 5.7; 95% CI 2.6 – 12.3; $p<0.001$) and further after 180 days (HR 2.2; 95% CI 1.1 – 4.5; $p=0.036$).

Impact of the number of infections

The likelihood of delisting or death for patients with 1 infection (n=65), 2 infections (n=39), or ≥ 3 infections (n=40) was compared with patients without infection (n=183). (Supplement 2) The cumulative number of infections showed an increased risk for delisting after one and two infections (HR 12.1; 95% CI 6.8 – 21.7; $p<0.001$ and HR 25.0; 95% CI 13.1 – 47.8; $p<0.001$, respectively). This effect was attenuated in patients with 3 or more infections (HR 3.3; 95% CI 0.8 – 14.7; $p=0.114$).

DISCUSSION

This is the first study, to our knowledge, describing the impact of infection on liver transplantation waiting list dynamics. In this cohort, 23.4% of the patients became too sick or died before transplantation. Infection occurred in almost half of the patients (44%) and was the primary cause for delisting. Patients with an infection are 5.2 times more likely to become delisted than non-infected patients. In the 30 days after the first infection patients are likely to migrate from the waiting list with a hazard of 33.8 for becoming delisted. High age, high MELD score, initial inappropriate antibiotic therapy and the presence of refractory ascites were significant predictors for delisting or death.

The results from our study indicate infection is the leading cause for delisting. This endorses the hypothesis that infection is the most important precipitating event for acute decompensation and acute on chronic liver failure resulting in (multi)organ

failure.(22) Interestingly, the risk for death or delisting attenuates after 3 infections. Most of these patients were listed for PSC or auto-immune hepatitis and experienced recurrent cholangitis, which does not lead to delisting. The high incidence of PSC could explain the relative high frequency of cholangitis compared to other studies.(23, 24) Cholangitis leads to delisting infrequently (as shown in Figure 3). We therefore postulate, that PSC-related cholangitis leads to an increased burden of disease but does not affect the rate of delisting.

The observed epidemiological change that bacterial infections are more often caused by Gram-positive and MDR bacteria was confirmed in this study.(11, 25-28) The rate of 25% MDR bacteria found in the study population was not expected from earlier studies in the Netherlands.(29, 30) This can be explained mainly by the difference of international guidelines and the Dutch national guideline to define MDR organisms.(19, 31) In particular, the definition for multidrug-resistant Enterococci spp. is much broader in international guidelines, which explains the majority of MDR organisms.

In contrast to earlier studies, the use of antibiotic prophylaxis did not significantly protect patients for delisting within 30 days following infection. In patients with advanced liver disease, long-term administration of norfloxacin reduces the incidence of SBP, prevents further decompensation and improves survival.(32, 33) Several studies have already demonstrated that the current recommended antibiotic prophylaxis occasionally fails due to norfloxacin-resistant organisms.(25, 34, 35)

In this cohort, 23.4% of the patients became too sick or died before transplantation, which is the unfortunate reality previously reported with data from transplant allocation programs.(4, 5, 36, 37) A recent prospective study by Reddy et al. discusses the impact of infection in hospitalized patients listed for LT on clinical outcome. This study only included infected patients and did not contain a control cohort of patients without infections.(14) Our study population consisted of all patients registered for LT, including patients with cirrhosis as well as patients with HCC. Naturally, HCC patients follow a different course in progress of liver disease, featured by lower MELD score, less liver-related comorbidities and less infections.

Although the study was carefully prepared, this study entailed limitations arising from the study design and daily clinical practice. First, the retrospective design encompassed data from hospitalization episodes in other centres, which was occasionally unavailable. It was not feasible to differentiate between nosocomial, health care-acquired and community-acquired infections, because patients were not prospectively and systematically screened for infection on hospital admission. Secondly, 83% of infections were classified by the standardized CDC criteria while an expert committee categorized the other proportion. This is inevitable in clinical practice when bacterial and fungal cultures are not standard performed or sometimes fail. Thirdly, the absence of predefined criteria for delisting patients is leading to subjective decision-making based on an expert opinion of the transplant hepatologist, which is representative of what occurs in daily clinical practice.

Fourthly, information about temporary delisting was unfortunately not at hand. This could have biased the results, since patients with a systemic infection acquire a temporarily inactive status on the waiting list and are not eligible for liver transplantation at that very moment. Lastly, we analysed patients and waiting list practices in the Netherlands. The Dutch population is presumably listed more often with PSC and with lower MELD scores compared to patients on the waiting list in the United States.(4) The results should be translated with care to other centers and geographical regions.

The results of this study underline the importance of appropriate and timely antimicrobial therapy once more. The clinical importance has been discussed in multiple cohort studies including cirrhotic patients with SBP or septic shock.(38-41) However, the significance of this issue has not yet been demonstrated for various infections in patients waiting for LT.

Emphasis should be directed on the prevention and treatment for infection by adequate antibiotic prophylaxis and immediate effective antibiotics, respectively. Knowledge about multidrug-resistant bacteria and geographical susceptibility patterns is crucial in order to address these issues. We hypothesize the implementation of periodically microbial colonization swabs in listed patients could support clinicians to prescribe effective antimicrobial prophylaxis and initiate immediate successful treatment. Improving clinical care regarding infection prevention and treatment would hypothetically lower waiting list mortality and could positively influence the patient's pre-transplantation and post-transplantation condition.

Future studies could focus on this window of opportunity for LT after the infection. It is necessary to gather more understanding when infection is likely to resolve or worsen, and which patients can benefit from early LT. Identifying biological and clinical parameters during the infection and the recovery could assist physicians in waiting list decision-making of re-activating patients' waiting list status or delisting. Additionally, prospective studies could benefit the knowledge about the pathophysiology of the clinical deterioration following the infection. Following this argument, research needs to be conducted whether infection might be considered as an exception in the transplantation priority algorithm, similar to patients with HCC and PSC. At last, outlining defined criteria for delisting could make the decision as objective and well-considerate as the prioritization for LT.

In conclusion, this study demonstrates a large proportion of patients on the liver transplantation waiting list have infections. Infections have a negative effect on the outcome for patients and therefore antimicrobial schedules should be properly individual adapted for effective prophylaxis and treatment of infections.

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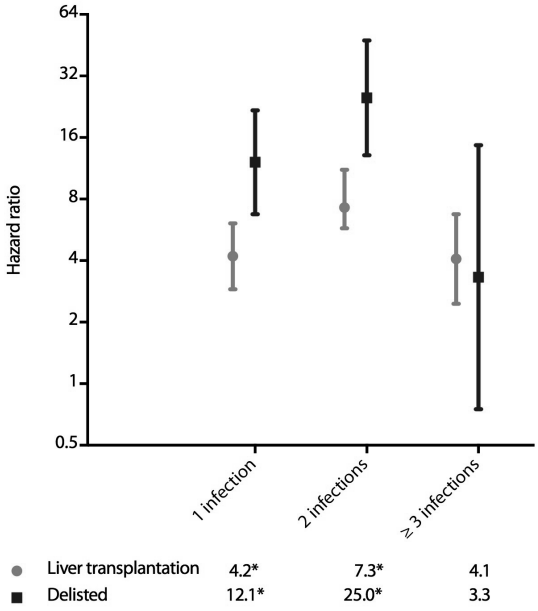
Supplement 1. Univariate regression analysis of risk factors for infection-related withdrawal from the waiting list.

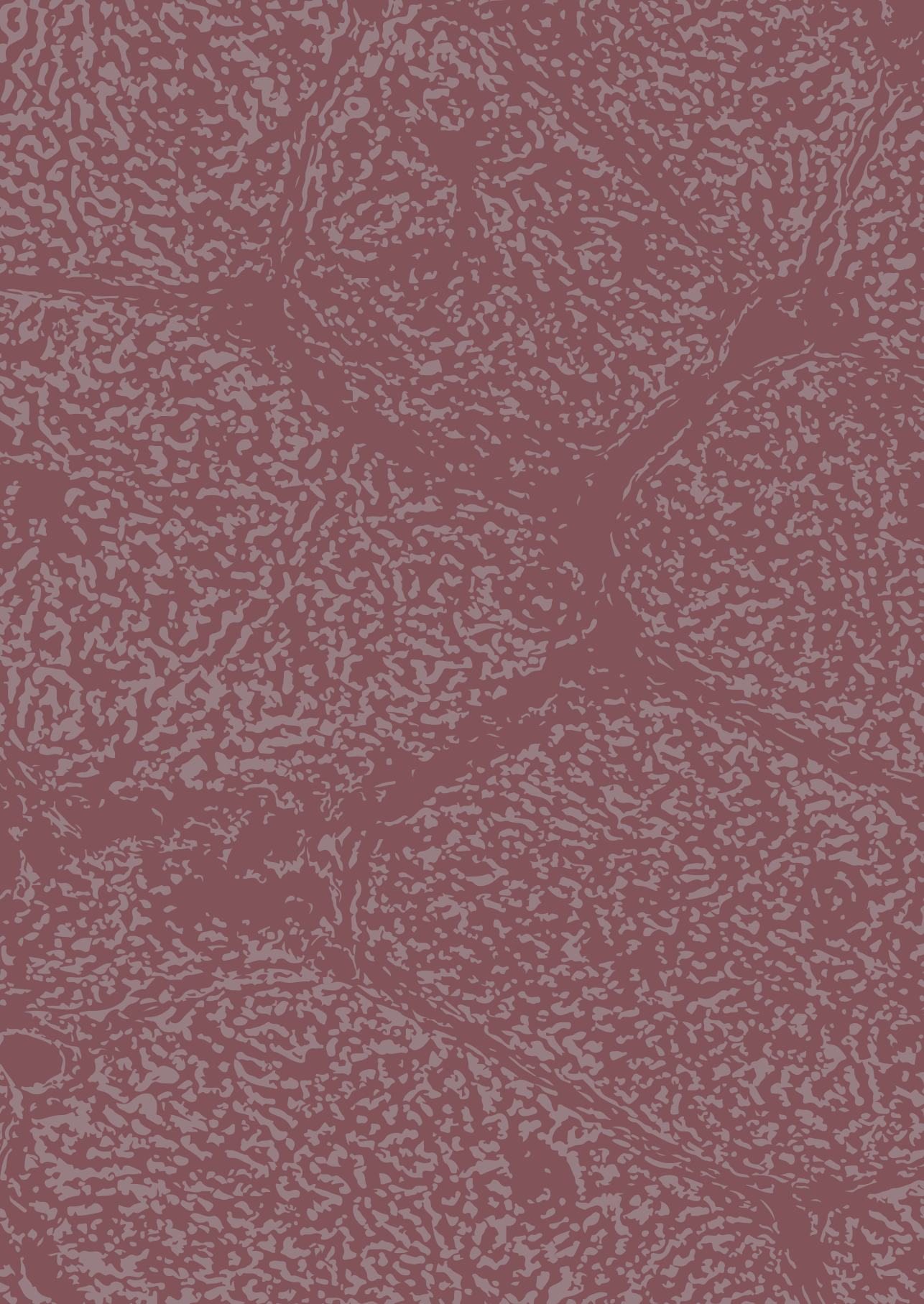
Risk factors	odds ratio	95% CI	*p*-value
Univariate model			
Age at time of infection (per year)	1.079	1.037 – 1.122	**<0.001**
Male gender	0.500	0.260 – 0.962	**0.038**
Aetiology of liver disease			
· Alcohol (n=33)	1 (ref.)		0.085
· Viral (n=52)	1.114	0.388 – 3.200	
· Auto-immune (n=147)	0.422	0.157 – 1.137	
· HCC (n=26)	0.310	0.058 – 1.639	
· Other (n=60)	0.413	0.126 -1.352	
MELD score (per point) at listing	1.102	1.049 – 1.157	**<0.001**
Medication use at listing			
· Diuretics (n=205)	4.757	1.813 – 12.482	**0.002**
· Laxatives (n=110)	1.679	0.870 – 3.241	**0.122**
Decompensation 30 days prior to infection			
· Kidney failure (n=55)	6.476	3.191 – 13.143	**<0.001**
· Refractory ascites (n=83)	3.590	1.829 – 7.049	**<0.001**
· Hepatic encephalopathy (n=83)	4.825	2.422 – 9.613	**<0.001**
· GI bleeding (n=35)	2.218	0.930 – 5.286	**0.072**
ICU admission 30 days prior to infection (n=29)	4.295	1.833 – 10.067	**0.001**
Invasive procedure 30 days prior to infection			
· Endoscopy (n=50)	1.933	0.877 – 4.262	**0.102**
· Drip feeding (n=29)	6.207	2.699 – 14.275	**<0.001**
· CVVH/MARS (n=10)	18.461	4.558 – 74.769	**<0.001**
· Mechanical ventilation (n=12)	10.994	3.305 – 36.569	**<0.001**
Type of infection			**0.003**
· SBP (n=58)	1 (ref.)		
· Cholangitis (n=75)	0.074	0.009 – 0.599	
· Urinary tract infection (n=39)	0.622	0.177 – 2.183	
· Respiratory infection (n=29)	2.450	0.849 – 7.073	
· Sepsis (n=22)	3.111	1.013 – 9.558	
· Gastro-intestinal infection (n=19)	0.302	0.036 – 2.559	
· Other (n=76)	0.825	0.312 – 2.184	
Microorganism			**0.001**
Gram-negative bacterium (n=73)	1 (ref.)		
Gram-positive bacterium (n=58)	2.326	0.792 – 6.836	
Fungus (n=15)	4.061	0.985 – 16.745	
Multiple organisms (n=22)	9.306	2.848 – 30.405	
Negative culture (n=78)	1.101	0.352 – 3.444	
MELD score (per point) during infection	1.218	1.150 – 1.291	**<0.001**
Antibiotic therapy inappropriate (n=58)	3.463	1.483 – 8.087	**0.004**

(CI, confidence interval; CVVH, continuous veno-venous hemofiltration; GI, gastro-intestinal; HCC, hepatocellular carcinoma; ICU, intensive care unit; MDR, multidrug-resistant; MARS, molecular adsorbent recirculating system; MELD, model for end-stage liver disease; SBP, spontaneous bacterial peritonitis)

Supplement 2. Hazard ratios for liver transplantation or delisting from the liver transplantation list in patients with 1 infection, 2 infections, and ≥ 3 infections compared with patients without infection. The hazard ratios are obtained using a time-dependent Cox proportional hazard model and the model was adjusted for age, gender, and MELD score at listing.



Results are presented as hazard ratio (HR) and 95% confidence interval (CI).
* *p*-value < 0.05.



Part III

FINDINGS AND IMPLICATIONS OF DIAGNOSTIC ASSESSMENTS DURING LIVER TRANSPLANTATION SCREENING

CHAPTER 7

The yield and safety of screening colonoscopy in patients evaluated for liver transplantation

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ABSTRACT

Background: Colorectal cancer screening with colonoscopy is commonly used in candidate patients for liver transplantation. We initiated this study to define the risk-benefit ratio of performing screening colonoscopy in this population.

Methods: A retrospective observational study of all consecutive patients undergoing colonoscopy during pre-liver transplantation screening between 2004-2017 was conducted. Endoscopic and pathological findings and clinical events potentially related to the colonoscopy in the 30 days after the procedure were registered and compared with a 30 days in-patient control time frame.

Results: A total of 858 colonoscopies were performed in 808 patients (65% male; median age 55 years (IQR 47-62); median MELD score 15 (IQR 11-18)). Colorectal cancer was found in 2 patients (0.2%) and advanced adenomas in 44 patients (5.4%). The only independent risk factor for an advanced neoplasm was age (OR 1.072 per year; 95%CI 1.031-1.115; $p<0.001$).

During the 30 days post-procedure period 178 clinical events occurred in 128 patients compared to 101 clinical events in 72 patients in the control time frames ($p<0.001$). After colonoscopy, there was a significant increased risk for renal failure ($p=0.001$) and gastro-intestinal bleeding ($p=0.023$). Presence of ascites and MELD score were identified as independent risk factors for acute renal failure and gastro-intestinal bleeding. During the study observation period 53.5% of the screened population actually underwent liver transplantation.

Conclusion: Colorectal cancer screening in pre-liver transplantation patients is associated with a relatively low prevalence of colorectal cancer, and an increased risk of post-colonoscopy complications such as acute renal failure and gastrointestinal bleeding, especially in patients with advanced liver disease. Since the risk-benefit ratio of standard performance of a screening colonoscopy in this population appears questionable, alternative screening strategies should be considered.

INTRODUCTION

Colonoscopy is commonly performed as part of the standard screening for neoplastic lesions in candidate patients for liver transplantation, although international guidelines do not clearly state in which patients it should be mandatorily performed or might be omitted.(1-3)

The prevalence of colorectal cancer (CRC) in liver transplantation candidates has not been well defined. Several studies have reported that the prevalence of premalignant colon lesions, i.e. advanced adenomas, in this patient population varies from 5.8 – 13.9%.(4-8) Removal of these precursor lesions is recommended, also considering the potentially accelerated rate of progression to CRC during long-term immunosuppressive therapy after transplantation.(9)

In addition, little quantitative data are available pertaining to the safety of colonoscopy in this population. Several case series have suggested that patients with end-stage liver disease undergoing colonoscopy are at increased risk for haemorrhage and perforation after polypectomy.(10, 11) Other reported complications include bacteraemia, peritonitis, and renal failure.(12-19)

The aim of the present study was to assess the yield and safety of screening colonoscopy in a large consecutive cohort of patients who underwent evaluation for liver transplantation, by investigating the prevalence and predictive factors for CRC and advanced adenomas and the incidence and predisposing factors for post-procedural complications.

METHODS

Study design and patients

All consecutive patients undergoing colonoscopy during pre-liver transplantation screening from 1st January 2004 - 1st May 2017 in the Erasmus MC, Rotterdam, the Netherlands, were retrospectively included. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in approval by the institution's human research committee on January 23rd 2017. Written informed consent was not necessary considering the nature of the study.

Colonoscopy procedure

Bowel preparation was achieved using polyethylene electrolyte glycol (PEG) solutions. Sedation, using midazolam and/or fentanyl, was given at the discretion of the patients' preference and physicians' judgement. Endoscopic reports were retrieved from the automated EndoALPHA reporting system (Endobase; Olympus Winter & Ibe, Hamburg, Germany). Specimens of resected colon tissue were processed and reviewed by specialized gastrointestinal pathologists using standard histologic methods.

Data collection

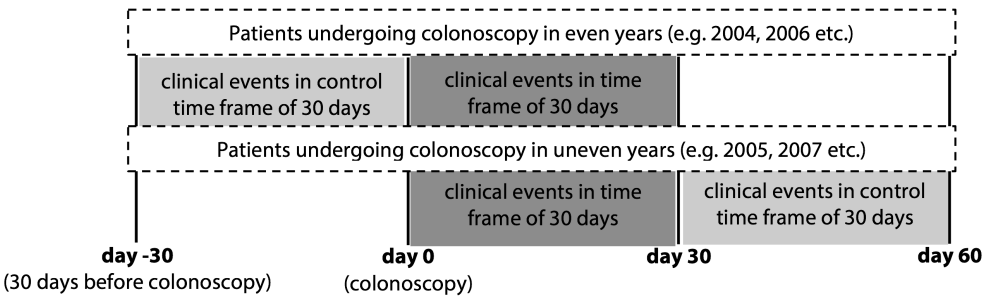
Clinical patient characteristics at the time of colonoscopy including gender, age, etiology

and severity of liver disease, presence of hepatocellular carcinoma (HCC), ascites (graded as none, diuretic-responsive and refractory), hepatic encephalopathy (HE) (graded according to the West Haven criteria) and laboratory values (creatinine, albumin, bilirubin and INR) were collected from electronic patients records. Data of the colonoscopy procedure, including use of premedication, adequacy of bowel preparation, cecal intubation rate, size, morphology, and histopathology of tumors, polyps and other endoscopic findings (e.g. inflammatory bowel disease (IBD), rectal varices and portal enteropathy) were recorded. In the context of this study, patients were followed till 1 year after liver transplantation, the date of death when not transplanted, or till the end of the study observation period (August 1st, 2018) when they were still on the waiting list.

Complications

All clinical events potentially related to the colonoscopy were registered in the 30 days period after the procedure. To assess colonoscopy associated risk in comparison with the general risk in this particular population, relevant clinical events were also registered in the 30 days period preceding the procedure, when this was performed in even years (e.g. 2004, 2006, etc.), and between day 31 to 60 after the procedure when this was performed in uneven years.(Figure 1) Patients who received a liver transplantation or died during the control time frame were not taken into account with respect to the assessment of complications. The following events were considered to be potentially related to colonoscopy: post-polypectomy haemorrhage, colon perforation, acute renal failure, gastrointestinal (GI) bleeding, new-onset or worsening of ascites and hepatic encephalopathy, bacterial infections (including bacteraemia, fever of unknown origin, spontaneous bacterial peritonitis (SBP), and respiratory, urogenital and other infections), cardiopulmonary events (including new-onset arrhythmias, myocardial infarction, congestive heart failure, aspiration pneumonia, and respiratory insufficiency), and significant rise in serum bilirubin.

Figure 1. Schematic view of the chosen control time frames in this study.



Definitions

Liver disease severity scores were calculated and patients were classified according to Child-Pugh (CP).(20) The model for end-stage liver disease (MELD) score was calculated with the formula: 0.957 × log(creatinine in mg/dL) + 0.378 × log(bilirubin in mg/dL) + 1.120 × log(INR) + 0.643.(21, 22) The adequacy of bowel preparation was classified as inadequate, poor, fair, good, or excellent using the Aronchick bowel preparation scale.(23, 24) Cecal intubation was defined as complete visualisation and intubation of the caecum, confirmed by the visual landmarks of the ileocecal valve and triradiate cecal fold.(25) Patients with an inadequate or poor bowel preparation were excluded from cecal intubation rate calculations. Colon tissue specimens were classified as normal colon tissue, hyperplastic polyps, inflammatory polyps, non-advanced adenomas, advanced adenomas, or CRC.(26) Non-advanced adenoma was defined as all tubular adenoma and serrated non-advanced adenoma.(26) Sessile serrated adenoma/polyp (SSA/P) was defined as predominantly architectural distortion with irregular dilated crypts that often have an L or T shape.(27) Traditional serrated adenoma (TSA) was defined as proburant or pedunculated grown pattern with distorted villiform configurations with columnar cells having abundant eosinophilic cytoplasm or centrally located elongated nuclei.(27) Advanced adenomas were defined as adenomas ≥ 10 mm, adenomas with high-grade dysplasia or with a villous component of at least 25%.(26, 28) Cancers were staged according to the 7th edition of the American Joint Committee on Cancer.(29) Advanced neoplasia was defined as advanced adenoma and/or colorectal cancer.

Acute renal failure was defined as a serum creatinine increase by 50% or more within 7 days or an increase of 0.3 mg/dL (26.5 µmol/L) within 2 days.(30, 31) GI bleeding was defined as all forms of variceal bleeding in the upper or lower gastrointestinal tract (thus excluding bleeding form a polypectomy site).(32) New-onset or worsening HE was defined as newly diagnosed or an increase of neurocognitive changes according to the West-Haven clinical criteria.(33, 34) New-onset ascites or worsening ascites was defined as a sudden increase of ascites and confirmation by ultrasound or fluid drainage by paracentesis. Bacterial infections were classified using Centers for Disease Control and prevention (CDC) criteria.(35, 36) Fever of unknown origin was defined as a prolonged febrile illness that persists without diagnosis after careful initial assessment.(37) SBP was defined as a polymorphonuclear (PMN) cell count in ascites ≥ 250/µL without a surgically treatable abdominal source of infection.(38) A bilirubin increase was defined as an increase of at least 5 mg/dL (85 µmol/L) within 2 days.

Statistical analysis

Continuous variables were reported as mean with standard deviation (SD), after visual confirmation of approximate normality, and compared using the Student's *T*-test. Continuous variables with a non-normal distribution were reported as median with an

interquartile range (IQR) expressed as the 25th to the 75th percentile. Categorical variables were reported as count with proportion and compared using the Chi-square test. A two-sided *p*-value <0.05 was considered significant.

Multivariable logistic regression, carried out to identify risk factors for cecal intubation failure, included the candidate predictor variables: gender, age, MELD score, ascites, HE, and sedation medication during colonoscopy. Multivariable logistic regression, carried out to identify risk factors for an advanced neoplasm, included the candidate predictor variables: gender, age, liver disease aetiology, HCC, MELD score, and colorectal cancer screening (i.e. colonoscopy, FOBT/FIT test, CT-colonography or barium enema examination) in the prior 5 years. Multivariable analyses for the logistic regression models were employed using the backward stepwise selection method with removal testing based on the significance of the likelihood-ratio statistic.

Kaplan-Meier analysis was used to estimate 1- and 2-year survival rates after liver transplantation.

All statistical analyses were performed using IBM® SPSS® Statistics for Windows, version 24.0.0.1 (IBM Corp., Armonk, NY, USA).

RESULTS

Patient characteristics

From 1st January 2004 – 1st May 2017, 1145 patients underwent pre-liver transplantation screening in the Erasmus MC, Rotterdam, the Netherlands. A total of 337 patients were not included in the study, mainly because colonoscopy was performed in a referring hospital (n = 90), patients were listed with high urgent priority (n=74), or the screening was prematurely terminated because of clinical deterioration of the patient condition (n = 41). The remaining 808 patients were included for the present analysis.(Figure 2) The study cohort included 524 men and 284 women with a median age 55 years (IQR 47 – 62) at the time of colonoscopy.(Table 1) The most frequent reason for pre-liver transplantation screening was alcoholic liver disease (22.9%), followed by viral hepatitis (21.4%), and primary sclerosing cholangitis (PSC) (17.6%).(Table 1) The prevalence of IBD in patients diagnosed with PSC was 59.9% (85/142 patients). HCC was present in 223 patients (27.6%). Median MELD score was 15 (IQR 11 – 18; range 6 – 40), 24.5% of patients had diuretic responsive ascites, 16.6% had refractory ascites and 97 (12%) patients had HE at the time of colonoscopy. Approximately 20% patients used at least one antibiotic agent during colonoscopy. One-fifth of patients received colorectal screening in the prior 5 years by colonoscopy; no cases were identified of patients undergoing colonoscopy after a positive screening with FOBT/FIT test, CT-colonography or barium enema examination.(Table 1)

Figure 2. Flow chart of study inclusion.

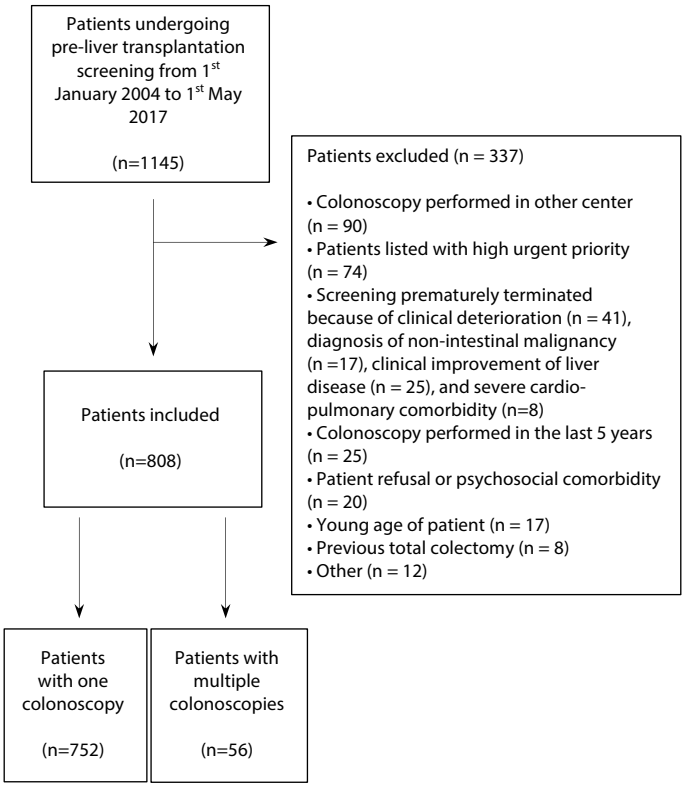


Table 1. Patient baseline characteristics at the time of screening colonoscopy.

	Patients (n= 808)
Male gender, *n (%)*	524 (64.9%)
Age in years, *median (IQR)*	55 (IQR 47 - 62)
Etiology of liver disease, *n (%)*	
Alcoholic liver disease	185 (22.9%)
Viral hepatitis	173 (21.4%)
PSC	142 (17.6%)
PBC/auto-immune hepatitis	65 (8.0%)
Cryptogenic liver cirrhosis	58 (7.2%)
NASH	41 (5.1%)
Other	144 (17.8%)
HCC, *n (%)*	223 (27.6%)
Blood serum parameters	
Creatinin (µmol/L), *median (IQR)*	71 (IQR 59 - 90)
Albumin (g/L), *mean (SD)*	33 (± 7)
Bilirubin (µmol/L), *mean (SD)*	87 (± 126)
INR, *mean (SD)*	1.4 (± 0.4)
Liver disease severity scores	
MELD score, *median (IQR)*	15 (IQR 11 - 18)
Child-Pugh class, *n (%)*	
A	473 (58.5%)
B	294 (36.4%)
C	41 (5.1%)
Ascites, *n (%)*	
None	476 (58.9%)
Diuretic responsive	198 (24.5%)
Refractory	134 (16.6%)
Hepatic encephalopathy, *n (%)*	
None	711 (88.0%)
West-Haven grade 1 - 2	71 (8.8%)
West-Haven grade 3 - 4	26 (3.2%)
Antibiotic use*, *n (%)*	162 (20.0%)
Norfloxacin	82 (10.1%)
Rifaximin	48 (5.9%)
Rifamipicin	9 (1.1%)
Ciprofloxacin	17 (2.1%)
Amoxicilin and clavulanic acid	5 (0.6%)
Other	12 (1.5%)
Colorectal screening in the prior 5 years, *n (%)*	193 (23.9%)

* 11 patients used multiple antibiotic agents.

Abbreviations: HCC: hepatocellular carcinoma; INR: International Normalized Ratio; IQR: interquartile range; MELD: Model For End-Stage Liver Disease; NASH: non-alcoholic steatohepatitis; PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis; SD: standard deviation.

Colonoscopy procedure

A total of 864 colonoscopies were performed in 808 patients. Bowel preparation adequacy was available in 663 patients and was scored excellent in 7.1%, good in 54.6%, fair in 16.1%, poor in 3.3%, and inadequate in 1%. Cecal intubation rate of the index colonoscopy was 96.7%. Cecal intubation failed due to loop formation in 15 patients and abortion due to patient discomfort/abdominal pain in 12 patients. Multivariable logistic regression, carried out for risk factors for cecal intubation failure, identified MELD score (OR 1.090 per point; 95% CI 1.015 – 1.170; p=0.018), as an independent risk factor.

In total, 56 re-colonoscopies were performed because of several reasons: poor bowel preparation (n=13), initial cecal intubation failure (n=25), anticoagulation status contra-indicated polypectomy (n=9), patients needed a re-colonoscopy after a period of 3 years on the liver transplantation waiting list or for re-liver transplantation (n=6), or for additional polypectomy or surveillance after polypectomy (n=3). In 6 patients with initial cecal intubation failure and in 5 patients with poor bowel preparation subsequent colonoscopy was postponed till after the transplantation.

In this cohort, 799/864 (92.4%) colonoscopies were performed under conscious sedation using intravenous midazolam and fentanyl. Sixty-five procedures were performed using fentanyl (n=25), remifentanil (n=7), or without any premedication (n=33). Patients did not receive standard peri-procedural antibiotic prophylaxis.

Diagnostic yield

In total, 625 polypectomies were performed during colonoscopy with an average of 2.3 polypectomies (± 1.3) per patient. At colonoscopy advanced neoplasia was found in 46 (5.6%) patients: advanced adenoma in 44 (5.4%) and CRC in 2 (0.2%). Non-advanced adenoma in 151 (18.7%) including SSA/P or TSA in 13 (1.6%), hyperplastic polyps in 130 patients (16.1%), inflammatory polyps in 3 (0.4%).(Table 2)

In 58 of the remaining 465 patients, a lesion was macroscopically present, but polypectomy was not attempted due to impaired coagulation and/or a macroscopically benign character (n=41), or histopathologic evaluation was not possible due to loss or insufficient yield of tissue (n=17). At a subsequent colonoscopy, advanced adenoma was diagnosed in 3/58 cases and CRC in none.

The only independent risk factor for advanced neoplasia was age (HR 1.072 per year; 95% CI 1.031 - 1.115; p<0.001). Advanced neoplasm was diagnosed in 5.6% of the patients aged 60 years or older, in 5.4% of the patients aged 50 – 59 years, in 1.8% of the patients aged 40 – 49 years, and no advanced neoplasm was diagnosed below the age of 40 years.(Figure 3) The two patients with CRC were a 62-year old female with a T2N0M0 rectal adenocarcinoma and a 64-year old female with a T2N0M0 adenocarcinoma of the sigmoid colon.

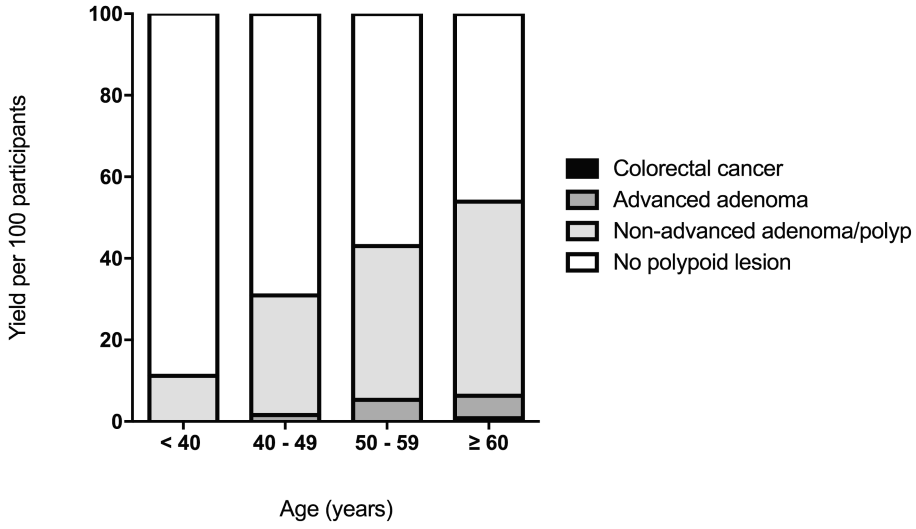
Table 2. Findings at colonoscopy.

	Patients (n= 808)
Diagnostic yield[I], *n (%)*	
Colorectal cancer	2 (0.2%)
Advanced adenoma	44 (5.4%)
≥ 10 mm	17
≥ 25% villous	9
high-grade dysplasia	4
≥ 10 mm + high-grade dysplasia	3
≥ 10 mm + ≥ 25% villous	8
≥ 25% villous + high-grade dysplasia	1
≥ 10 mm + ≥ 25% villous + high-grade dysplasia	2
Non-advanced adenoma	164 (20.3%)
SSP/A or TSA	13
Hyperplastic polyp	130 (16.1%)
Inflammatory polyp	3 (0.4%)
No lesions	465 (57.6%)
Other pathologies*, *n (%)*	
Inflammatory bowel disease	92 (11.4%)
Rectal varices	72 (8.9%)
Angiodysplasia	61 (7.5%)
Portal hypertensive colopathy	58 (7.2%)
Diverticulosis	57 (7.1%)
Hemorrhoids	42 (5.2%)

[I]According to the most advanced lesion.
*Patients could have multiple other pathologies.

Abbreviations: SSP/A: sessile serrated polyp/adenoma; TSA: traditional serrated adenoma.

Figure 3. Most advanced lesion per participant found during colonoscopy.



Other colon abnormalities

In 294 patients (36.4%) other colon abnormalities were reported.(Table 2) There were no cases of newly diagnosed IBD.

Complications after colonoscopy

During the 30 days period after colonoscopy 178 clinical events occurred in 128 (14.9%) patients compared to 101 clinical events in 72 (8.6%) patients with an event in the control time frames (p<0.001).(Table 3)

Table 3. Frequency of complications in patients undergoing screening colonoscopy.

	Complications during 30 days post-colonoscopy (n=858)	**Complications during 30 days control time frame (n=835)**	***p*-value**
Acute renal failure, *n (%)*	33 (3.8%)	10 (1.2%)	**0.001**
Gastro-intestinal bleed, *n (%)*	25 (2.9%)	11 (1.3%)	**0.023**
Bacterial infection, *n (%)*	54 (6.3%)	37 (4.4%)	0.089
SBP	18	17	
Fever of unknown origin	15	6	
Bloodstream infection	12	3	
Respiratory infection	4	-	
Gastro-intestinal infection	4	7	
Urogenital infection	3	3	
Other	0	1	
Hepatic encephalopathy, *n (%)*	47 (5.5%)	32 (3.8%)	0.109
Pulmonary complications, *n* (%)	10 (1.2%)	3 (0.4%)	0.058
(Aspiration) pneumonia	3	-	
Respiratory insufficiency	7	3	
Cardiac complications, *n* (%)	7 (0.8%)	4 (0.5%)	0.391
New-onset arrhythmia	5	1	
Congestive heart failure	2	2	
Myocardial ischemia	-	1	
Ascites (new-onset or worsening), *n (%)*	1 (0.1%)	4 (0.5%)	0.169
Bilirubin increase, *n* (%)	1 (0.1%)	-	0.324

Abbreviation: SBP: spontaneous bacterial peritonitis.

After colonoscopy, there was a significant increased risk for acute renal failure (33 vs. 10; p=0.001). Patients with acute renal failure had an average creatinin rise of 66 µmol/L; 35 were treated with volume expansion, albumin and/or vasopressors and 8 with dialysis. The presence of ascites (diuretic responsive ascites OR 1.199; 95% CI 0.356 – 4.038, refractory ascites OR 5.384; 95% CI 1.935 – 14.978, p=0.001) and high MELD score (OR 1.265 per

point; 95% CI 1.180 – 1.356; $p<0.001$) were independent risk factors for post-colonoscopy renal failure.

The risk for GI bleeding not originating from polypectomy sites was also significantly elevated after colonoscopy (25 vs. 11; $p=0.023$). MELD score (OR 1.127 per point; 95% CI 1.061 – 1.197; $p<0.001$) was found to be an independent risk factor for post-colonoscopy GI bleeding.

Furthermore, there was a non-significant increase in bacterial infections (54 vs. 37 cases; $p=0.089$), HE (47 vs. 32 cases; $p=0.109$), pulmonary complications (10 vs. 3 cases; $p=0.058$), and cardiac complications (7 vs. 4 cases; $p=0.391$), in the post-colonoscopy and control time frames, respectively.(Table 3)

Post-polypectomy haemorrhage occurred in two patients, both could be endoscopically managed. In one patient colon perforation occurred after polypectomy, which was successfully treated conservatively.

Since the study duration was 13.5 years, the impact of the time of screening was measured on the detection of advanced adenomas and complication occurrence. There were no statistical significant differences regarding the time of screening on these outcome measures.(Supplement 1)

Clinical course

The median follow-up time was 285 days (IQR 106 – 636). In this cohort, 260 patients (32.2%) died, 432 patients (53.3%) received a liver transplant, and 116 patients (14.4%) were waiting for a liver transplant at the end of the observation period. The 1-year survival rate after liver transplantation was 91%, and the 2-year survival rate 88%.

DISCUSSION

In this study, we assessed the diagnostic yield and safety of performing a screening colonoscopy in patients evaluated for liver transplantation. We found that CRC was diagnosed in 0.2% of the population and advanced adenoma in 5.4%. Age was the only significant predictive factor for advanced neoplasia. Furthermore, colonoscopy with standard PEG bowel preparation was associated with a significantly increased risk for renal failure and non-polypectomy GI bleeding, especially in patients with most severe liver disease.

A key finding of the present study is the relatively low prevalence of CRC in this patient population. These results are consistent with those of comparable studies that did not find any case of CRC in patients undergoing pre-transplantation evaluation.(6, 8, 39) The 5.4% prevalence of advanced adenomas in our study was comparable to that reported by Weismuller et al. (prevalence 5.8%; 243 patients with a mean age of 53 years), but differs markedly from the 13.9% prevalence (567 patients; median age of 54 years) reported by Jeschek et al.(6, 8) These diverging results may be related to differences in study

methodology since the latter study results were not based on the actual diagnosed rate of advanced adenomas, but rather to a statistical adjustment of this number assuming the same rate of advanced adenomas among resected and non-resected polyps.(8) The results of large cohort studies assessing the prevalence rate of advanced neoplasms in unselected healthy subjects, although of slightly older age, are in line with those in our study. Imperiale et al. reported a CRC rate of 0.6% and an advanced adenoma rate of 5.6% in 1994 patients with a mean age of 60 years and Stoop et al. found a rate of 0.5% and 8.2%, respectively, in 1276 patients with a mean age of 61 years.(40, 41)

Another important finding is that 53.5% of the population undergoing screening actually underwent liver transplantation. Although 14.4% are still waiting for a liver transplant, a substantial proportion of patients have died on the waiting list or were not placed on the waiting list due to contra-indications for liver transplantation.(42) Moreover, 9% of the 432 transplanted patients died within 1 year and an additional 3% in the second year after transplantation. Thus, the number of patients that could theoretically benefit from screening colonoscopy is further decreased by the operative and post-operative mortality.

Our results indicate that colonoscopy increases the risk for complications such as acute renal failure and GI bleeding. We hypothesize that this may be related to bowel preparation with an inherent substantial fluid load that may induce unwanted circulatory alterations and fluid shifts. Indeed, water retention in patients with a pre-existent hyperdynamic circulation, increasing the portal venous pressure, has been previously reported in patients undergoing bowel preparation with decompensated liver disease, congestive heart failure and chronic renal insufficiency.(13, 43) Our study result that MELD score is the most important predictive factor for these complications after colonoscopy supports this theory.

The cecal intubation rate of 96.7% in our study is fairly similar to the rate of 83 – 96% found in other cohorts of patients evaluated for liver transplantation.(6, 39) Multiple explanations are proposed for the slightly lower cecal intubation rate in this population compared to that in healthy subjects. The presence of ascites may lead to more mobile bowel loops floating in ascitic fluid, and may lower the efficacy of external abdominal pressure in order to reduce loop formation.(39) Also, the cecal intubation rate may be adversely affected by other factors including overall poor general condition and reduced possibilities for effective use of premedication.(39) In the present study, MELD score was identified as an independent predictor for cecal intubation failure, which may support these hypotheses.

Currently, the American Association for the Study of the Liver (AASLD) clinical practice guideline recommends that liver transplantation candidates should undergo an age and risk factor-appropriate cancer screening including colonoscopy without further specifications.(2) The European Association for the Study of the Liver (EASL) clinical

practice guideline states that CRC screening is mandatory for candidates older than 50 years.(3) However, considering the currently available data, the indication for standard pre-liver transplantation screening colonoscopy may be questioned considering the balance between yield and associated risks and costs, also considering important other factors such as the substantial waiting list and perioperative mortality.

We suggest that other screening strategies should be considered. A possible alternative approach could be the use of a fecal immunochemical test (FIT) as a general first line screening test in subjects aged 50 years or older, and to consider colonoscopy only in FIT-positive patients. Patients with inflammatory bowel disease, primary sclerosing cholangitis and other conditions associated with an increased risk for CRC should be managed according to generally accepted guidelines.

This is the first study, to the best of our knowledge, that systemically assessed the complication risk of pre-liver transplantation screening colonoscopy, taking into account the underlying general risk for unwanted events associated with the liver disease. The considerable size of the study population and the completeness of data are other factors likely contributing to the reliability of our results. A limitation is that patients who underwent colonoscopy in another centre before referral were not taken into account. However, this was a relatively small group and our study design reflects the real world situation in a referral hospital for liver transplantation. Also, due to the retrospective design of the study, not all relevant factors, such as adequacy of bowel preparation, could be fully analysed.

Future research projects regarding CRC screening in transplant candidates could focus on the assessment of factors relevant for more refined risk stratification in this population, such as age, gender, aetiology of liver disease, family history of CRC, body mass index, smoking and drinking habits, and comorbidities such as diabetes. It may be equally important to prospectively assess the results of alternative screening strategies.

In conclusion, this study describes the yield and safety of colonoscopy in patients evaluated for liver transplantation screening and provides arguments why a reconsideration of guidelines regarding the necessity of colonoscopy in unselected patients seems appropriate. We propose that alternative colorectal screening strategies should be considered and further explored.

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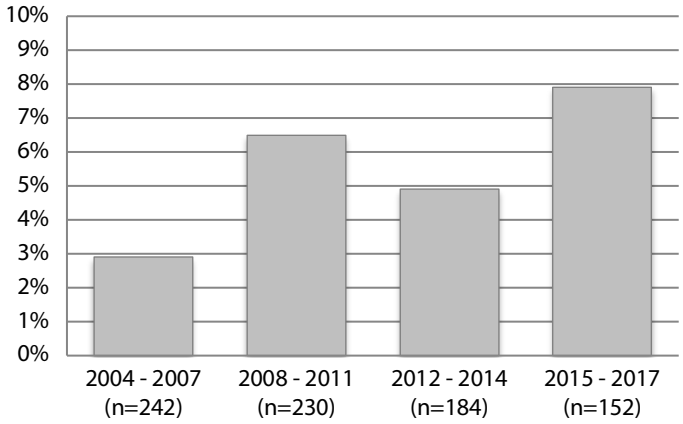
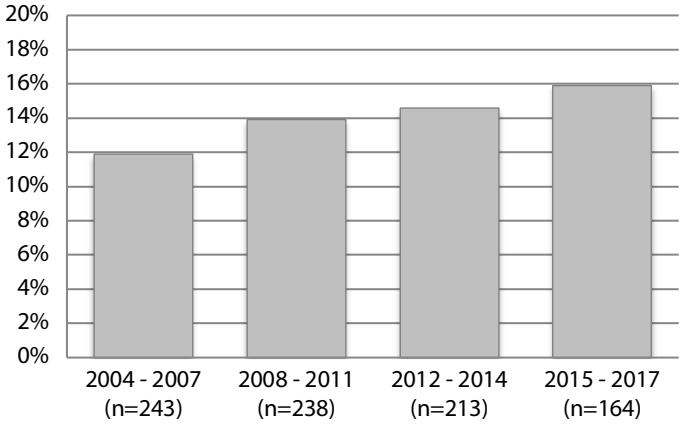
Supplement 1. Sensitivity analyses of the time period of screening on the primary endpoints.

A. Detection of advanced neoplasms during colorectal screening, separately shown for each time period of screening (p=0.135).



B. Prevalence of complications after colonoscopy during colorectal screening, separately shown for each time period of screening (p=0.709).



CHAPTER 8

Screening colonoscopy in patients evaluated for liver transplantation: a closer look in a defined population.

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We thank the colleagues for their interest in our study.(1) Although the risk for colorectal carcinoma after liver transplantation may be increased, our study shows that in this particular, vulnerable population the yield of advanced neoplasia detected by colonoscopy is low and is associated with an elevated risk of complications. In addition, we should like to stress the fact that only just over 50% of the screened patients actually underwent liver transplantation. Based on these data the timing of performing a screening colonoscopy may be reconsidered, e.g. performing screening colonoscopies post liver transplantation in a subset of patients.

Although the sensitivity of FIT is low for adenoma and serrated lesions, sensitivity for CRC is around 80%. Therefore, FIT may be used as an alternative to screen patients pre-liver transplantation. We agree that if FIT is chosen the cut-off used will be essential to assure an optimal benefit- risk balance.

We continue to believe that the benefit-harm ratio of screening colonoscopy in all potential candidate patients for liver transplantation is questionable and that other strategies should be considered and further explored.

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CHAPTER 9

Identification and prognostic impact of malnutrition in a population screened for liver transplantation

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ABSTRACT

Background: Sarcopenia is prevalent in patients with liver cirrhosis and is negatively associated with clinical outcomes. In a population screened for liver transplantation we aimed to assess the prevalence of abnormal nutritional status and to what extent a clinical screening tool is able to reliably select patients for extensive nutritional assessment including CT. We also evaluated which nutritional parameters are independently associated with clinical outcomes.

Methods: Analysis of consecutive patients undergoing detailed nutritional assessment during pre-liver transplantation screening from October 2015 to April 2017.

Results: In 102 included patients (66.7% male; median age of 56.3 years (IQR 43.9-64.0); median MELDNa score of 14.7 (IQR 9.4-19.0)), presarcopenia was diagnosed in 30/102 patients (29.4%), sarcopenia in 20/102 (19.6%), and impaired muscle quality in 19/102 (18.6%). Application of the EASL rapid screen tool as the primary instrument for nutritional assessment would have resulted in selection of 40/69 cases, thus 42.0% of patients with actual muscle mass depletion and/or impaired muscle function would not have been selected for further nutritional evaluation. In contrast to muscle mass depletion, impaired muscle function was a significant predictor for 6-month decompensation-free (p=0.006) and hospitalization-free (p=0.003) survival, when adjusted for age and MELDNa score.

Conclusion: In our population the efficacy of a clinical screening tool for malnutrition was unsatisfactory. A detailed nutritional assessment is therefore recommended in all patients undergoing liver transplantation screening. Impaired muscle function might be clinically more relevant than muscle mass depletion, and muscle function testing should be considered an integral part of nutritional assessment in chronic liver disease.

INTRODUCTION

Malnutrition – a nutrition-related disorder resulting from lack of intake or uptake of nutrition – is a frequent complication of advanced chronic liver disease and has been reported in 22-70% of patients awaiting liver transplantation.(1-4) Sarcopenia, characterized by a reduction in muscle mass and function, is a major component of malnutrition.(5) It is associated with a higher rate of complications (including infections, hepatic encephalopathy, and ascites), in addition to waiting list and transplantation-related mortality.(2-6)

The recent European association for the Study of the Liver (EASL) clinical practice guideline for nutrition recommends detailed multicomponent nutritional evaluation compromising assessment of 1) muscle mass by CT, 2) muscle function by handgrip strength or short physical performance battery, and 3) dietary intake including the quality and quantity of food and supplements.(Supplement 1)(4) Although the EASL and the European Society of Clinical nutrition and metabolism (ESPEN) emphasize that the definition sarcopenia includes both muscle mass and function, most recently published papers regarding sarcopenia in advanced chronic liver disease focus solely on muscle mass depletion by analysing skeletal muscle mass on the third lumbar level using CT.(1, 2, 4, 7-9)

Performing a detailed nutritional assessment in all patients is resource and time consuming, and could be unnecessary. The EASL guideline proposes a rapid screen in all patients with severe advanced liver disease.(4) Patients with Child–Pugh class C liver disease, a body mass index (BMI) below 18.5 kg/m^2, or with a medium or high risk for malnutrition according to a screening tool (e.g. the Royal Free Hospital-nutritional prioritizing tool) should undergo more detailed assessment.(4) Currently, there are no published studies evaluating the adequacy of this stepwise approach in a patient population screened for liver transplantation.

The primary aim of the present study was to assess, in a population of patients with advanced liver disease, to what extent the EASL rapid screen reliably identifies patients screened for liver transplantation who could benefit from a more detailed nutritional assessment. Furthermore, we aimed to assess which nutritional findings are independently associated with the risk for complications and survival.

MATERIALS AND METHODS

Study design and data collection

All patients undergoing liver transplantation screening between 1st October 2015 to 1st April 2017 in the Erasmus MC, Rotterdam, the Netherlands, were eligible for inclusion. Patients were excluded if liver transplantation screening terminated prematurely, screening was performed in a referring hospital, patients were listed with high urgency priority, or a

detailed nutritional assessment was not performed. The study protocol conforms to the ethical guidelines of the in 2013 revised Declaration of Helsinki as reflected in approval by the institution's human research committee on 12th June 2017 (MEC-2017-290), with the determination that written informed consent was not necessary considering the design of the study.

Data were collected from the electronic patient records at time of liver transplantation screening and during at least 6 months follow-up. Demographic, biochemical, nutritional and clinical characteristics as well as clinical outcome parameters were collected for each patient. Patients were followed till 1) liver transplantation, 2) death on the liver transplantation waiting list or permanent delisting from the liver transplantation waiting list, or 3) end of the observation period (1st March 2018).

Radiographical assessment of muscle mass

Each patient routinely underwent abdominal computed tomography (CT) during liver transplantation screening as part of the surgical screening. The cross-sectional skeletal muscle area was determined on a single abdominal cross-sectional image at the third lumbar vertebral level by the identification of the psoas muscle, paraspinal muscles (erector spinae, quadratus lumborum), and abdominal wall muscles (transversus abdominus, external and internal obliques, rectus abdominus) by two research physicians, and automatically all tissue with a radiodensity between -30 to +150 Hounsfield units was calculated. The skeletal muscle index in cm^2/m^2 was calculated using the cross-sectional skeletal muscle area, as previously described,(10) and adjusted for body height. The calculation of cross-sectional skeletal muscle area and skeletal muscle index were performed using FatSeg®, an in-house software program, developed by Erasmus MC, University Medical Center, Rotterdam, the Netherlands.(11)

Detailed nutritional assessment

A dietician experienced in managing patients with liver disease assessed dietary intake (protein intake in g/kg/day, energy intake percentage of requirement) and the nutritional status by anthropometry (weight [kg], height [cm], handgrip strength [kg]), and bioelectrical impedance analysis (phase angle, fat-free mass [kg, %]). The handgrip strength was measured using the handgrip dynamometer (Jamar) and reported as percentile reference values adjusted for age and gender.(12) Fat free mass and phase angle were measured using bioelectrical impedance analysis (BIA, Bodystat). Phase angle measurements were done as previously reported.(13, 14) Fat-free mass was reported as percentile reference values adjusted for age and gender.(12)

Definitions

The model for end-stage liver disease (MELD) score was calculated with the formula: 0.957

× log(creatinine in mg/dL) + 0.378 × log(bilirubin in mg/dL) + 1.120 × log(INR) + 0.643, and MELD sodium (MELDNa) score by the formula: MELD – Na (in mmol/L) – [0.025 × MELD × (140 – Na)] + 140 with Na.(15) Decompensation was defined as occurrence of one of the following clinical events: acute renal failure, new-onset or worsening of ascites and hepatic encephalopathy, and bacterial infections. Acute renal failure was defined as a serum creatinine increase by 50% or more within 7 days or an increase of 0.3 mg/dL (26.5 µmol/L) within 2 days.(16, 17) Ascites was classified as none, mild-moderate in case of diuretic responsive ascites, and refractory if additional large-volume paracentesis was necessary during an optimized treatment with diuretic agents. New-onset or worsening hepatic encephalopathy was defined as newly diagnosed or an increase of neurocognitive changes according to the West-Haven clinical criteria.(18, 19) Diagnosis of bacterial infection was made according to Centers of Disease Control and Prevention criteria.(20, 21)

Body mass index (BMI) was calculated by dividing weight in kg by square height in m^2, and adjusted for ascites by subtracting 5% of weight in mild/moderate ascites, and 15% in refractory ascites.(4) Inadequate dietary intake was defined as a protein intake below 1.2 g/kg/day,(22) or an energy intake below 50% of requirement.(23, 24) The Royal Free Hospital-nutritional prioritizing tool classified patients as medium and high risk for malnutrition.(25) Muscle function loss was defined as a handgrip strength below the 10^{th} percentile.(12) A phase angle equal or below 5.4 was considered to be prognostic unfavourable.(13, 14) Patients were classified as having muscle mass depletion if skeletal muscle index was below 43 cm^2/m^2 in males with a body mass index (BMI) below 25 kg/m^2, and below 53 cm^2/m^2 in males with a BMI equal or above 25 kg/m^2, and for females a skeletal muscle index below 41 cm^2/m^2 regardless of BMI.(8) Sarcopenia was defined as muscle mass depletion and muscle function loss.(1, 4) Presarcopenia was defined as muscle mass depletion with normal muscle function.(26) Impaired muscle quality was defined as muscle function loss with normal muscle mass.(27)

Statistical analysis

Continuous variables were reported as mean with standard deviation (SD), after visual confirmation of approximate normality, and compared using the Student's *T*-test. A median and range from the first to the third quartile (IQR) was computed for continuous variables with a non-normal distribution. Categorical variables were reported as count with proportion and compared using the Chi-square test. A two-sided *p*-value <0.05 was considered significant.

Multiple multivariable logistic regression analyses were carried out to identify predictive factors for inadequate dietary intake, muscle mass depletion, impaired muscle function, and muscle mass depletion or impaired muscle function, using the following candidate predictor variables: age, gender (female; male), liver cirrhosis (none; present), MELDNa score, alcohol use (none; <14 IU for women or <21 IU for men; ≥14 units IU for

women or ≥21 IU for men), and BMI adjusted for ascites. The multivariable analysis for the logistic regression models were employed using the backward stepwise selection method with removal testing based on the probability of the likelihood-ratio statistic of the maximum partial likelihood estimates.

The actuarial probabilities of decompensation-free survival, hospitalization-free survival and transplant-free survival (death and liver transplantation as event) after detailed nutritional assessment were estimated using the Kaplan-Meier method and a comparison was made in patients with muscle mass depletion and impaired muscle function using log-rank tests.

Multivariable Cox regression analyses were carried out to identify whether muscle mass depletion and impaired muscle function were, independent of age and MELDNa score, significant predictors for 6-month decompensation-free, 6-month hospitalization-free and 6-month transplant-free survival after detailed nutritional assessment.
All statistical analyses were performed using IBM® SPSS® Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patients

Between 1st October 2015 to 1st April 2017 137 patients were evaluated for liver transplantation. We could include 102 patients in the present study.(Figure 1) The population comprised of 68 men and 34 women with a median age of 56.3 years (IQR 43.9 - 64.0).(Table 1) Cirrhosis was diagnosed in 80.4% cases and hepatocellular carcinoma (HCC) in 37.3%. Nearly fifty percent of patients suffered from chronic cholestatic, autoimmune or viral liver disease. The median MELDNa score was 14.7 (IQR 9.4 - 19.0).

Figure 1. Flow chart of study population.

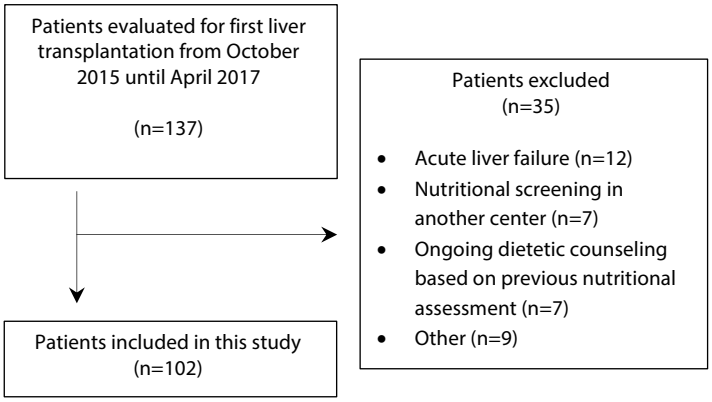


Table 1. Demographic and clinical patient characteristics.

	Included patients (n=102)
Male, *n (%)*	68 (66.7%)
Age (years), *median (IQR)*	56.3 (43.9 – 64.0)
Etiology of liver disease, *n (%)*	
Biliary and auto-immune disease	28 (27.5%)
Viral hepatitis	18 (17.6%)
Alcohol	17 (16.7%)
Non-alcoholic steatohepatitis	10 (9.8%)
Metabolic disorder	8 (7.8%)
Polycystic liver disease	7 (6.9%)
Other	14 (13.8%)
Concomitant hepatocellular carcinoma, *n (%)*	38 (37.3%)
Liver cirrhosis, *n (%)*	82 (80.4%)
MELDNa score, *median (IQR)*	14.7 (9.4 – 19.0)
Child-Pugh score, *median (IQR)*	7 (5 – 9)
Child-Pugh class, *n (%)*	
A	44 (43.1%)
B	35 (34.3%)
C	23 (22.5%)
Ascites, *n (%)*	
Diuretic-responsive	19 (18.6%)
Diuretic-refractory	20 (19.6%)
BMI adjusted for ascites (kg/m^2), *mean (SD)*	26.9 (5.1)
Hepatic encephalopathy, *n (%)*	
None	93 (91.2%)
West Haven grade 1 - 2	9 (8.8%)
Blood serum parameters‡	
Creatinin (µmol/L), *median (IQR)*	74.5 (63.0 – 90.0)
Sodium (mmol/L), *median (IQR)*	140.0 (137.0 – 142.0)
Albumin (g/L), *median (IQR)*	34.5 (30.8 – 42.2)
Bilirubin (µmol/L), *median (IQR)*	33.0 (13.8 – 71.0)
INR, *median (IQR)*	1.3 (1.2 – 1.6)
ASAT (U/L), *median (IQR)*	57.5 (35.8 – 96.3)
ALAT (U/L), *median (IQR)*	46.5 (27.8 – 79.0)
ɣ-glutamyltransferase (U/L), *median (IQR)*	128 (52.5 – 191.8)
Alkaline phosphatase (U/L), *median (IQR)*	151.5 (106.8 – 270.0)
Haemoglobin (mmol/L), *median (IQR)*	7.5 (6.4 – 8.4)
Thrombocytes (10^9/L), *median (IQR)*	118 (86 – 203)
Leucocytes (10^9/L), *median (IQR)*	5.7 (3.8 – 8.0)
Medical history with chronic disorder, *n (%)*	
Diabetes	18 (17.6%)
Renal disease	17 (16.7%)
Cardiovascular disease	10 (9.8%)
Pulmonary disease	10 (9.8%)

‡ Normal laboratory reference ranges: Creatinin 55 – 115 µmol/L, Sodium 136 – 145 mmol/L, Albumin 35 – 50 g/L, Bilirubin 0 – 16 µmol/L, INR <1.7, ASAT 0 – 34 U/L, ALAT 0 – 44 U/L, ɣ-glutamyltransferase 0 – 54 U/L, Alkaline phosphatase 0 – 114 U/L, Haemoglobin 7.5 – 10.5 mmol/L, Thrombocytes 150 – 370 109/L, Leucocytes 3.5 – 10 109/L.

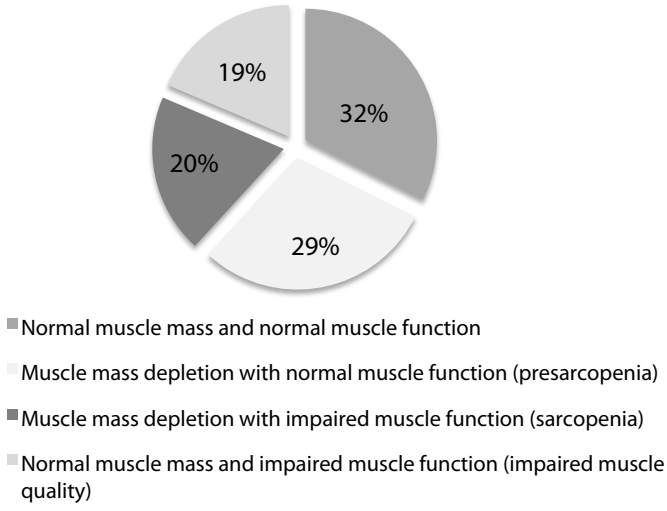
Table 1. Demographic and clinical patient characteristics. *(continued)*

Abbreviations: ALAT: alanine transaminase, ASAT: aspartate transaminase, BMI: body mass index, INR, international normalized ratio, IQR: interquartile range, MELDNa: model for end-stage liver disease model including sodium.

Prevalence of abnormal nutritional findings

Dietary intake assessment revealed that protein intake was below 1.2 kg/g/day in 64.7% patients and energy intake was below 50% requirement in 1.0%. The prevalence of inadequate dietary intake, defined as either inadequate protein or caloric intake, was 64.7%, because inadequate energy intake always co-existed with inadequate protein intake. Presarcopenia (low muscle mass and normal muscle function) was diagnosed in 30/102 patients (29.4%), sarcopenia (low muscle mass and low muscle function) in 20/102 (19.6%), and impaired muscle quality (normal muscle mass and low muscle function) in 19/102 (18.6%). Thus, sixty-nine patients (67.6%) were diagnosed with either presarcopenia, sarcopenia, or impaired muscle quality).(Figure 2) There was no association between inadequate dietary intake and presarcopenia (p=0.851), sarcopenia (p=0.311), or impaired muscle quality (p=0.364).

Figure 2. Prevalence of muscle mass depletion and impaired muscle function.



Evaluation of EASL nutritional screening protocol

Figure 3 summarizes the results of the work-up according to the recently proposed EASL rapid screen for identifying patients with liver disease at risk for malnutrition/sarcopenia. (Supplement 1) In our liver transplantation screening cohort, 49 patients (48.0%) fulfilled at least one of the criteria for an extensive nutritional assessment including CT: 23 patients had Child–Pugh class C liver disease, an additional 5 patients had a BMI <18.5 kg/m^2, and another 21 patients were at medium or high risk according to evaluation using the Royal Free Hospital-nutritional prioritizing tool. Applying the EASL screen to our population resulted in identification of 11/30 (36.7%) cases with presarcopenia, 16/20 (80%) with sarcopenia, and 13/19 (68.4%) with impaired muscle quality. The EASL algorithm resulted in identification of 27/50 (54.0%) patients with muscle mass depletion, 29/39 (74.4%) patients with impaired muscle function, and 40/69 (58.0%) patients with at least one muscle abnormality (i.e. presarcopenia, sarcopenia, or impaired muscle quality).

Figure 3. Flow chart for rapid screen protocol in this population.

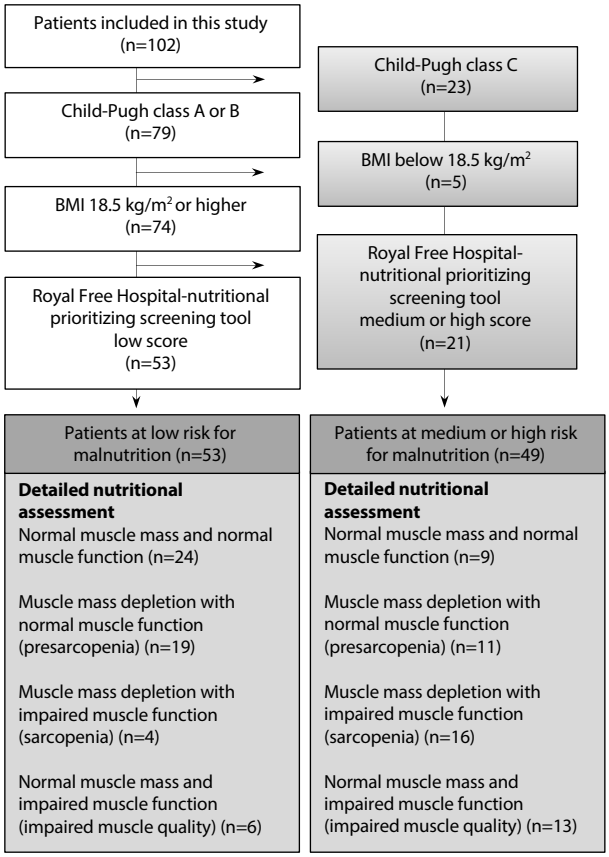


Table 2. Results of bioelectrical impedance analysis according to muscle mass and function.

Muscle mass (skeletal muscle index)	**Normal (n=52)**	**Presarcopenia, or sarcopenia (n=50)**	***p*-value**
Phase angle (°), (%) ≤ 5.4	16 (30.8%)	29 (58.0%)	0.006
Fat–free mass, (%) < 10th percentile	1 (2.0%)	6 (12.0%)	0.044
Muscle function (handgrip strength)	**Normal (n=63)**	**Sarcopenia or impaired muscle quality (n=39)**	
Phase angle (°), (%) ≤ 5.4	23 (36.5%)	22 (56.4%)	0.049
Fat–free mass, (%) < 10th percentile	4 (6.3%)	3 (7.7%)	0.794
Muscle mass and function	**Normal (n=33)**	**Presarcopenia, sarcopenia or impaired muscle quality (n=69)**	
Phase angle (°), (%) ≤ 5.4	7 (21.1%)	38 (55.1%)	0.001
Fat–free mass, (%) < 10th percentile	1 (3.0%)	6 (8.7%)	0.290

Bioelectrical impedance analysis

A phase angle ≤ 5.4° was measured in 39.4% patients with inadequate dietary intake. (Table 2) Patients with muscle mass depletion (p=0.006), impaired muscle function (p=0.049), and either muscle mass depletion or impaired muscle function (p=0.001) had significantly more often a phase angle ≤ 5.4°. However, not more than 60% of the population was correctly identified with a phase angle ≤ 5.4°. Fat-free mass below the 10th percentile was diagnosed in 12.0% of patients with muscle mass depletion, 7.7% patients with impaired muscle function, and 8.7% patients with presarcopenia, sarcopenia or impaired muscle quality.

Predictive factors for inadequate dietary intake, muscle mass depletion, and impaired muscle quality

Inadequate dietary intake was found more often in patients with higher age (OR per year: 1.035, 95%CI 1.001 – 1.070, p=0.046) and high BMI (OR per kg/m^2: 1.173, 95%CI 1.044 – 1.319, p=0.007). Muscle mass depletion was independently associated with low BMI (OR per kg/m^2: 0.898, 95%CI 0.819 – 0.986, p=0.024), impaired muscle function with female gender (OR: 2.868, 95%CI 1.119 – 7.349, p=0.028), higher MELDNa score (OR per point: 1.095, 95%CI 1.010 – 1.187, p=0.027), and low BMI (OR per kg/m^2: 0.894, 95%CI 0.806 – 0.992, p=0.034). Presarcopenia, sarcopenia or impaired muscle quality was likewise independently associated with female gender (OR: 3.199, 95%CI 1.031 – 9.929,

p=0.044), higher MELDNa score (OR per point: 1.101, 95%CI 1.004 – 1.207, *p*=0.041), and low BMI (OR per kg/m^2: 0.850, 95%CI 0.759 – 0.953, *p*=0.005).(Table 3)

Table 3. Independent predictive factors for inadequate dietary intake, muscle mass depletion and/or impaired muscle quality identified by multivariable logistic regression analysis.

	OR	95% CI	*p*-value
Inadequate dietary intake			
Age (per year)	1.035	1.001 – 1.070	**0.046**
BMI adjusted for ascites (per kg/m2)	1.173	1.044 – 1.319	**0.007**
Muscle mass depletion			
BMI adjusted for ascites (per kg/m2)	0.898	0.819 – 0.986	**0.024**
Impaired muscle function			
BMI adjusted for ascites (per kg/m2)	0.894	0.806 – 0.992	**0.034**
MELDNa score (per point)	1.095	1.010 – 1.187	**0.027**
Gender male (reference) female	 1 2.868	 - 1.119 – 7.349	**0.028**
Presarcopenia, sarcopenia or impaired muscle quality			
BMI adjusted for ascites (per kg/m2)	0.850	0.759 – 0.953	**0.005**
MELDNa score (per point)	1.101	1.004 – 1.207	**0.041**
Gender male (reference) female	 1 3.199	 - 1.031 – 9.929	**0.044**

Abbreviations: BMI: body-mass index; CI: confidence interval, MELDNa score: Model for End-Stage Liver Disease-Sodium score; OR: odds ratio.

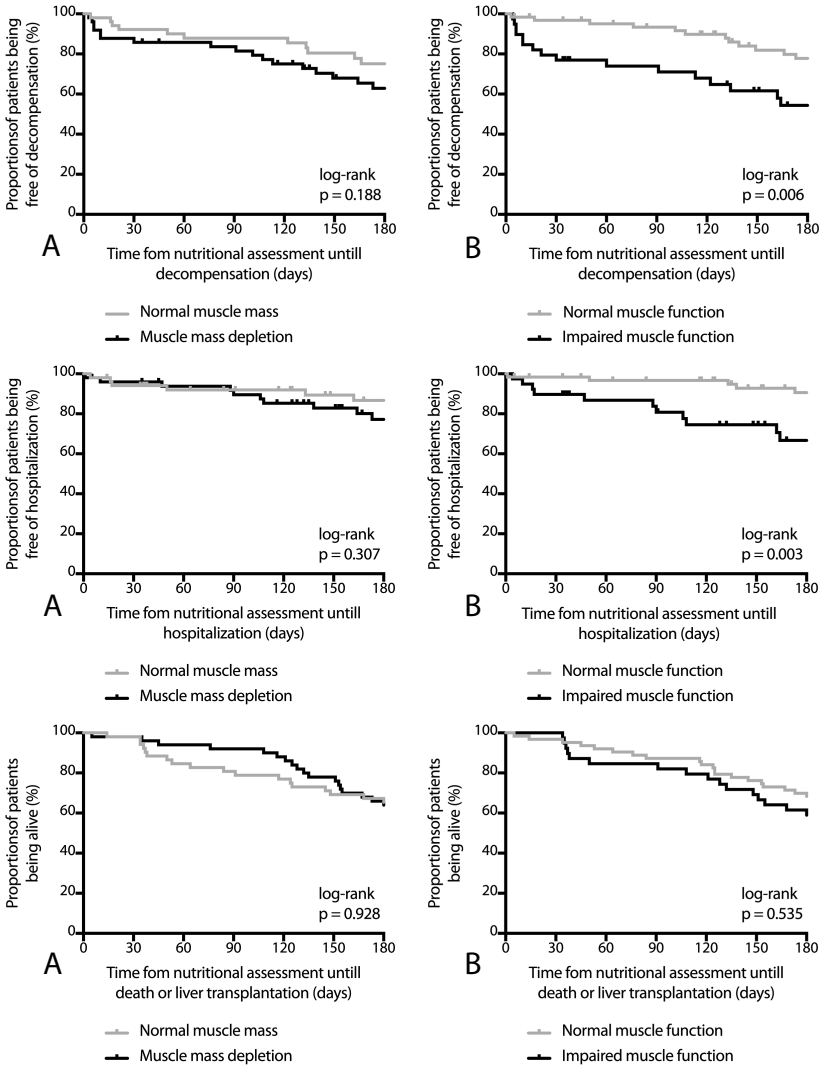
Clinical outcome

At the end of follow-up, 33 patients (32.4%) were still registered on the liver transplantation waiting list with a median follow-up time of 362.0 days (IQR 272.0 – 436.0), 55 patients (53.9%) had received a liver transplant after a median waiting time of 167.0 days (IQR 116.0 – 315.0), and 14 patients (13.7%) were removed on the waiting list due to death, clinical deterioration or hepatocellular carcinoma progression after a median time of 199.0 days (IQR 114.5 – 316.5).

Comparing patients with normal muscle mass and muscle mass depletion showed no significant differences with respect to 6-month decompensation-free survival (63% vs 75%, *p*=0.188), hospitalization-free survival (77% vs 87%, *p*=0.307), and transplant-free survival (62% vs 64%, *p*=0.928).(Figure 4A) However, when patients with or without impaired muscle function were compared, significant differences were found in 6-month decompensation-free survival (54% vs 78%, *p*=0.006), and hospitalization-free survival (67% vs 91%, *p*=0.003), but not in transplantation-free survival (62% vs 70%, *p*=0.535).(Figure 4B)

Cox-regression analysis revealed that muscle mass depletion was not an independent predictor for 6-month decompensation-free, hospitalization-free, or transplant-free survival. Impaired muscle function was a predictor for worse decompensation-free survival (HR 2.171, 95%CI 1.010 – 4.666, p=0.047) and hospitalization-free survival (HR 3.319, 95%CI 1.433 – 7.690, p=0.005), independent of age and MELDNa score, but not for transplant-free survival.

Figure 4. Kaplan-Meier estimates of decompensation-free survival (upper row), hospitalization-free survival (middle row), and transplantation-free survival (lower row), according to abnormalities in muscle mass (A) and muscle function (B).



DISCUSSION

In this cohort study a detailed nutritional assessment was performed in patients evaluated for liver transplantation. We noted muscle mass depletion and/or impaired muscle function in two thirds of patients, thereby confirming previous studies that this is highly prevalent in populations with advanced liver disease. If a detailed nutritional assessment would have been restricted to medium- or high-risk patients according to the rapid screen as proposed by the EASL, 42% of patients screened for liver transplantation with muscle mass depletion and/or impaired muscle function would have been missed. Furthermore, in contrast to muscle mass depletion, impaired muscle function was a significant predictor, independent of liver disease severity, of decompensation and mortality.

In this study 49% of patients were diagnosed with muscle mass depletion based on the third lumbar level skeletal muscle index, which is in line with other studies reporting a prevalence of 38-45%.(3, 8, 28) Similarly, the prevalence of 38% of impaired muscle function as assessed by handgrip strength was comparable to the findings of another study (30%) in 292 liver transplant candidates.(28)

Muscle function was a better predictor of 6-month decompensation-free and hospitalization-free survival than muscle mass in the present study. The key prognostic impact of muscle function has previously been reported by a prospective study including 373 patients. Physical performance, assessed by handgrip strength and gait speed (walking pace over a 5 meter distance), was found to predict cirrhosis-related complications requiring hospitalization independently from liver disease severity.(29) Similarly, two prospective studies including 213-292 patients both concluded that impaired muscle function, in contrast to muscle mass depletion, predicted waiting list mortality, independently from liver disease severity.(28, 30)

Altogether, these data indicate that decreased muscle function, possibly related to structural and functional changes such as fat accumulation, decreased muscle fiber volume or contractility and mitochondrial dysfunction, more than muscle mass or volume, is strongly correlated with outcome in advanced liver disease.(27, 31) Consequently, for prognostication measuring muscle function, such as with the simple handgrip strength test, seems more relevant than CT-based muscle volumetry.

Interestingly, in our population a high BMI was associated with inadequate dietary intake, whereas a low BMI was a risk factor for muscle mass depletion and impaired muscle function. Possibly, for patients with a high BMI it is more challenging to reach a protein intake of 1.2 g/kg/day, which is important to keep in my mind in relation to lifestyle and nutritional treatment programs in sarcopenic obesity. Our patients with a low BMI and of female gender were more prone to have muscle mass and function abnormalities, which is in line with findings of other studies.(3, 7, 8)

In addition, a phase angle ≤ 5.4°, assessed by bioelectrical impedance analysis, was significantly associated with a diagnosis muscle mass depletion and impaired muscle

function, but this finding had a low sensitivity with 56-58% for both muscle abnormalities. Measurements of fat-free mass were not associated with muscle abnormalities, which might be explained by the interference of ascites or oedema. Therefore, the data supports the conclusion that BIA does not have additional beneficial diagnostic value in this particular population.

This study has several limitations related to the study design. The size of the study population could possibly have been too small to detect a significant relationship between muscle mass and the clinical outcome and our liver transplantation screening cohort might demographically differ in MELD score and liver disease aetiology compared to other countries. Furthermore, in this study an observer effect might have occurred. Results of impaired muscle function were known by clinicians, and could have influenced clinical decisions, whereas muscle mass tests were only retrospectively performed for research purposes and unknown to clinicians. However, this is an unlikely explanation, since the main reasons for delisting liver transplant candidates are clinical deterioration and hepatocellular carcinoma progression, and not nutritional status.(32, 33)

Future prospective studies in this population are necessary to investigate whether reversibility of impaired muscle function and muscle mass depletion is possible, which nutritional and exercise programs are effective, and whether this subsequently positively influences clinical outcome. In addition, although oncologic and geriatric studies found sarcopenia is a measure of frailty (i.e. the physiological condition to have a reduced capacity to withstand environmental stresses), knowledge of the exact pathophysiologic mediators of this reduced capacity and worse clinical outcome is missing.(27, 34, 35)

In conclusion, among patients with advanced liver disease the results of nutritional screening according to the recent EASL practice guideline were suboptimal since this instrument would not lead to identify a substantial proportion of individuals with muscle mass depletion and/or impaired muscle function (sarcopenia, pre-sarcopenia and diminished muscle strength). Our data suggest a detailed nutritional assessment should be performed in all patients with advanced liver disease. We confirm previous studies that muscle function, rather than muscle mass, has major prognostic significance. Therefore, testing muscle function may be more relevant than assessing muscle mass.

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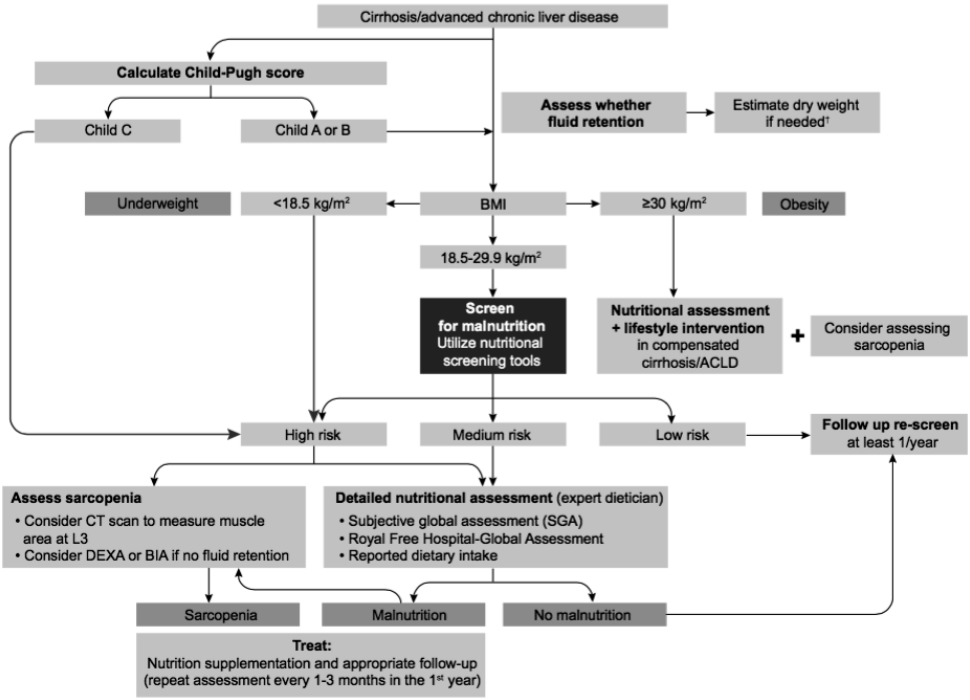
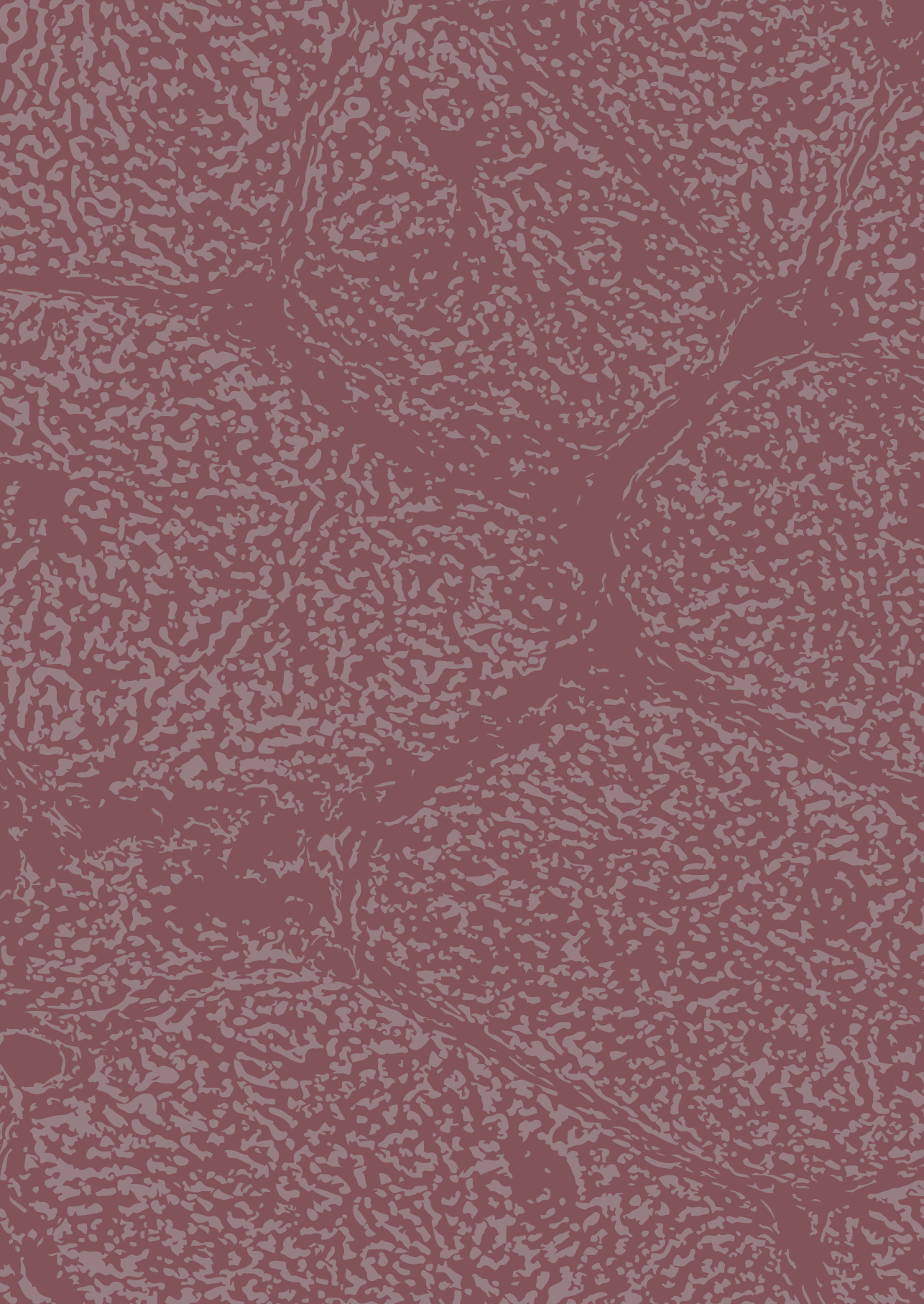
Supplement 1. Identification of patients with rapid screen for detailed nutritional screening and sarcopenia assessment in patients with advanced chronic liver disease as proposed by the EASL Clinical Practice Guidelines on nutrition in chronic liver disease.





Part IV

TREATMENT EVALUATION OF ECTOPIC VARICEAL BLEEDING AND HEPATIC ENCEPHALOPATHY

CHAPTER 10

Variable efficacy of transjugular intrahepatic portosystemic stent shunt (TIPSS) in the management of ectopic variceal bleeding: a multicenter retrospective study

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ABSTRACT

Background: Evidence for the efficacy of transjugular intrahepatic portosystemic stent shunt (TIPSS) in ectopic variceal bleeding (EctVB) is largely based on relatively small series.

Aims: To define the efficacy of TIPSS in EctVB.

Methods: Retrospective analysis of consecutive patients with chronic liver disease who presented with EctVB and received TIPSS in three tertiary centers in 1992-2016.

Results: The study included 53 patients (70% male, median age 61 years, median model for end-stage liver disease (MELD) score 11). The ectopic varices were located around the insertion of stomas (40%), duodenum (23%), rectum (17%), and at other sites (20%). Three-quarter of the patients had received previously unsuccessful medical, endoscopic or surgical therapy.

The median follow-up time was 14.0 months. Following TIPSS bleeding recurred in 12 cases: 6/12 (50%) with duodenal varices, 2/9 (22%) with rectal varices, and one each case with stomal (1/21), intraperitoneal (1/3), hepaticojejunostomy (1/2), and ascending colon varices (1/2).

The risk factors for rebleeding were MELD score at TIPSS placement (HR: 1.081 per point; 95% CI: 1.012 - 1.153; p=0.034), varices located at another site than an enterostomy (HR: 9.770; 95% CI: 1.241 - 76.917; p=0.030), and previous local therapy (HR: 5.710; 95% CI: 1.211 - 26.922; p=0.028). The estimated cumulative rebleeding rate was 23% at 1 year, 26% at 3 years and 32% at 5 years. Post-TIPSS hepatic encephalopathy manifested or worsened in 16/53 patients (30%).

Conclusion: TIPSS provides long-term control of bleeding in the majority of cirrhotic patients with EctVB. TIPSS is particularly effective in stomal EctVB, the most frequent cause of EctVB, but might not be as effective in duodenal EctVB.

INTRODUCTION

Approximately 5% of variceal bleedings occur outside the cardio-esophageal junction and are denoted as ectopic.(1) Ectopic varices are predominantly located in the small and large intestine and around enterocutaneous stomas, but can also be present in the peritoneum, biliary tree, and pelvic organs.(2) Abdominal and pelvic surgery is a well-known risk factor because post-operative adhesions and the creation of an enterostomy facilitate the formation of porto-systemic collaterals.(1, 2)

The management of ectopic variceal bleeding (EctVB) is challenging and not based on the results of controlled trials. Local endoscopic treatment modalities (band ligation, injection sclerotherapy, clips, argon plasma coagulation) and selective variceal embolization frequently fail to prevent rebleeding with reported recurrence rates up to 80% within 6 months.(3, 4) Surgical treatment, such as local sutures, devascularization procedures, or stoma revision with resiting, will only occasionally provide long-term control of bleeding in selected patients. The creation of surgical portosystemic shunts is associated with significant morbidity and mortality, particularly in patients with decompensated cirrhosis, and is rarely performed nowadays.(5-7)

Transjugular intrahepatic portosystemic stent shunt (TIPSS) creation is used to treat patients suffering from EctVB.(8) Although evidence suggests that TIPSS is usually effective to prevent recurrent bleeding, research publications are restricted to patient series including only 8 – 28 patients.(9-13) Also, variable results have been published with respect to concomitant variceal embolization, and the additional therapeutic value of embolization combined with TIPSS placement remains unclear.(9, 11-14)

We therefore aimed, in a multicenter cohort of patients with EctVB, to further determine the efficacy of TIPSS, and to evaluate outcomes in subgroups with different types of ectopic varices. We also intended to explore the benefit of concomitant vascular embolization of collateral vessels feeding the ectopic varices.

PATIENTS AND METHODS

Study design and data collection

We included all consecutive patients with advanced chronic liver disease, who underwent TIPSS placement for EctVB using bare metal stents or expanded polytetrafluoroethylene (e-PTFE)–covered nitinol stents (Viatorr, W.L. Gore & Associates Inc, Flagstaff, AZ, USA) in three tertiary referral centers: Erasmus MC, Rotterdam, the Netherlands, between January 1992 – December 2016; Academic Medical Center (AMC), Amsterdam, the Netherlands, between January 1998 – December 2016; and UZ Leuven, Leuven, Belgium, between January 2000 – December 2013. Demographic, biochemical clinical, and survival data were collected from patient hospital records and entered into a database for statistical analysis. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in approval by the Medical Ethics Committee Erasmus MC, Rotterdam, the

Netherlands, on April 18th, 2017 (MEC-2017-217), stating that written informed consent was not necessary considering the retrospective study design.

Definitions

The diagnosis of advanced chronic liver disease was based on liver histology, or a combination of clinical, biochemical, and radiologic findings.(15) The model for end-stage liver disease (MELD) score was calculated with the formula**:** 0.957 × log(creatinine in mg/dL) + 0.378 × log(bilirubin in mg/dL) + 1.120 × log(INR) + 0.643.(16, 17) Comorbidity with a cardiovascular condition, pulmonary condition, or renal condition was defined as a condition requiring long-term medical treatment for which regular specialist follow-up care was necessary. Early TIPSS was defined as TIPSS insertion within 72 hours after an EctVB episode.(18) Bleeding was defined as a decrease in hemoglobin (Hb) by 2 g⁄dL (1.24 mmol/L), or the requirement of more than 2 units of packed red cells within 24 hours to stabilize hemoglobin concentration or signs of volume depletion (systolic blood pressure below 100 mmHg and/or heart rate above 100⁄min).(13) Rebleeding was defined as a single episode of clinical significant recurrent melena or hematemesis from portal hypertensive sources after day 5 that resulted in any of the following: a) hospital admission, b) blood transfusion, c) drop in Hb of 3 g/dL (1.86 mmol/L), or d) death within 6 weeks.(19)

The standard follow-up protocol for stent function differed per center: in the Erasmus MC a functional assessment of bare TIPSS stents with Doppler ultrasound (US) was performed 2, 7, and 30 days after placement, at 3-month intervals during the first year of follow-up, and every 6 months thereafter. Following TIPSS with covered stents standard follow-up imaging was not performed. In the AMC all stents were assessed at 3-7 days, 3 months, 6 months, and 12 months after placement, and every year thereafter. In UZ Leuven stent function was assessed every 6 months. In all centers, patients received an angiography with venous portal pressure measurements when shunt dysfunction was suspected based on findings during Doppler ultrasound or clinical symptoms. Shunt dysfunction was defined as shunt stenosis greater than 50% of the shunt and/or hepatic venous portal gradient higher than 12 mmHg.(20)

Statistical analysis

Continuous variables were reported as mean with standard deviation (SD), after visual confirmation of approximate normality, and compared using a T-test. A median and range from the first to the third quartile (IQR) was computed for continuous variables with a non-normal distribution, and compared using a Mann-Whitney test. Categorical variables were reported as count with proportion and compared using the Chi-square test.

The actuarial probabilities of being free of shunt dysfunction (shunt dysfunction as event, censoring at death or liver transplantation), being free of rebleeding (rebleeding as event, censoring at death or liver transplantation), and transplant-free survival (death

as event, censoring at liver transplantation) after TIPSS creation were estimated using the Kaplan-Meier method and compared using log-rank tests.

A univariable Cox regression analysis was carried out to identify risk factors for rebleeding at TIPSS placement using candidate predictor variables, hereinafter mentioned, as described in the literature and based on the clinical and research experiences of co-investigators: MELD score, location of EctVB, local treatment of the EctVB, urgency placement of TIPSS, type of stent used during TIPSS, portal-pressure gradient after TIPSS placement above 12 mmHg, and concomitant embolization.(2, 11-13, 18, 21, 22) The univariable Cox regression models were adjusted with a propensity score to take into account differences in MELD score at TIPSS placement for each individual covariate.

Furthermore, the effect of concomitant embolization during the TIPSS procedure compared to TIPSS alone on rebleeding and mortality was analysed. For this analysis as well, a propensity score was calculated using a logistic regression model, estimating the probability to receive concomitant embolization given the following observed baseline characteristics: MELD score, location of EctVB, type of stent used during TIPSS and urgency placement of TIPSS as predictor variables.

A two-sided p-value <0.05 was considered significant for descriptive statistics and a p-value <0.10 was considered significant for univariable regression models. All statistical analyses were performed using IBM® SPSS® Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

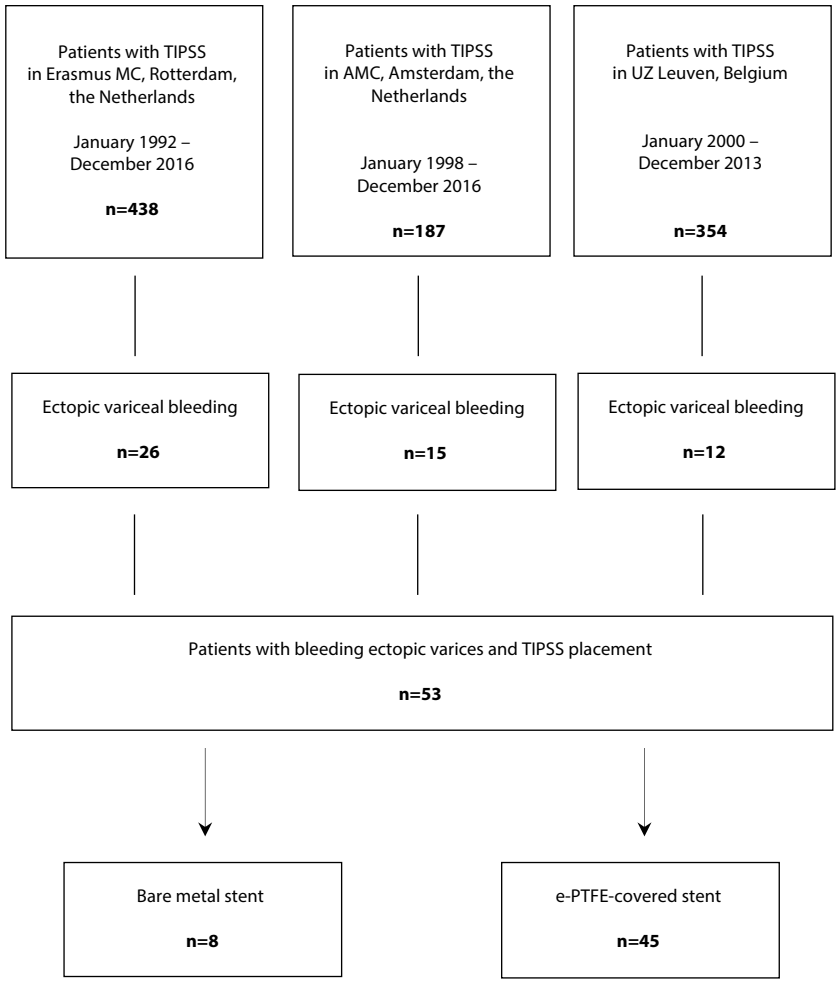
RESULTS

Patient characteristics and TIPSS procedures

In the three centers, 53 patients received TIPSS for EctVB during the study period, representing 5.4% of the total population (n=979) undergoing a TIPSS procedure. (Figure 1) The study population consisted predominantly of males with a median age of 61 years (IQR 51 – 66), and a median MELD score of 11 (IQR 9 – 17).(Table 1) The ectopic varices were most often located near the mucocutaneous junction of stomas (40%), followed by the duodenum (23%), rectum (17%), and other sites (20%). TIPSS placement was the initial treatment for EctVB in 23% of the patients. 77% of the patients had been unsuccessfully treated for EctVB with one or multiple modalities; 24 patients (45%) had undergone previous endoscopic treatment (band ligation, injection therapy, coagulation), 22 (42%) had received vasoactive medication (non-selective beta-blockers, somatostatin, terlipressin), 9 (17%) had undergone abdominal surgery (stoma revision or relocation, bowel resection), and 1 patient (2%) had received endovascular treatment (embolization).

TIPSS were created with a bare metal stent in 8 patients and with an e-PTFE–covered stent in 45 patients.(Table 2) Hemodynamic measurements showed that the median pre-TIPSS portosystemic gradient (PSG) decreased from 14 mmHg (IQR 10 – 20) to 6 mmHg (IQR 4 – 7) after TIPSS placement. The post-TIPSS PSG remained above 12 mmHg in 4 patients. Eighteen (34%) patients received early TIPSS and initial hemostasis was achieved in all cases.

Figure 1. Flow diagram of inclusion.



Abbreviations: e-PTFE: expanded polytetrafluoroethylene; TIPSS: transjugular intrahepatic portosystemic stent shunt.

Table 1. Clinical characteristics of the study population.

	Patients with ectopic variceal bleeding (n= 53)
Male gender, *n (%)*	37 (69.8%)
Age in years, *median (IQR)*	61 (51 - 66)
Etiology of portal hypertension, *n (%)*	
Alcoholic liver cirrhosis	25 (47.2%)
PSC/PBC/auto-immune hepatitis	11 (20.8%)
Cryptogenic liver cirrhosis	7 (13.2%)
Viral hepatitis	2 (3.8%)
Other	8 (15.0%)
Child-Pugh class†, *n (%)*	
A	34 (65.4%)
B	15 (28.8%)
C	3 (5.8%)
MELD score†, *median (IQR)*	11 (9 - 18)
Portal vein thrombosis, *n (%)*	5 (9.4%)
Comorbidity‡, *n (%)*	
Previous medical history of malignancy§	11 (20.8%)
Colorectal cancer	5
Urothelial carcinoma	3
Pancreatic cancer	2
Hepatocellular cancer	1
Lung cancer	1
Hodgkin's disease	1
Cardiovascular condition	9 (17.0%)
Inflammatory bowel disease	8 (15.1%)
Diabetes	6 (11.3%)
Pulmonary condition	4 (7.6%)
Renal condition	4 (7.6%)
Medical history of gastro-esophageal variceal bleeding, *n (%)*	8 (15.1%)
Number of previous episodes of gastro-esophageal variceal bleeding, *n* (%)	
1 – 3	6 (11.3%)
4 – 6	1 (1.9%)
7 or more	1 (1.9%)
History of abdominal surgery, *n (%)*	36 (67.9%)
Location of bleeding ectopic varices, *n (%)*	
Enterostomal¶	21 (39.7%)
Colostomy	11
Ileostomy	8
Urostomy	3
Duodenum	12 (22.6%)
Rectum	9 (17.0%)
Intraperitoneal	3 (5.7%)
Hepaticojejunostomy	2 (3.8%)
Ascending colon	2 (3.8%)
Jejunum	1 (1.9%)
Caecum	1 (1.9%)

Table 1. Clinical characteristics of the study population. *(continued)*

Number of previous episodes of ectopic variceal bleeding, *n (%)*	
1 – 3	26 (49.1%)
4 – 6	7 (13.2%)
7 or more	20 (37.7%)
Previous treatment of ectopic variceal bleeding, *n (%)*	
None	12 (22.6%)
Medication‡	22 (41.5%)
Non-selective β-blocker	14
Somatostatin	12
Terlipressin	1
Endoscopic‡	24 (45.3%)
Band ligation	9
Injection therapy	17
Coagulation	2
Endovascular embolization‡	1 (1.9%)
Surgery‡	9 (16.9%)

† Data regarding liver disease severity missing in 1 case.

‡ Patients could have multiple concomitant comorbidities or received multiple treatment modalities, either concomitant or successive.

§ Two patients had a history with two malignancies.

¶ One patient presented with concomitant colostomy and urostomy bleeding.

Abbreviations: IQR: interquartile range; MELD: model for end-stage liver disease.

Table 2. TIPSS procedural data.

	All patients (n=53)	**Patients with bare metal stents (n= 8)**	**Patients with e-PFTE–covered stents (n= 45)**
pre-TIPSS placement PSG (mmHg), *median (IQR)*	14 (10 - 20)	22 (12 - 26)	14 (9 - 19)
post-TIPSS placement PSG (mmHg), *median (IQR)*	6 (4 - 7)	12 (7 - 16)	5 (4 - 7)
Decrease in PSG (mmHg), *median (IQR)*	8 (6 - 13)	8 (6 - 12)	8 (6 - 13)
Concomitant embolization, *n (%)*	13 (24.5%)	1 (12.5%)	12 (26.7%)
Early TIPSS placement, *n (%)*	18 (34%)	4 (50%)	14 (31%)
Diameter stent (mm), *median (IQR)*	9 (8 - 10)	9 (8 - 10)	9 (8 - 10)

Abbreviations: e-PTFE: expanded polytetrafluoroethylene; IQR: interquartile range; PSG: portosystemic gradient; TIPSS: transjugular intrahepatic portosystemic stent shunt.

Clinical outcome

The median follow-up time was 14.0 months (IQR 3.8 – 45.9). Following TIPSS, EctVB from the same site occurred in 12/53 (23%) patients.(Figure 2) Bleeding recurred in 1/21 (5%) cases with stomal varices, 6/12 (50%) cases with duodenal varices, in 2/9 (22%) with rectal varices, in 1/3 cases with intraperitoneal varices, in 1/2 cases with varices in the ascending colon, and in 1/2 cases with varices located at the hepaticojejunostomy. The four patients with jejunal, cecal, sigmoid, or umbilical vein ectopic varices remained free of rebleeding.

Most rebleeds were diagnosed shortly after TIPSS creation; in 8 patients (4 with duodenal varices, 1 with varices at the hepaticojejunostomy, 1 with intraperitoneal varices, 1 with rectal varices, and 1 with ascending colon varices) in the first month after the TIPSS procedure (15%), in 2 patients (1 with duodenal varices and 1 with rectal varices) after 1 – 6 months (4%), and in 2 patients (1 with duodenal varices and 1 with urostomal varices) after 6 months (4%). In 9 of these 12 patients, rebleeding was associated with shunt dysfunction. After TIPSS placement, the estimated cumulative ectopic variceal rebleeding rate was 23% at 1 year, 26% at 3 years, and 32% at 5 years.(Figure 3) Rebleeding from other sources occurred in 4 patients: 3 from gastro-oesophageal varices and 1 from haemorrhagic gastropathy. The univariable Cox regression to identify risk factors for rebleeding found three predicting variables: high MELD score (HR: 1.081 per point; 95% CI: 1.012 – 1.153; p=0.020), EctVB located at another site than an enterostomy (HR: 9.770; 95%CI: 1.241 – 76.917; p=0.030), and local treatment preceding TIPSS (HR: 5.710; 95% CI: 1.211 – 26.922; p=0.028).(Table 3)

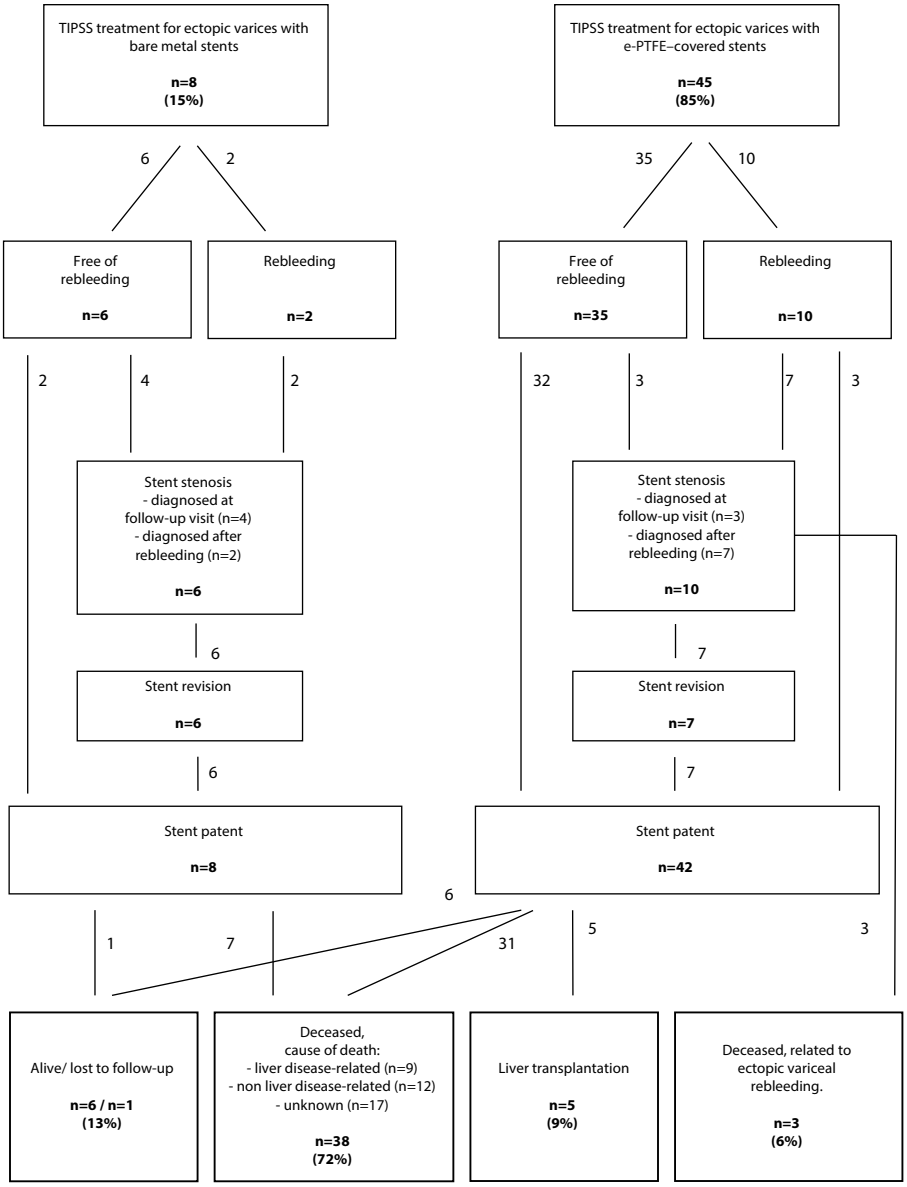
The rebleeding risk in the two main subcategories of EctVB – stomal and duodenal varices – differed markedly. A comparison of these groups with respect to aetiology of liver disease, MELD score, type of stent, concomitant embolization, post-TIPSS PSG > 12 mmHg, and established stent dysfunction, did not reveal significant differences. However, age was significantly lower in patients with duodenal EctVB (54 vs. 65 years, p=0.016), and 11/12 duodenal EctVB had been treated endoscopically before TIPSS, while local endoscopic or other procedures were performed in only 2/21 cases with stomal EctVB (p<0.001).(Supplement 1)

TIPSS dysfunction was diagnosed in 6/8 patients with bare metal stents (75%) compared to 10/45 with e-PTFE–covered stents (22%) (p=0.011). In 7 patients shunt dysfunction was diagnosed at an elective follow-up visit and in 9 patients after a rebleed. Most shunt dysfunctions were diagnosed in the first 6 months after TIPSS creation; in 7 patients in the first month (13%), in 6 after 1 – 6 months (11%), and in 3 after 6 months (6%). The estimated cumulative TIPSS dysfunction rate significantly differed (p=0.003) for bare metal stents (1 year: 76%; 3 years: 100%) compared to e-PTFE–covered stents (1 year: 23%; 3 years: 24%; 5 years: 31%).(Figure 4)

A total of 31 shunt revisions were performed in the first two years after TIPSS creation in 13 patients. An additional stent was placed in 8 patients, in 4 patients

angioplasty was performed followed in two cases by additional stent placements, and in 1 patient local thrombolysis was accomplished. Three patients with shunt dysfunction and rebleeding died.

Figure 2. Rebleeding, stent patency and clinical outcome in patients with bare metal stents and e-PTFE–covered stents.



Post-TIPSS hepatic encephalopathy (HE) manifested or worsened in 16/53 patients (30%). HE could be managed medically in 12 patients, however, in 4 patients radiological re-intervention was performed reducing the TIPSS diameter and improving or resolving in all cases the symptoms of hepatic encephalopathy. In no cases a complete shunt occlusion was performed.

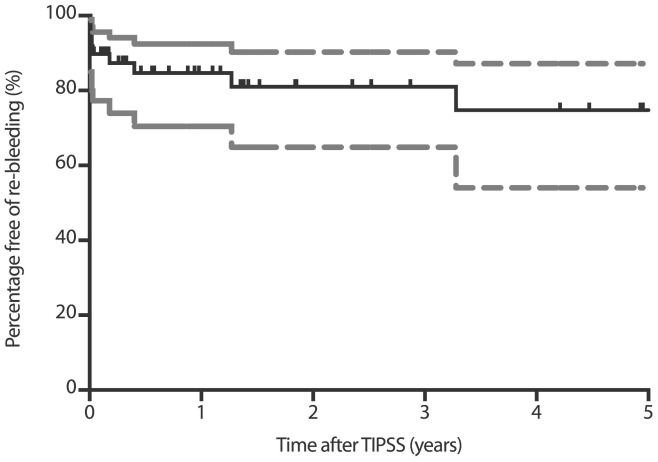
Table 3. Univariable analysis of ectopic variceal rebleeding.

	HR	**95% CI**	***p*-value**
MELD score (per point)	1.081	1.012 – 1.153	**0.020**
Location of ectopic varices†			**0.030**
Enterostomal	1		
Other site	9.770	1.241 – 76.917	
Previous local therapy†	5.710	1.211 – 26.922	**0.028**
Early placement of TIPSS†			0.653
≤ 72 hours after EctVB episode (reference)	1		
> 72 hours after EctVB episode	0.737	0.195 – 2.787	
Type of TIPSS†			0.887
Bare (reference)	1		
e-PTFE–covered	0.9894	0.193 - 4.148	
Post-TIPSS PSG†			0.884
≤ 12 mmHg (reference)	1		
> 12 mmHg	1.171	0.141 – 9.735	
Concomitant embolization†	1.133	0.304 – 4.221	0.852

† Hazard ratio adjusted with propensity score for MELD score at TIPSS placement. Abbreviations: CI: confidence interval; e-PTFE: expanded polytetrafluoroethylene; EctVB: ectopic variceal bleeding; HR: hazard ratio; MELD: model for end-stage liver disease; PSG: portosystemic gradient; TIPSS: transjugular intrahepatic portosystemic stent shunt.

In this cohort, 41 patients died, 5 underwent liver transplantation, 6 were alive at the end of follow-up and 1 was lost to follow up. The causes of death were liver disease-related in 12 patients (29.3%) including 3 patients dying of EctVB, not liver disease-related in 12 patients (29.3%), and unknown in 17 patients (41.4%).(Figure 2) The estimated 30-day, 1-year, and 5-year mortality rates were 11%, 41% and 75%, respectively.(Supplement 2)

Figure 3. Actuarial probability (black solid line) with 95% confidence interval (grey dashed lines) of remaining free of rebleeding following TIPSS.

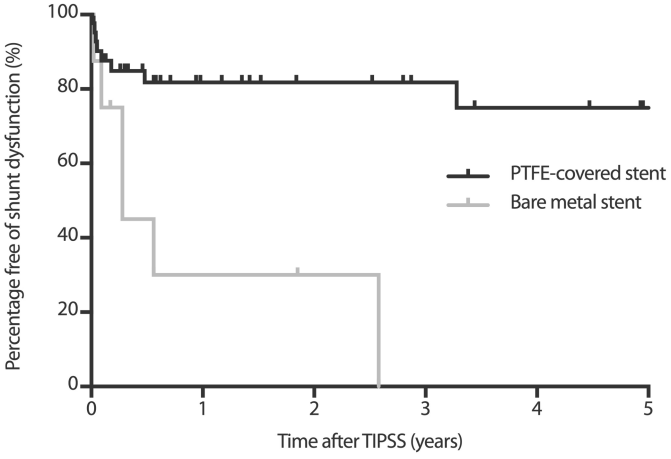


Number of patients at risk

53	25	16	13	12	8

Abbreviations: TIPSS: transjugular intrahepatic portosystemic stent shunt.

Figure 4. Actuarial probability of being free of shunt dysfunction: shunt dysfunction was diagnosed more often in patients with bare metal stents (grey line) compared to patients with e-PTFE–covered stents (black line) (log rank p=0.003).



Number of patients at risk

PTFE–covered stent					
45	20	15	12	10	7
Bare metal stent					
8	2	1	-	-	-

Concomitant embolization during TIPSS

Concomitant embolization during the TIPSS procedure was performed in 13 patients: 4 patients had varices located near enterocutaneous stomas, 4 had duodenal varices, 2 had rectal varices, 2 had intraperitoneal varices, and 1 had varices in the ascending colon. Concomitant embolization was performed in 4/9 patients with an acute bleeding and in 9/44 patients as a secondary prophylactic measure. There were no statistically significant differences between patients receiving embolization and TIPSS alone with respect to age, gender, MELD score, location of varices, presentation with acute bleeding, or treatment center. After propensity score adjustment, the hazard ratio for rebleeding of concomitant embolization compared to TIPSS alone was 0.701 (95% CI: 0.145 – 3.390; p=0.659) and the hazard ratio for mortality was 0.776 (95% CI: 0.281 – 2.148; p=0.626).

DISCUSSION

This multicenter cohort study evaluated the efficacy of TIPSS with predominantly e-PTFE–covered stents in subgroups of patients with bleeding from ectopic varices. The present study confirms that TIPSS was an effective treatment by completely preventing rebleeding in the large majority (77%) of cases. TIPSS was particularly effective in patients with less severe liver disease and with varices located at enterostomas. In contrast, the rebleeding risk in patients with duodenal varices was unexpectedly high.

The observed cumulative 23% rebleeding rate at 1 year is comparable with previously reported rates varying from 23% to 39%,(11-13) while the 26% rate at 2 years was considerably lower than previously reported.(12) It seems likely that the superior long-term bleeding control is attributable to the use of e-PTFE–covered stents in the large majority of cases. The actuarial risk of remaining free from rebleeding in the present series in comparison with the risk observed in TIPSS-treated gastro-oesophageal bleeding reported in two recently published studies originating from the participating centers was 77% vs. 94-100% at 1 year, 74% vs. 92-94% at 3 years, and 68% vs. 90-92% at 5 years, respectively.(22, 23) Thus, the overall rebleeding risk in TIPSS-treated EctVB appears to be higher than that in gastro-oesophageal bleeding. Our data indicate that this seems attributable to the relevant high rebleeding risk in TIPSS-treated duodenal EctVB.

In our cohort, shunt dysfunction was diagnosed in three-quarter of the patients with rebleeding and occurred three times more often in bare metal stents compared to e-PTFE–covered stents. In total, TIPSS dysfunction occurred in 23% at 1-year follow-up compared to approximately 20% in the study from Kochar et al. and 49% in the study from Vidal et al.(12, 13) Although these rates vary notably, the trend that e-PTFE–covered stents have improved shunt patency is in line with widely reported experience.(24, 25)

In our series the efficacy of TIPSS in patients with duodenal EctVB, who had a disappointing 50% rebleeding risk, was relatively poor. A potential explanatory

factor may be that local, but ultimately unsuccessful endoscopic therapies frequently preceded TIPSS. In our experience endoscopic treatment, in particular repeated tissue glue injections, may lead to significant duodenal ulcerations that can be the cause of repeated bleeding in their own right. In such cases it may be very difficult to distinguish portal hypertensive related bleeding from other causes, and management may be troublesome. Another possibility is that local tumorous vascular ingrowth or thrombosis could cause (re)bleeding unrelated to portal hypertension. However, in our two cases with duodenal variceal bleeding and a previous diagnosis of pancreatic cancer, there was no evidence for residual or recurrent tumour. Also with respect to other malignancies there was no indication that these were of etiological importance. Further studies in this type of EctVB are required to further address the timing of TIPSS and whether alternative therapeutic approaches, in particular balloon-occluded retrograde-transvenous-obliteration (BRTO) may be a preferable strategy.(26)

The efficacy of TIPSS has to be balanced against the risk of serious side effects, in particular hepatic encephalopathy. Post-TIPSS HE manifested or worsened in 30% of our patients, which was comparable with other reported experience.(18, 23, 27, 28) The majority of post-TIPSS HE could be managed medically, but in some cases a stent diameter reduction was necessary. A recent report suggests that there might be an optimum of 8 mm TIPSS diameter to effectively decompress the portal system in relation to the encephalopathy risk.(29) With the knowledge that the diameter of TIPSS can passively increase after placement, improved results regarding post-TIPSS HE may be expected in the future for diameter controlled expansion stents.(30, 31)

A recent meta-analysis found a non-significant trend towards a beneficial effect of variceal embolization in addition to TIPSS.(14) Our data are in line with these results as embolization did not significantly improve the probability of remaining free of rebleeding or survival. However, considering the potential selection bias occurring when embolization of the culprit varix is not feasible and the limited number of patients treated, we were unable to reliably assess the value of embolization as an adjunctive measure.

To the best of our knowledge, we report the largest multicenter cohort of patients with TIPSS for EctVB with predominantly e-PTFE–covered stents and our data reflect real life practice in three university hospitals. Despite the retrospective study design, only 1 patient was lost to follow-up. This is the first study allowing a preliminary assessment of the efficacy of TIPSS in subgroups of EctVB, although the results should be interpreted cautiously considering the size of the patient population. Ideally, prospective trials could provide more clarity about the role of TIPSS in subgroups of EctVB as well as on the role of concomitant embolization. However, such studies may never be performed considering the low prevalence of the disease, the heterogeneity in varices location, and the technical inability to embolize all culprit collateral vessels.

In conclusion, our study demonstrates that TIPSS effectively prevents rebleeding in the majority of patients presenting with EctVB. TIPSS is particularly effective in bleeding from enterostomas, the most frequent type of EctVB. However, the results in duodenal EctVB, with a 50 percent rebleeding rate, were disappointing and highlight the need for alternative therapeutic approaches.

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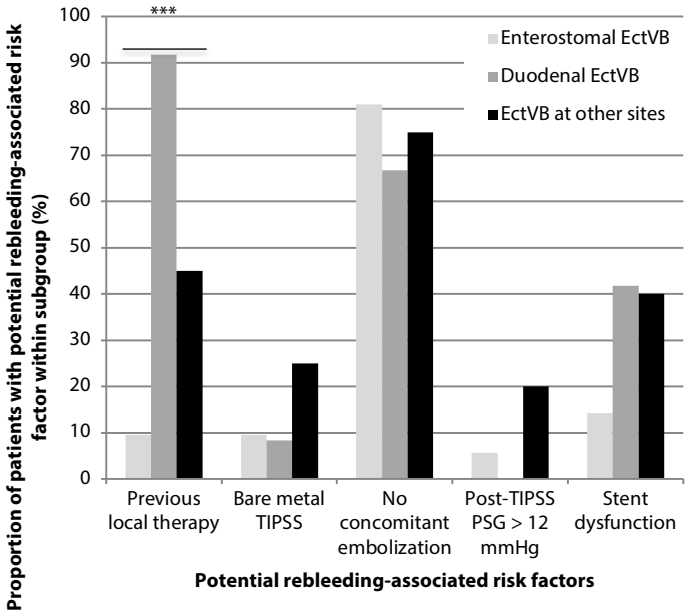
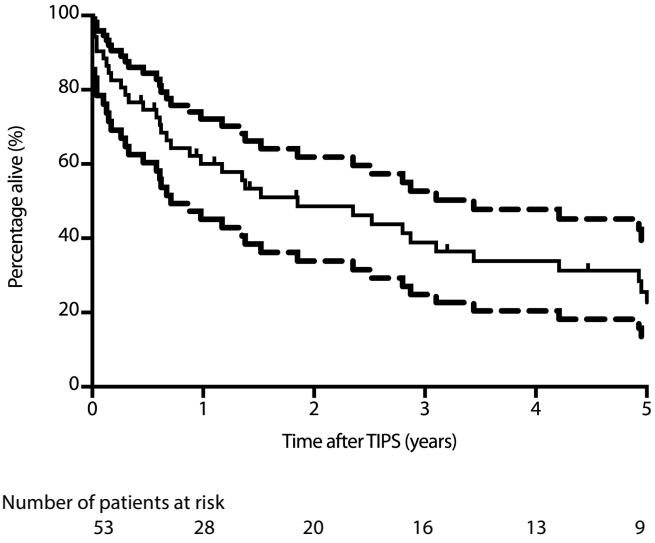
Supplement 1. Potential risk factors associated with rebleeding present at time op TIPSS placement in different subgroups: enterostomal EctVB (1 rebleeding in 21 cases), duodenal EctVB (6 rebleedings in 12 cases), and EctVB at other sites (5 rebleedings in 20 cases).



Abbreviations: EctVB: ectopic variceal bleeding; PSG: portosystemic gradient; TIPSS: transjugular intrahepatic portosystemic stent shunt.

*** = *p*-value <0.001

Supplement 2. Actuarial probability (solid line) with 95% confidence interval (dashed lines) of transplant free-survival following TIPSS.



Abbreviations: TIPSS: transjugular intrahepatic portosystemic stent shunt.

CHAPTER 11

The efficacy and safety of rifaximin-α: a 2-year observational study of overt hepatic encephalopathy

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ABSTRACT

Background: Five years after rifaximin-α registration as secondary prophylaxis for overt hepatic encephalopathy (HE) in the Netherlands, we aimed to evaluate the use of hospital resources and safety of rifaximin-α treatment in a real-world setting.

Methods: Prospective identification of all patients using rifaximin-α for overt HE. Assessment of hospital resource use, bacterial infections, and adverse events during 6-month episodes before and after rifaximin-α initiation.

Results: During 26 months we included 127 patients (71.7% male; median age 60.8 years (IQR 56.2-66.1); median MELD score 15.0 (IQR 12.1-20.4); 98% using lactulose treatment). When comparing the first 6 months after rifaximin-α initiation to the prior 6 months, HE-related hospital admissions decreased (0.86 to 0.41 admissions/patient; $p<0.001$), as well as the mean length of stay (8.85 to 3.79 bed days/admission; $p<0.001$). No significant differences were found regarding HE-related intensive care unit admissions (0.09 to 0.06 admission/patient; $p=0.253$), stay on the intensive care unit (0.43 to 0.57 bed days/admission; $p=0.661$), emergency department visits (0.66 to 0.51 visit/patient; $p=0.220$), outpatient clinic visits (2.49 to 3.30 bed visit/patient; $p=0.240$), or bacterial infections (0.41 to 0.35 infection/patient; $p=0.523$). Adverse events were recorded in 2.4% of patients.

Conclusions: The addition of rifaximin-α to lactulose treatment was associated with a significant reduction in the number and length of HE-related hospitalizations for overt HE. Rifaximin-α treatment was safe and well tolerated.

INTRODUCTION

Hepatic encephalopathy (HE) is a neuropsychiatric complication of advanced liver disease characterized by indiscernible changes (covert HE) to clinically obvious changes (overt HE) in intellect, behaviour, motor function and consciousness.(1) Overt HE affects approximately 30 – 40% of patients with cirrhosis,(2) is the most lethal cirrhosis complication with a survival rate between 40 – 55% at 6 months after diagnosis,(3, 4) and negatively affects quality of life.(5, 6)

Rifaximin-α is a poorly adsorbed antimicrobial agent and has been registered since 2013 as secondary prophylaxis for overt HE in the Netherlands.(7) The pharmacological effect of rifaximin-α has been attributed to a reduction in gut absorption and production of ammonia.(8) A meta-analysis of randomized controlled trials of rifaximin-α treatment in HE found that rifaximin-α had a beneficial effect on the secondary prevention of overt HE, increased the proportion of patients who recovered from HE, and reduced mortality.(9)

At present, the impact of rifaximin-α has not been extensively studied in a real-world setting (i.e. medical data outside controlled research study protocols in a heterogenous patient population). Recently, a cohort study of 114 patients concluded that rifaximin-α significantly reduced hospitalizations, critical care admissions, and accident and emergency (A&E) department attendances in patients using rifaximin-α for at least 6 months.(10) However, a potential beneficial effect of rifaximin-α on liver transplantation waiting list mortality or overall mortality has not been clearly established.

The primary aim of this study was to assess the impact of rifaximin-α treatment by evaluating the effect on hospitalizations, A&E department visits, outpatient clinic visits, and bacterial infections in the first 6 months after initiation compared to the prior 6 months. Secondarily, we evaluated the treatment duration and safety profile of rifaximin-α.

METHODS

Study design and patients

We aimed to identify all individuals who were treated with rifaximin-α between the 1st of September 2015 and the 1st of November 2017 at Erasmus MC, University Medical Center, Rotterdam, the Netherlands. The researchers were immediately informed the electronic medical record computer software via email when rifaximin-α was prescribed in the Erasmus MC or when a patient using this agent was registered in the hospital. All patients using rifaximin-α as secondary prophylaxis for overt HE, irrespective of the use of lactulose at that time, were prospectively included in the study. Patients were excluded when rifaximin-α was prescribed in absence of (a history of) HE, clinical data was incomplete, or when non-adherence to rifaximin-α treatment was reported. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in approval by the institution's human research committee (MEC-2015-394) with the determination that written or oral informed consent was not required considering the design of the study.

Data collection

Data regarding demographics (age; gender), clinical characteristics (aetiology of liver disease; presence of hepatocellular carcinoma; presence of HE; presence of ascites; concomitant lactulose and norfloxacin use; and blood serum values), rifaximin-α use (duration of exposure; dosage; temporary and permanent discontinuation; (serious) adverse events), and clinical outcome (number of HE-related hospital admissions and bed days on a general ward and the intensive care unit; number of liver-related hospitalizations and bed days; number of A&E department and outpatient clinic visits; number and type of infections) were retrospectively collected from electronic patient hospital records. Patients were followed for at least 6 months after rifaximin-α initiation (last data collection on 1st of May 2018), or until death, liver transplantation, or permanent discontinuation of rifaximin-α occurred.

Definitions

The model for end-stage liver disease (MELD) and the model for end-stage liver disease including sodium (MELDNa) scores were calculated with formulas used by the OPTN and Eurotransplant.(11, 12) Ascites was classified as diuretic responsive or refractory, and HE was graded according to West Haven criteria.(13, 14) The Child Pugh score and classification were calculated with the HE West Haven grade, severity of ascites, bilirubin level (μmol/L), INR and albumin level (g/L).(15) A liver-related hospital admission was defined as a hospitalization with the primary reason of admission being related to the chronic liver disease: HE, variceal bleeding, new-onset or worsening of ascites, infection, hepatorenal syndrome, hepatocellular carcinoma, or general deterioration. Infection diagnosis and determination of infection type were determined following definitions formulated by the Centers for Disease Control.(16-19) All liver-related hospital admission comprises of both HE-related and liver-related non-HE hospital admissions.

Statistical analysis

Continuous variables were reported as mean with standard deviation (SD), after visual confirmation of approximate normality. A median and interquartile range (IQR), the range between the 25th to the 75th percentile, was computed for continuous variables with a non-normal distribution. Continuous variables were analysed using a paired t-test. Categorical variables were reported as count with proportion and compared using the Chi-square test, or the McNemar's test when comparing paired outcomes. A two-sided *p*-value <0.05 was considered significant.

The actuarial probabilities of rifaximin-α use after therapy initiation were estimated using Kaplan-Meier analysis. Death, liver transplantation, and rifaximin-α discontinuation were counted as event in these analyses. All data analyses were performed using IBM SPSS statistics for Windows, Version 24.0.

RESULTS

Patient characteristics

Between 1st of September 2015 and 1st of November 2017, 151 patients were identified with rifaximin-α treatment in the Erasmus MC. A total of 24 patients were excluded: 14 patients were prescribed rifaximin-α for other indications than HE; data regarding clinical endpoints was incomplete in 6, non-adherence to rifaximin-α was reported in 3 and 1 received rifaximin-α as primary prophylaxis. The remaining 127 patients using rifaximin-α as secondary prophylaxis for overt HE were included in the study analysis.(Figure 1) The study cohort included 91 males and 36 females with a median age of 60.8 years (IQR 56.2 – 66.1). The median MELD score among patients was 15.0 (IQR 12.1 – 20.4). At time of rifaximin-α initiation, 49.6% of patients were classified as having HE West Haven grade 1, 31.5% with West Haven grade 2, 13.4% with West Haven grade 3, and 5.5% with West Haven grade 4. Lactulose was used by 124 (97.6%) patients and norfloxacin by 33 (26.0%) patients.(Table 1)

Figure 1. Flow chart of study inclusion.

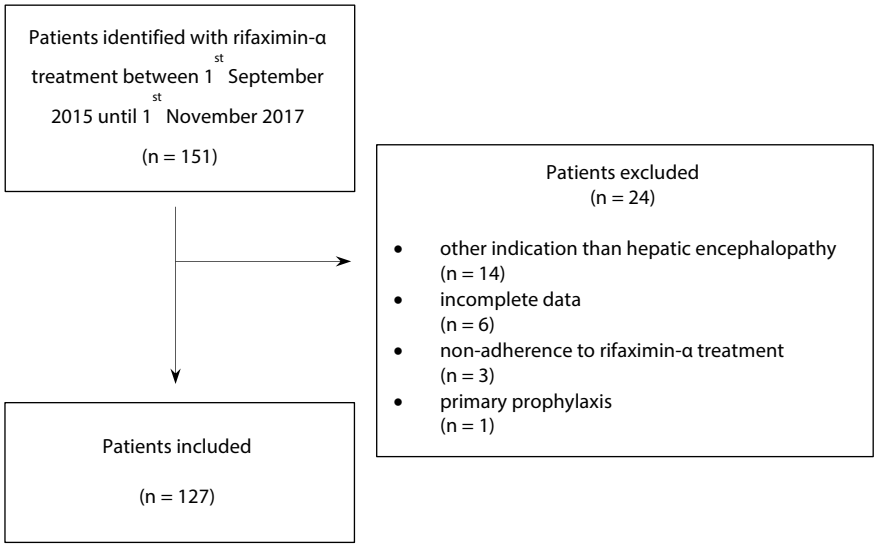


Table 1. Patient baseline clinical characteristics at the time of rifaximin-α initiation.

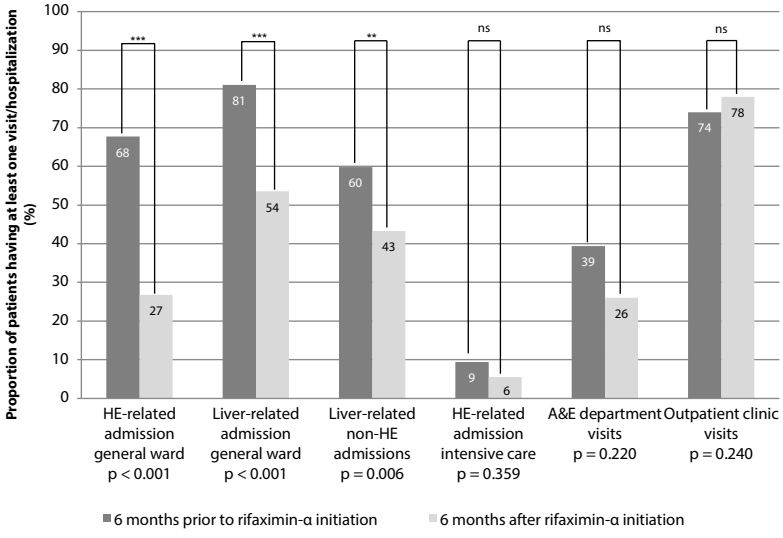
	Patients (n = 127)
Male gender, *n (%)*	91 (71.7%)
Age in years, *median (IQR)*	60.8 (IQR 56.2 – 66.1)
Etiology of liver disease, *n (%)*	
Alcoholic liver disease	43 (33.9%)
Viral hepatitis	25 (19.7%)
NASH	17 (13.4%)
Cryptogenic	15 (11.8%)
PSC/PBC/auto-immune hepatitis	15 (11.8%)
Other	5 (3.9%)
Unknown	2 (1.6%)
HCC, *n (%)*	27 (21.3%)
Liver disease severity scores	
MELD score, *median (IQR)*	15.0 (IQR 12.1 – 20.4)
MELDNa score, *median (IQR)*	16.8 (IQR 12.4 – 24.2)
Child-Pugh number, *median (IQR)*	8.0 (IQR 7.0 – 10.0)
Child-Pugh class, *n (%)*	
A	20 (15.7%)
B	45 (35.4%)
C	37 (29.1%)
HE severity classification, *n (%)*	
West Haven grade 1	63 (49.6%)
West Haven grade 2	40 (31.5%)
West Haven grade 3	17 (13.4%)
West Haven grade 4	7 (5.5%)
Ascites, *n (%)*	
None	21 (16.5%)
Diuretic responsive	36 (28.3%)
Refractory	70 (55.1%)
Blood serum parameters	
Creatinin (mmol/L), *median (IQR)*	86.5 (IQR 70.7 – 126.0)
Ammonia (µmol/L), *median (IQR)*[†]	84.0 (IQR 64.0 – 121.7)
Sodium (mmol/L), *median (IQR)*	138.5 (IQR 134.0 – 142.0)
Albumin (g/L), *median (IQR)*[‡]	32.0 (SD 28.0 – 36.0)
CRP (mg/L), *median (IQR)*[§]	16.0 (IQR 8.0 – 32.5)
ASAT (U/L), *median (IQR)*	58.0 (IQR 43.5 – 87.5)
ALAT (U/L), *median (IQR)*	40.0 (IQR 26.5 – 62.0)
Gamma-GT (U/L), *median (IQR)*[¶]	88.0 (IQR 52.5 – 163.5)
Alkaline phosphatase (U/L), *median (IQR)*	144.0 (IQR 108.0 – 210.5)
Total bilirubin (µmol/L), *median (IQR)*	35.0 (IQR 19.0 – 69.5)
Haemoglobin (mmol/L), *median (IQR)*	6.8 (IQR 5.9 – 8.0)
Platelet count (x10^9/L), *median (IQR)*	100.0 (IQR 65.5 – 146.0)
Leukocyte count (x10^9/L), *median (IQR)*	6.0 (IQR 4.2 – 8.3)
INR, median (IQR)	1.5 (IQR 1.3 – 1.7)
Lactulose use, *n (%)*	124 (97.6%)
Norfloxacin use, *n (%)*	
None	94 (74.0%)
400 mg, once daily	31 (24.4%)
400 mg, twice daily	2 (1.6%)

Table 1. Patient baseline clinical characteristics at the time of rifaximin-α initiation. *(continued)*
[†] Data was missing for 65 patients; [‡] Data was missing for 7 patients; [§] Data was missing for 39 patients; [¶] Data was missing for 10 patients.
Abbreviations: ALAT: alanine transaminase; ASAT: aspartate transaminase; CRP: C-reactive protein; Gamma-GT: gamma-glutamyl transferase; HCC: hepatocellular carcinoma; HE: hepatic encephalopathy; INR: International Normalized Ratio; IQR: interquartile range; MELD: Model For End-Stage Liver Disease; MELDNa: Model For End-Stage Liver Disease Sodium; NASH: non-alcoholic steatohepatitis; PBC: primary biliary cholangitis; PSC: primary sclerosing cholangitis.

Clinical parameters and resource use in the 6 months prior to and after rifaximin-α initiation

Figure 2 shows the proportion of patients having a hospital admission or visit in the 6 months prior to and after rifaximin-α initiation. The proportion of patients with HE-related hospital admissions to a general ward decreased from 67.7% patients prior to rifaximin-α initiation to 26.8% patients after rifaximin-α initiation (p<0.001). Similarly, the proportion of patients with liver-related hospital admissions to a general ward decreased (81.1% to 53.5%; p<0.001), as well as all liver-related non-HE hospital admissions to a general ward (59.8% to 43.3%; p=0.006). There were no significant changes in HE-related intensive care unit admissions (9.4% to 5.5%; p=0.359), A&E department visits (39.4% to 26.0%; p=0.220), or outpatient clinic visits (74.0% to 78.0%; p=0.240) between the 6 months prior to and after rifaximin-α initiation.

Figure 2. Differences in proportion of patients with at least one hospital visit or hospitalization during 6-month episodes before and after initiation of rifaximin-α treatment



The total mean number of HE-related hospital admission to the general ward decreased from 0.86 admission/patient (SD 0.81) to 0.41 (SD 0.80) (p<0.001). Also, the mean length of stay shortened from 8.85 bed days/admission (SD 11.20) to 3.79 (SD 9.37) (p<0.001). The total mean number bed days during liver-related admissions decreased from 17.18 bed days/patient (SD 18.68) to 10.16 (SD 14.81) (p=0.021) and the total mean number of bed days during non-liver related hospital admissions did not differ with 0.55 bed days/patient (SD 2.27) to 0.40 (SD 1.44) (p=0.585).

No significant differences were found in the mean number of HE-related intensive care unit admissions (0.09 to 0.06 admission/patient; p=0.253), or the mean length of stay on the intensive care unit (0.43 to 0.57 bed days/admission; p=0.661).(Table 2)

Table 2. Hospital visits, admissions and length of stay during 6-month episodes before and after rifaximin-α initiation

	6 months prior to rifaximin-α initiation	**6 months after rifaximin-α inittion**	***p*-value**
HE-related admissions on the general ward per patient in 6 months, *mean (SD)*	0.86 (0.81)	0.41 (0.80)	<0.001
HE-related hospital bed days on the general ward per admission in 6 months, *mean (SD)*	8.85 (11.20)	3.79 (9.37)	<0.001
HE-related admissions on the intensive care unit per patient in 6 months, *mean (SD)*	0.09 (0.29)	0.06 (0.23)	0.253
HE-related hospital bed days on the intensive care unit per admission in 6 months, *mean (SD)*	0.43 (1.64)	0.57 (3.17)	0.661
Liver-related hospital bed days in 6 months, *mean (SD)*	17.18 (18.68)	10.15 (14.81)	0.021
Non liver-related hospital bed days in 6 months, *mean (SD)*	0.55 (2.27)	0.40 (1.44)	0.585
A&E department visits per patient in 6 months, *mean (SD)*	0.66 (1.06)	0.51 (1.11)	0.220
Outpatient clinic visits per patient in 6 months, *mean (SD)*	2.94 (2.64)	3.30 (3.21)	0.240

Abbreviations: A&E: accident and emergency; HE: hepatic encephalopathy; SD: standard deviation.

There were no significant changes in the proportion of patients having a bacterial infection in the 6 months before or after the initiation of rifaximin-α for patients without systemic antibiotic use (25.5% to 22.3%; *p*=0.690) or patients using norfloxacin prophylaxis (39.4% to 30.3%; *p*=0.629).(Table 3)

Table 3. Bacterial infections during 6-month episodes before and after rifaximin-α initiation

	Patients in analysis	**Bacterial infections in 6 months prior to rifaximin-α initiation**	**Bacterial infections in 6 months after rifaximin-α-initiation**	***p*-value**
All study patients	127			
Number of infections per patient in 6 months, *mean (SD)*		0.41 (0.75)	0.35 (0.76)	0.523
Patients not using norfloxacin	94			
Number of infections per patient in 6 months, *mean (SD)*		0.41 (0.75)	0.35 (0.76)	0.751
Number of infections, *n (%)*		24 (25.5%)	21 (22.3%)	0.690
Bacteremia, *n (%)*		9 (9.6%)	8 (8.5%)	
SBP, *n (%)*		6 (6.4%)	6 (6.4%)	
Respiratory, *n (%)*		3 (3.2%)	4 (4.3%)	
Urogenital, *n (%)*		9 (9.6%)	4 (4.3%)	
Patients using norfloxacin	33			
Number of infections per patient in 6 months, *mean (SD)*		0.39 (0.79)	0.30 (0.70)	0.320
Number of infections, *n (%)*		13 (39.4%)	10 (30.3%)	0.629
Bacteremia, *n (%)*		1 (3.0%)	2 (6.1%)	
SBP, *n (%)*		12 (36.4%)	7 (21.2%)	
Respiratory, *n (%)*		-	1 (3.0%)	
Urogenital, *n (%)*		1 (3.0%)	-	

Abbreviations: SBP: spontaneous bacterial peritonitis; SD: standard deviation.

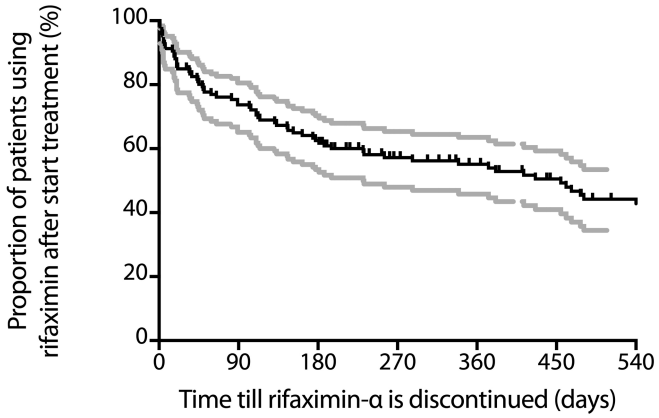
Rifaximin-α treatment duration and safety profile

The median treatment duration of rifaximin-α was 232 days (IQR 65.0 - 579.0). Figure 3 shows the estimated rifaximin-α users' rate until discontinuation. The rifaximin-α users rate after initiation was 74% at 3 months, 63% at 6 months, 55% at 1 year, and 44% at 18 months. The reasons for stopping rifaximin-α treatment in the first 6 months were: death in 24 (18.9%) patients, liver transplantation in 16 (12.6%) patients, and temporarily or permanently discontinuation in 8 (6.3%) patients for other reasons.

In the long-term follow up (until end of study observation, death, liver transplantation, or rifaximin-α discontinuation), rifaximin-α was temporarily discontinued in 7 (5.5%) patients: due to long-term HE resolution in 5 patients, adverse events in 1 patient and without any documented reason in 1 patient, but re-initiated after recurrence of overt HE. Rifaximin-α treatment was permanently discontinued in 8 (6.3%) patients: in 3 patients

prescription was discontinued without a documented reason, 2 patients had adverse events, in 2 patients treatment was withdrawn in the terminal phase of the underlying disease and in one case due to non-adherence. In total 3 patients reported an adverse event: nausea assumed to be related to rifaximin-α, rash assumed to be related to rifaximin-α, and polyneuropathy assumed to be non-related to rifaximin-α. Rifaximin-α dosage was raised to 1650 mg per day in 11 (8.7%) patients due to recurrence of overt HE while on 1100 mg per day.

Figure 3. Kaplan-Meier curve showing the proportion (solid line) of patients using rifaximin-α after initiation of treatment



DISCUSSION

The present study shows that treatment with rifaximin-α was associated with a reduction in the number of HE- and liver-related hospitalizations on the general ward and the median length of hospitalization. No evidence was found for a significant impact on intensive care unit hospitalizations, A&E department and outpatient clinic visits, or bacterial infections in the first 6 months after initiation compared to the prior 6 months. Treatment with rifaximin-α was well tolerated and rarely discontinued for other reasons than liver transplantation or death.

This study confirms earlier reports that rifaximin-α can reduce the number of HE- and liver-related hospitalisations and bed days.(10, 20) However, the finding that this treatment was associated with a significant reduction in intensive care unit hospitalizations or bed days, or A&E department visits could not be confirmed in the

present study.(10) Factors that could potentially explain these contrasting results may include differences in local treatment protocols, varying criteria for intensive care unit admissions and differences in study population characteristics, especially with respect to liver disease aetiology and severity.(10)

We found no evidence for an effect of rifaximin-α treatment on the incidence of bacterial infections, neither in patients not receiving antibiotic treatment nor in patients using continuous antibiotic treatment for the prophylaxis of spontaneous bacterial peritonitis (SBP). Previous studies have shown that rifaximin-α is an effective antibiotic prophylaxis for spontaneous bacterial peritonitis (SBP).(21) This infection is the most common precipitating factor for overt HE.(22) Although there was a non-significant decrease in SBP in our population, the power of the data might not be sufficient to draw conclusions regarding bacterial infections.

The safety profile of rifaximin-α was considered to be excellent with only 2.4% patients experiencing an adverse event of which none was considered to be serious. This is comparable to other observational cohort studies reporting adverse events in 4% of rifaximin-α users; however, these were mainly Clostridium difficile infections, an important clinical problem.(10, 23, 24)

This is the first study evaluating the efficacy of rifaximin-α with a pre-post study design that did not select solely patients that were alive and without a liver transplantation at 6 months. Approximately one-third of the patients dies or undergoes liver transplantation in the first 6 months after rifaximin-α initiation. Therefore, this study better reflects the efficacy of rifaximin-α in general practice. However, the pre-post observational study design has several limitations, as it is not possible to control all elements in the clinical course, such as the natural progression of the underlying liver disease or for instance a change in diuretic treatment. This is a general difficulty when evaluating the efficacy of treatment for overt HE, as the disease has often an episodic character and does not always present in the same severity. Therefore, hard endpoints as hospitalizations, bed days, and hospital visits were chosen.

Future studies in overt HE management are necessary to individualize treatment strategy. For example, it has not been determined which factors influence rifaximin-α treatment success, the effectiveness of high dose rifaximin-α treatment as previous shown for acute HE, and in which patients treatment can be safely withdrawn.(25)

In conclusion, this study found an association between a reduction in the number and length of HE and liver-related hospitalizations and the initiation of rifaximin-α treatment. The benefit of rifaximin-α on other types of hospital resources was less clear. Our data support the additional use of rifaximin-α in patients with recurrent overt HE already receiving standard (lactulose) treatment. No evidence was found for an adverse effect on the risk of bacterial infections and treatment was very well tolerated.

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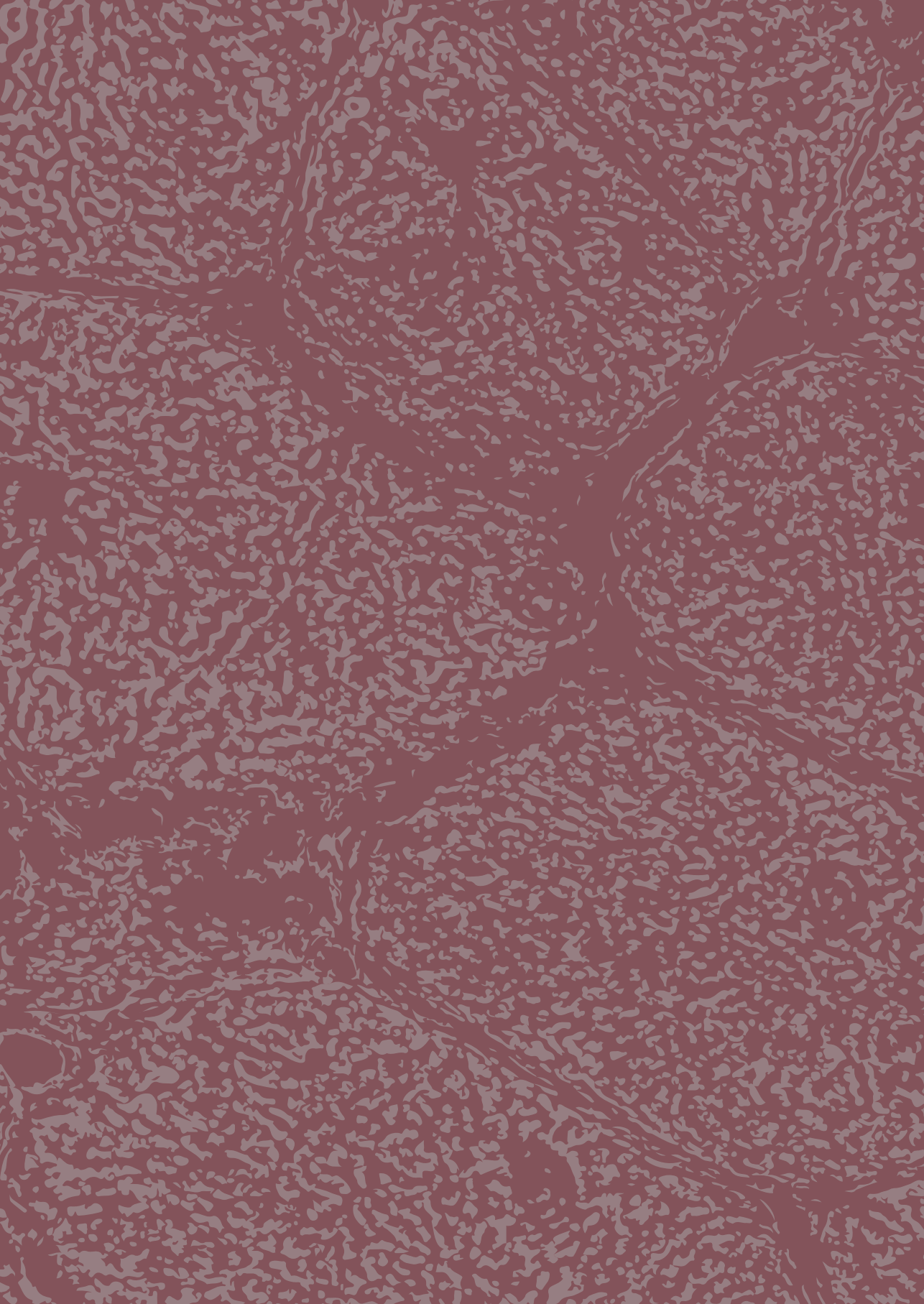

Part V
DISCUSSION

CHAPTER 12
Summary and discussion

SUMMARY AND DISCUSSION

The aim of this thesis was to study clinical, diagnostic and therapeutic aspects of frequent complications in patients with severely advanced liver disease, including spontaneous bacterial peritonitis, bacterascites, other infections, (ectopic) variceal bleeding, hepatic encephalopathy and malnutrition, with the general aim to evaluate current and new diagnostic and therapeutic strategies and with the ultimate aim to optimize patient management.

A general introduction to advanced chronic liver disease is presented in **Chapter 1**. The natural course, pathophysiology, frequent complications, and management are discussed.

The second part of this thesis – **Ascites and infections** – addresses general aspects of ascites, shows the results of studies evaluating etiological, diagnostic and therapeutic aspects of ascitic infections, and the clinical significance and impact of these and other infections in patients listed for liver transplantation.

Chapter 2 provides a review of the initial evaluation, differential diagnosis, and diagnostic approach of patients presenting with new-onset ascites. Measuring the serum ascites albumin gradient (SAAG) is the recommended initial diagnostic step when the cause of ascites is unknown. Additional ascitic biochemical, cytological and microbiologic testing, in particular determination of the polymorphonuclear white blood cell count, is usually indicated, but should take into account the clinical background, the results of other diagnostic studies and the likelihood of potential causes. This chapter also discusses a number of novel biomarkers in ascitic fluid such as bacterial DNA and proteins involved in the inflammatory response. Although some studies have found associations between bacterial translocation markers and inflammatory state or rate of complications, data on diagnostic accuracy and cost-effectiveness regarding this and other potential new biomarkers are yet insufficient or missing.(1-4)

Chapter 3. Reagent dipstick strips are widely used as a bedside tool to detect leukocyte esterase in urine for the diagnosis of urinary tract infection. This chapter presents the results of a prospective cross-sectional study evaluating the accuracy of leukocyte esterase reagent strips for diagnosing spontaneous bacterial peritonitis (SBP). In 157 ascites samples of patients with cirrhosis, the diagnostic accuracy of the reagent strip was comparable to the gold standard test (manual polymorphonuclear neutrophil count) with regard to the negative predictive value and sensitivity. Therefore, the leukocyte esterase reagent strip was considered to be an excellent tool to exclude SBP. The reagent strips used in this study were not specifically designed for ascitic fluid

analysis indicating different cut-off levels for leukocyte concentration and possible interference of proteins present in ascites. More recently, a large French multicenter study has shown that a strip with specific characteristics for ascitic fluid analysis, the Periscreen strip, has a 100% sensitivity and negative predictive value in 803 ascitic fluid samples of outpatients.(5) Although data support the reagent strip as a valuable and cheap tool to exclude SBP in patients with a low pre-test probability (outpatients), the use of reagents strips is currently not included in international guidelines, probably mainly due to reported variability in diagnostic accuracy.(6-8)

In **Chapter 4**, changes in the causative pathogens of SBP in our center were assessed. In several countries changes in microbiology have been reported and attributed to long-term widespread quinolone use and increased prevalence of hospital admissions.(9-14) These findings have raised doubts about the currently recommended prophylactic and therapeutic antibiotic strategy in SBP. In the current study, a comparison was made between the results of ascitic bacterial cultures in two cohorts of patients diagnosed with SBP in the years 2003–2005 and 2013–2014, respectively. We found a modest but non-significant increase of Gram-positive bacteria and multidrug-antibiotic–resistant bacteria causing SBP, which differs from data reported in other geographical areas. We concluded that microbiology and susceptibility patterns' differences can be region dependent and empiric antibiotic prophylaxis and treatment of SBP should be based on national or regional microbiological data. The latest European guideline emphasizes that antibiotic treatment for SBP should be guided by regional microbiological data, together with the mode of acquisition (community-acquired, healthcare-associated, or nosocomial), and the infection severity.(6)

The clinical characteristics, microbiological findings and prognosis of 123 patients with bacterascites are described and compared to patients with SBP in **Chapter 5**. It is remarkable that bacterascites has remained a relatively infrequently studied clinical entity. Bacterascites is a different clinical entity than SBP and defined by an ascitic fluid polymorphonuclear neutrophil count below 250/μL and a positive ascitic fluid culture.(15) Bacterascites was more often caused by Gram-positive bacteria in comparison to SBP, however, patients with bacterascites and SBP were highly comparable with respect to severity of liver disease and mortality rate. Bacterascites was likely to persist or to evolve to SBP in a significant proportion of patients if left untreated. We found that discrimination between asymptomatic and symptomatic patients, as proposed in current guidelines, was not an optimal indicator to predict clinical outcome.(6, 7) Our results suggest that the threshold to initiate antibiotic treatment should be low, particularly in patients with severely advanced liver disease. Other recent studies have reported that the relative ascites PMN count (the absolute PMN count divided by the absolute leukocyte count) and

clinical characteristics, particularly presence of hepatocellular carcinoma and MELD-scores above 17, provide information with respect to the risk of progression of bacterascites to SBP.(16, 17) Future studies are urgently required to establish with more certainty clinical and/or biochemical markers enabling reliable identification of patients with bacterascites who may benefit from antibiotic treatment, in order to minimize antibiotic under- and overtreatment in this population.

Chapter 6 reports a cohort study evaluating the frequency of infections in 327 patients awaiting liver transplantation and assessing the impact of infections on clinical outcome. Infections occurred in 44% of patients. The results of our study indicate that infection is the leading cause for delisting from the liver transplantation waiting list, increasing the chance by 5.2 times to become delisted from the waiting list compared to non-infected patients. In particular patients with advanced age, severely advanced liver disease, or receiving inappropriate antibiotic therapy were susceptible for delisting due to infection. The results of this study underline the importance of appropriate and timely antimicrobial therapy once more in order to prevent and treat infections before patients lose their suitability for liver transplantation. Similarly, a multicenter North American consortium found that patients with infections are at a much higher risk to become delisted.(18) In addition, an association was found between delisting and the number and type of organ failures caused by the infection. These data support the importance to recognize acute-on-chronic liver failure, a relatively recent distinguished clinical identity, in which the type and number of organ failures is directly associated with an increased risk of short-term mortality.(6, 19)

The third part – **Findings and implications of diagnostic assessments during liver transplantation screening** – covers studies assessing the results and impact of colorectal cancer screening and nutritional evaluation during liver transplantation screening.

In **Chapters 7 and 8**, the results of colonoscopy during pre-liver transplantation screening in 808 patients were presented. The main finding of this study was that colorectal cancer (CRC) screening in this subpopulation was associated with a relatively low prevalence of colorectal cancer (0.2%) and advanced adenomas (5.4%). Patients had an increased post-procedural risk of complications, such as acute renal failure and gastrointestinal bleeding, especially patients with severely advanced liver disease. The indication for standard pre-liver transplantation screening colonoscopy may be questioned considering the balance between the yield and associated risks and costs, also taking important competing mortality risks, such as the substantial waiting list and perioperative mortality, into account. Alternative strategies for colorectal cancer screening should be considered. Future research projects regarding CRC screening in

transplant candidates could focus on the assessment of factors relevant for more refined risk stratification in this population. It may be equally important to prospectively assess the results of alternative CRC screening strategies.

Chapter 9 presents a study reporting the results from 102 patients during liver transplantation screening undergoing detailed nutritional assessment. Sarcopenia, characterized by a reduction in muscle mass and function, is a major component of malnutrition and is associated with a higher rate of complications and mortality. Identification of nutrition-related disorders can be beneficial in order to be able to optimize patient condition before liver transplantation.(20-24) Our study confirms that two-thirds of patients had muscle mass depletion and/or impaired muscle function. The clinical screening tool, as proposed in a recent international guideline, had a poor efficacy in discriminating patient with low or high risk on malnutrition in this subpopulation.(25) Until there is a discriminative parameter identifying, which patients should undergo detailed nutritional assessment, we propose all patients screened for liver transplantation should be screened for malnutrition with a detailed nutritional assessment. Furthermore, data of this study suggests that impaired muscle function might be clinically more relevant than muscle mass depletion. This is in line with several other studies indicating that impaired muscle function has a stronger correlation with clinical outcome in advanced liver disease than muscle mass or volume.(26, 27) Consequently, for prognostication measuring muscle function, such as with the simple handgrip strength test, seems more relevant than CT-based muscle volumetry.

Future prospective studies in this population are necessary to investigate whether reversibility of impaired muscle function and muscle mass depletion is possible, which nutritional and exercise programs are effective, and whether this subsequently positively influences clinical outcome. In addition, although oncologic and geriatric studies found sarcopenia is a measure of frailty (i.e. the physiological condition to have a reduced capacity to withstand environmental stresses), knowledge of the exact pathophysiologic mediators of this reduced capacity and worse clinical outcome is missing.(27-29)

In the fourth part – **Treatment evaluation of ectopic variceal bleeding and hepatic encephalopathy** – of this thesis, the efficacy of treatment strategies for ectopic variceal bleeding and hepatic encephalopathy were assessed.

Chapter 10 includes a multicenter retrospective cohort study evaluating the long-term control of bleeding in 53 patients with ectopic variceal bleeding using transjugular intrahepatic port-systemic shunt (TIPSS). We found that TIPSS provides long-term control of bleeding in the majority of patients with ectopic variceal bleeding with a rebleeding rate of 23% at 1 year, 26% at 3 years and 32% at 5 years. The efficacy of shunt placement

to avoid rebleeding seemed to differ for the location of the bleeding ectopic varices. Patients with stomal variceal bleeding had a 95% success rate, but patients with duodenal variceal bleeding a 50% success rate. A potential explanatory factor may be that local, but ultimately unsuccessful endoscopic therapies, in particular repeated tissue glue injections, may lead to significant duodenal ulcerations that can be the cause of repeated bleeding in their own right. Further studies in duodenal variceal bleeding are required to further address the timing of TIPSS and whether alternative therapeutic approaches might be a more preferable strategy. The efficacy of TIPSS has to be balanced against the risk of serious side effects, in particular hepatic encephalopathy, manifesting or worsening in 30% of our patients, which was comparable with other reported experience.(30-33) We found a non-significant trend towards a beneficial effect of variceal embolization in addition to TIPSS. This was in line with the results of a recent meta-analysis that showed concomitant embolization did not significantly improve the probability of remaining free of rebleeding or survival.(34)

In **Chapter 11**, the addition of rifaximin to lactulose treatment in the secondary prevention of hepatic encephalopathy was evaluated in 127 patients in a pre-post study design. In the six months following initiation of rifaximin, patients had a significantly lower number of hospitalizations for hepatic encephalopathy and a reduced length of admission, compared with the preceding six months. This study confirms results from earlier reports, however, we did not find a significant reduction in intensive care unit hospitalizations or emergency room department visits.(35, 36) Previous studies have shown that rifaximin is an effective antibiotic prophylaxis for SBP.(37, 38) Although there was a non-significant decrease in SBP in our population, the power of the data was not sufficient to draw conclusions regarding bacterial infections. Lastly, we found the safety profile of rifaximin to be excellent with a low number of reported adverse events. Future studies in hepatic encephalopathy management are necessary to individualize treatment strategy. For example, it has not been determined which factors influence rifaximin treatment success, the effectiveness of off-label high dose rifaximin treatment, and in which patients treatment can be safely withdrawn.(39)

General conclusion

This thesis focuses on various deleterious complications of chronic advanced liver disease. The vulnerability of the patient with chronic advanced liver disease is reflected by the significant risk of developing (further) complications and death. Not only the disease itself, but also the implications of diagnostic and therapeutic interventions can potentially be harmful for the patient, such as colorectal cancer screening in patients with advanced chronic liver disease or TIPSS in the management of ectopic variceal bleeding. Furthermore, the landscape in which the patient presents is dynamic: microbiologic patterns change, antibiotic resistance emerges, and biotechnology and laboratory research advances.

Our understanding of the complex problems and many threats encountered in patients with advanced chronic liver disease gradually deepens. Complications may involve any organ system, may occur consecutively or simultaneously and are often clearly related, such as variceal bleeding and renal failure complicating infections and encephalopathy and ascites following gastrointestinal bleeding. Current evidence suggests that addressing or strongly focus on individual complications, for instance prophylaxis of variceal bleeding or detection and early treatment of hepatocellular carcinoma, is unlikely to have a major effect on overall prognosis. Therefore, a multi-focussed approach is essential. However, much uncertainty exists with respect to the potential impact of certain specific interventions. For instance, whether (early) detection and attempts to treat malnutrition will ultimately influence prognosis is unclear. Also, much has to be learned about the potential importance of complications, such as variceal bleeding, SBP or malnutrition, in risk stratification. In this thesis, the importance of mainly individual patient strategies is considered, such as the approach to a patient with ectopic variceal bleeding from the duodenum versus bleeding originating from stomal varices, or the antibiotic strategy in bacterascites as compared to SBP.

Taken together, the results of the presented studies shed light on certain aspects in the complex management of patients with advanced chronic liver disease, and will hopefully contribute to improve outcome in this population at high risk for fatal and non-fatal complications.

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CHAPTER 13

Samenvatting en discussie

SAMENVATTING EN DISCUSSIE

Dit proefschrift is gewijd aan klinische, diagnostische en therapeutische aspecten van veel voorkomende complicaties bij patiënten met gedecompenseerde levercirrose, waaronder infecties, met name spontane bacteriële peritonitis (SBP) en bacterascites, (ectopische) varicesbloedingen, hepatische encefalopathie en ondervoeding. Het doel van de opgenomen studies was om huidige en nieuwe diagnostische en therapeutische strategieën te evalueren teneinde de patiëntenzorg te optimaliseren.

Hoofdstuk 1 geeft een algemene inleiding tot gedecompenseerde levercirrose. De pathofysiologie en het natuurlijk beloop, veel voorkomende complicaties en de behandeling van deze ziekte worden geïntroduceerd en toegelicht.

Het tweede deel van het proefschrift – **Ascites en infecties** – betreft studies naar etiologische, diagnostische en therapeutische facetten van ascites, met de nadruk op infecties in ascites (bacterascites en spontane bacteriële peritonitis) en de klinische impact van het infecties bij patiënten op de levertransplantatie wachtlijst.

Hoofdstuk 2 bevat een literatuur synopsis van de initiële evaluatie, differentiaal diagnose en de diagnostische benadering van patiënten met ascites. Het meten en berekenen van de serum ascites albumine gradiënt (SAAG) is de eerste aanbevolen diagnostische stap wanneer de oorzaak van ascites onduidelijk is. Additionele biochemische, cytologische en microbiologische analyses van ascites, met name het bepalen van het aantal polymorfonucleaire leukocyten (PMN's), is vaak geïndiceerd, maar moet worden afgewogen tegen de klinische achtergrond, zoals de resultaten van andere diagnostiek en de waarschijnlijkheid van de mogelijke oorzaken. In dit hoofdstuk worden nieuwe biomarkers in ascites besproken, zoals de detectie van bacterieel DNA en eiwitten betrokken bij de inflammatoire respons. Hoewel sommige studies een verband hebben gevonden tussen deze markers van bacteriële translocatie en inflammatie of het aantal complicaties, zijn er onvoldoende data over de diagnostische accuratesse en kosteneffectiviteit van deze biomarkers.(1-4)

Hoofdstuk 3. Teststroken, ook wel dipsticks of teststrips genoemd, worden in de alledaagse medische praktijk gebruikt als eenvoudig screenend onderzoek van urine voor het diagnosticeren van een urineweginfectie middels de detectie van onder andere leukocyten esterase. Dit hoofdstuk toont de resultaten van een prospectieve cross-sectionele studie naar de diagnostische accuratesse van teststroken in ascites voor het diagnosticeren van spontane bacteriële peritonitis (SBP). De diagnostische accuratesse van de teststroken was vergelijkbaar met de gouden standaard (handmatige PMN telling)

met betrekking tot de negatief voorspellende waarde en sensitiviteit, gemeten in 157 ascites monsters van patiënten met levercirrose. Derhalve werd de teststrook geacht een excellent middel te zijn om SBP te excluderen. De teststrip in deze studie is ontworpen voor urine en niet specifiek voor het gebruik in ascites. Het toont andere afkapwaarden voor leukocyten concentraties dan gebruikelijk zijn voor de diagnose SBP en mogelijk interfereren eiwitten in ascites met de test. Onlangs heeft een groot Frans multicenter onderzoek aangetoond dat de Periscreen strip, een teststrook specifiek ontworpen voor analyse van ascites, een 100% sensitiviteit en negatief voorspellende waarde had in 803 ascites monsters van poliklinische patiënten.(5) Alhoewel data tonen dat teststroken een waardevol en goedkoop hulpmiddel zijn om SBP te excluderen in patiënten met een lage pre-test waarschijnlijkheid van ziekte (poliklinische patiënten), is het gebruik van teststroken niet geïncludeerd in internationale richtlijnen, waarschijnlijk door de hoge variëteit in diagnostische accuratesse.(6-8)

In **Hoofdstuk 4** wordt onze studie weergegeven die erop gericht was na te gaan in hoeverre in ons centrum de aard van de micro-organismen, die ten grondslag ligt aan SBP, in de loop van de tijd is veranderd. In verschillende landen zijn er verschuivingen in type SBP-ziekteverwekkers gerapporteerd en dit verschijnsel wordt toegeschreven aan het langdurig en wijdverspreid gebruik van (fluoro)quinolone antibiotica en een toename van ziekenhuisopnames.(9-14) Deze bevindingen doen de vraag rijzen of het huidige aanbevolen profylactische en therapeutische antibiotische beleid voor SBP accuraat is. In de beschreven studie werd een vergelijking gemaakt tussen de microbiologische resultaten van asciteskweken in patiënten met SBP in de jaren 2003–2005 en 2013–2014. Er werd een milde niet-significante toename van Gram-positieve en multiresistente bacteriën die SBP veroorzaakten waargenomen, een bevinding die verschilt van andere geografische gebieden. We concludeerden dat microbiologische en antibioticumresistentie patronen tussen regio's kunnen verschillen en empirisch antibiotisch beleid voor SBP gebaseerd moet zijn op nationale of regionale microbiologische data. De laatst gepubliceerde Europese richtlijn benadrukt dat de keuze van antibiotische behandeling van SBP gefundeerd moet zijn op regionale microbiologische data, samengaand met waar de infectie is verworven (gemeenschap of ziekenhuis) en de ernst van de infectie.(6)

De klinische kenmerken, microbiologische bevindingen en de prognose van 123 patiënten met bacterascites werden in **Hoofdstuk 5** beschreven en vergeleken met patiënten met SBP. Het is opmerkelijk dat bacterascites een relatief weinig bestudeerd ziektebeeld is gebleven. Bacterascites is anders dan SBP en wordt gekenmerkt door een laag aantal PMN's (<250/µL) in ascites, maar een positieve asciteskweek.(15) Bacterascites werd vaker veroorzaakt door Gram-positieve bacteriën vergeleken met SBP, maar patiënten met bacterascites en SBP waren zeer vergelijkbaar met betrekking tot de ernst van de cirrose en het mortaliteitsrisico. Bacterascites bleef voortbestaan of ontwikkelde

zich vaak tot SBP wanneer er geen antibiotische behandeling werd ingesteld. We vonden dat het discrimineren tussen patiënten met en zonder symptomen, zoals in de huidige internationale richtlijn staat beschreven, geen optimale indicator is om de klinische uitkomst te voorspellen.(6, 7) Onze resultaten suggereren dat de drempel om antibiotische behandeling te starten laag zou moeten zijn, zeker bij patiënten met ernstige gedecompenseerde cirrose. Andere recente studies rapporteerden dat het relatieve PMN aantal in ascites (het totale PMN aantal gedeeld door het totale leukocyten aantal) en klinische kenmerken, waaronder de aanwezigheid van levercelkanker en een MELD-score boven de 17, goede voorspellers waren voor de progressie van bacterascites naar SBP.(16, 17) Toekomstige studies naar klinische en/of biochemische voorspellende kenmerken voor patiënten met bacterascites die baat hebben bij antibiotische behandeling zijn hard nodig, met als doel om antibiotische onder- of overbehandeling te minimaliseren.

Hoofdstuk 6 bevat een studie naar het optreden van infecties bij 327 patiënten op de levertransplantatiewachtlijst en de impact van de infecties op het klinisch beloop. Infecties deden zich voor in 44% van deze patiënten. De resultaten van onze studie lieten zien dat infecties de meest voorkomende oorzaak waren van uitval op de levertransplantatie wachtlijst. Het relatief risico op uitval van de wachtlijst nam toe met een factor 5.2 ten opzichte van patiënten zonder een infectie. Met name patiënten met een hogere leeftijd, ernstige cirrose, of patiënten die empirisch antibiotica ontvingen waar de verwekker van de infectie niet gevoelig voor was, liepen risico op uitval van de levertransplantatie wachtlijst door infectie. De resultaten van deze studie benadrukken het belang van tijdige en geschikte antibiotische behandeling om infectie te voorkomen en te behandelen, voordat patiënten hun geschiktheid om een levertransplantaat te ontvangen verliezen. Een Noord-Amerikaanse multicenter studie vond dezelfde associatie dat patiënten met infecties een groter risico liepen om uit te vallen van de levertransplantatie wachtlijst.(18) Tevens werd er een associatie gevonden tussen uitval van de levertransplantatie wachtlijst en het aantal en type orgaanfalen veroorzaakt door de infectie. Deze data ondersteunen het belang om acuut op chronisch leverfalen te herkennen, een relatief nieuwe ziekte entiteit, waarbij het aantal en type orgaanfalen direct geassocieerd zijn met het mortaliteitrisico.(6, 19)

Het derde deel van het proefschrift – **Bevindingen en implicaties van diagnostische onderzoeken tijdens screening voor levertransplantatie** – bevat studies met resultaten van de darmkankerscreening en de evaluatie van de voedingsstatus ter beoordeling van de geschiktheid voor het ontvangen van een levertransplantaat.

In **Hoofdstuk 7 en 8**, worden de resultaten en complicaties van protocollaire colonoscopie in het kader van levertransplantatie screening gepresenteerd. De belangrijkste bevinding van deze studie, waarin 808 patiënten waren opgenomen, was dat darmkankerscreening

in deze subpopulatie een relatief lage prevalentie van darmkanker (0.2%) en advanced adenomen (5.4%) toonde. Patiënten hadden na colonoscopie een groter risico op complicaties, zoals acute nierinsufficiëntie en gastro-intestinale bloedingen, met name patiënten met ernstige cirrose. De indicatie voor het standaard uitvoeren van een colonoscopie tijdens levertransplantatie screening is daarom betwistbaar, in ogenschouw nemende de opbrengst en de geassocieerde risico's en kosten. Alternatieve strategieën voor darmkankerscreening moeten worden overwogen. Toekomstige studies betreffende darmkankerscreening bij levertransplantatiekandidaten zijn gewenst en zouden zich moeten richten op het identificeren van factoren op grond waarvan een betrouwbare risicostratificatie in deze populatie mogelijk is. Tevens is het minstens zo belangrijk om prospectief alternatieve darmkankerscreening strategieën te evalueren.

In **Hoofdstuk 9** zijn de resultaten van een gedetailleerde voedingsevaluatie opgetekend die verricht werd bij 102 patiënten tijdens protocollaire evaluatie voor levertransplantatie. Sarcopenie, gekenmerkt door een reductie in spiermassa en –functie, is een belangrijke component van ondervoeding en wordt geassocieerd met een toename van complicaties en mortaliteit. Het identificeren van voedingsgerelateerde ziekte kan bevorderlijk zijn om de conditie van de patiënt te verbeteren voordat zij een levertransplantatie ondergaan.(20-24) Onze studie toonde dat twee derde van de patiënten een verminderde spiermassa en/of –functie had. Het screenende algoritme, zoals recent voorgesteld in een internationale richtlijn, was weinig efficiënt voor het identificeren van patiënten met een laag of hoog risico op ondervoeding in onze populatie.(25) Totdat er een efficiënter algoritme is gevonden, stellen wij voor om bij alle levertransplantatie kandidaten een gedetailleerde voedingsevaluatie te verrichten. Onze studie toont tevens dat spierfunctie een klinisch meer relevante voorspellende parameter is dan spiermassa. Ook andere studies vonden dat een verminderde spierkracht een sterkere correlatie had met klinische uitkomsten van gedecompenseerde cirrose dan spiermassa of –volume.(26, 27) Derhalve lijkt het meten van spierfunctie, bijvoorbeeld met de handgriptest, relevanter voor het voorspellen van het klinisch beloop dan spiervolumetrie middels CT. Toekomstige prospectieve studies zijn noodzakelijk om vast te stellen of een verminderde spierfunctie/-massa reversibel is, welke voedings- en oefentherapieën efficiënt zijn, en of reversibiliteit ook de klinische uitkomst positief kan beïnvloeden. Alhoewel er binnen de oncologie en geriatrie overeenstemming is bereikt dat sarcopenie een maatstaf is voor 'frailty' (i.e. de fysiologische conditie van een verminderde capaciteit om zich stand te houden bij stressoren uit de omgeving), is er vraag naar kennis over de exacte pathofysiologische mediatoren van deze verminderde capaciteit en slechtere klinische uitkomsten.(27-29)

In het vierde deel van dit proefschrift – **De evaluatie van de behandeling naar ectopische varicesbloedingen en hepatische encefalopathie** – wordt de effectiviteit

van enkele behandelstrategieën bij deze ziektebeelden geëvalueerd.

Hoofdstuk 10 bevat een multicenter retrospectieve cohortstudie naar het beloop van 53 patiënten met ectopische varicesbloedingen die behandeld werden met een transjugulaire intrahepatische portosytemische shunt (TIPSS). TIPSS was in het merendeel van de patiënten met ectopische varicesbloedingen langdurig succesvol in het voorkomen van bloedingen met een recidief risico van 23% na 1 jaar, 26% na 3 jaar en 32% na 5 jaar. De effectiviteit van TIPSS leek afhankelijk van de aard van de ectopische varicesbloeding. Bij patiënten met stomabloedingen trad in slechts 5% van de gevallen een recidief bloeding op tegen 50% bij patiënten met duodenum varicesbloedingen. Een potentiele verklaring hiervoor is dat plaatselijke endoscopische therapieën, die op lange termijn onsuccesvol blijken te zijn, bijvoorbeeld recidiverende cyano-acrylaatlijm injecties, kunnen leiden tot significante duodenale ulcera, welke zelf opnieuw een bloeding kunnen veroorzaken. Verdere studies naar duodenale varicesbloedingen zijn nodig om de timing van TIPSS behandeling te onderzoeken en na te gaan of andere behandelstrategieën de voorkeur verdienen. De effectiviteit van TIPSS moet worden afgewogen tegen de serieuze bijwerkingen van de behandeling, met name hepatische encefalopathie, welke zich klinisch manifesteerde of verergerde bij 30% van de patiënten. Dit percentage is vergelijkbaar met ervaringen van andere studies.(30-33) Als laatste werd er een gunstige, maar niet statistisch significante, trend waargenomen wanneer de varices gelijktijdig werden geëmboliseerd. Dit resultaat komt overeen met een recent gepubliceerde meta-analyse welke aantoonde dat gelijktijdige varicesembolisatie geen significante meerwaarde had bij het voorkomen van nieuwe bloedingen of het verbeteren van de overleving.(34)

In **Hoofdstuk 11** zijn de resultaten van een studie weergegeven betreffende karakteristieken en beloop van 127 patiënten met hepatische encefalopathie die naast lactulose behandeld werden met rifaximine. In de zes maanden na start van rifaximine hadden patiënten een significant lager aantal opnames voor hepatische encefalopathie en een kortere opnameduur, vergeleken met de zes maanden voorafgaand aan de start met rifaximine. Deze studie bevestigt de bevindingen van eerdere studies, echter vonden we geen significante daling van intensive care opnames of spoedeisende hulp presentaties.(35, 36) Eerdere studies hebben aangetoond dat rifaximine een effectief antibioticum is als SBP profylaxe.(37, 38) Alhoewel er een niet-significante vermindering was van SBP in onze populatie, had onze studie te weinig power om hier betrouwbare uitspraken over te doen. Ten slotte had rifaximine een goed veiligheidsprofiel met een laag aantal gerapporteerde bijwerkingen. Toekomstige studies bij patiënten met hepatische encefalopathie zijn noodzakelijk om de behandeling te kunnen individualiseren. Het is tot op heden niet duidelijk door welke factoren de behandeling met rifaximine succesvol is, wat de effectiviteit van rifaximine in

hogere doseringen is en bij welke patiënten veilig gestopt kan worden met rifaximine.(39)

Algemene conclusie

Dit proefschrift is gewijd aan verschillende complicaties van gedecompenseerde levercirrose. De kwetsbaarheid van de patiënt met gedecompenseerde cirrose ziet men gereflecteerd in het significante risico om verdere complicaties te ontwikkelen of te overlijden. Niet alleen de ziekte zelf, maar ook invasieve diagnostische en therapeutische interventies kunnen potentieel schadelijk zijn voor de patiënt, bijvoorbeeld darmkankerscreening bij levertransplantatie kandidaten of TIPSS voor de behandeling van ectopische varicesbloedingen. Daarnaast bevindt de patiënt met gedecompenseerde levercirrose zich in een dynamisch landschap: microbiologische patronen veranderen, antibiotica resistentie is wereldwijd een toenemend probleem en nieuwe biotechnologische ontwikkelingen vragen om evaluatie en validatie.

Ons begrip van de complexe problematiek en de vele bedreigingen die patiënten met gedecompenseerde levercirrose treffen wordt geleidelijk groter. Complicaties kunnen zich voordoen in elk orgaan, opeenvolgend of tegelijkertijd, en zijn vaak evident aan elkaar gerelateerd, zoals varicesbloedingen en acute nierinsufficiëntie tijdens een infectie of hepatische encefalopathie en ascites na een gastro-intestinale bloeding. Huidig wetenschappelijk bewijs suggereert dat de behandeling van individuele complicaties, bijvoorbeeld profylaxe van varicesbloedingen, zeer waarschijnlijk geen groot effect heeft op de algehele prognose. Derhalve is een multifactoriële benadering essentieel. Echter, of (vroege) detectie en pogingen om bijvoorbeeld ondervoeding te behandelen uiteindelijk de prognose beïnvloeden is onduidelijk. Verdere studies zijn noodzakelijk om tot een betere risicostratificatie te komen, zoals bij patiënten met bacterascites of ten aanzien van invasieve procedures als screenings colonoscopie bij kandidaat patiënten voor levertransplantatie.

De resultaten van de gepresenteerde studies hebben licht geworpen op enkele aspecten van de complexe behandeling van patiënten met gedecompenseerde levercirrose, en zullen hopelijk bijdragen aan het verbeteren van de klinische uitkomst in deze populatie die bedreigd wordt door multipele fatale en niet-fatale complicaties.

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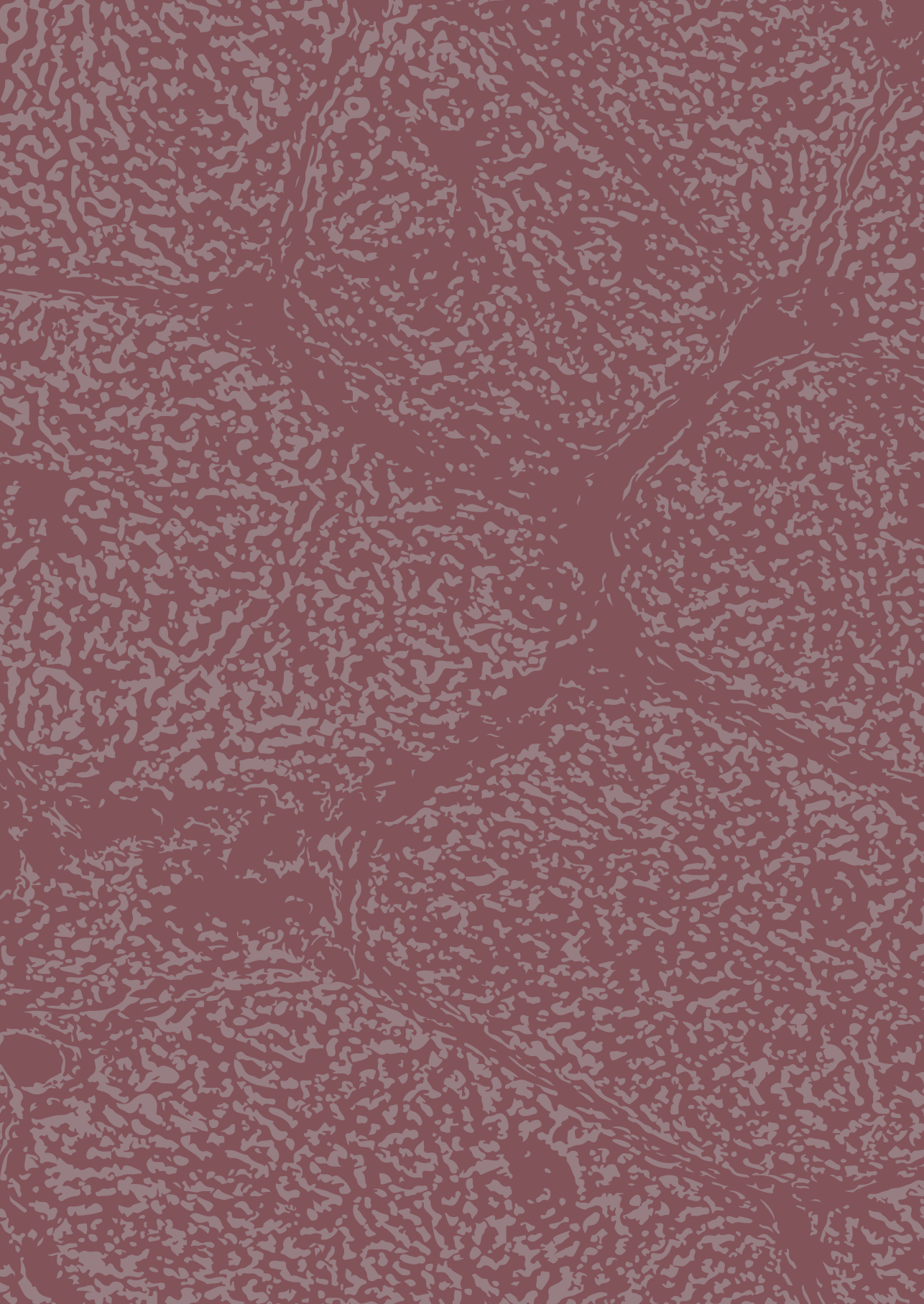

APPENDICES

Abbreviations

Contributing authors

Publication list

Portfolio

About the author

Dankbetuiging (acknowledgements)

ABBREVIATIONS

A&E	accident and emergency
AASLD	American association for the study of the liver
ADA	adenosine deaminase activity
AFP	α-fetoprotein
ALAT	alanine transaminase
ASAT	aspartate transaminase
BIA	bioelectrical impedance analysis
BMI	body mass index
BRTO	balloon-occluded retrograde-transvenous-obliteration
CA-125	cancer antigen 125
CA19-9	cancer antigen 19-9
CDC	centers for disease control
CEA	carcinoembryonic antigen
CI	confidence interval
CP	Child-Pugh
CRC	colorectal cancer
CRP	C-reactive protein
CT	computed tomography
CVVH	continuous veno-venous hemofiltration
DNA	deoxyribonucleic acid
EASL	European association for the study of the liver
EctVB	ectopic variceal bleeding
e-PTFE	expanded polytetrafluoroethylene
ESBL	extended-spectrum beta-lactamase
ESPEN	European society of clinical nutrition and metabolism
FIT	fecal immunochemical test
FOBT	fecal occult blood test
Gamma-GT	gamma-glutamyl transferase
GI	gastrointestinal
Hb	hemoglobin
HCC	hepatocellular carcinoma
HE	hepatic encephalopathy
HIV	human immunodeficiency virus

HR	hazard ratio
IBD	inflammatory bowel disease
ICU	intensive care unit
INR	international normalized ratio
IQR	interquartile range
LT	liver transplantation
MARS	molecular adsorbent recirculating system
MDR	multidrug-antibiotic–resistant
MELD	model for end-stage liver disease
MELDNa	model for end-stage liver disease including sodium
MRSA	methicillin-resistant Staphylococcus aureus
NASH	non-alcoholic steatohepatitis
NSBB	non-selective beta-blocker
OPTN	organ procurement and transplantation network
OR	odds ratio
PBC	primary biliary cholangitis
PCR	polymerase chain reaction
PEG	polyethylene electrolyte glycol
PMN	polymorphonuclear neutrophil
PPI	proton-pump inhibitor
PSC	primary sclerosing cholangitis
PSG	portosystemic gradient
ROC	receiver operating characteristic
SBP	spontaneous bacterial peritonitis
SSA/P	sessile serrated adenoma/polyp
TIPS/TIPSS	transjugular intrahepatic portosystemic shunt placement
SAAG	serum-ascites albumin gradient
SD	standard deviation
TSA	traditional serrated adenoma
US	ultrasound
VEGF	vascular endothelial growth factor
VRE	vancomycin-resistant Enterococci
WBC	white blood cell

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1. R.C. (Rosalie) Oey, P. (Pim) Aarts, N.S. (Nicole) Erler, H.J. (Herold) Metselaar, P.L.M. (Patty) Lakenman, S. (Saskia) Riemslag Baas-van der Ree, M.C. (Monique) van Kemenade, H.R. (Henk) van Buuren, R.A. (Robert) de Man. Identification and prognostic impact of malnutrition in a population screened for liver transplantation. Clinical Nutrition ESPEN. 2020.

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Metselaar. The impact of infections on delisting patients from the liver transplantation waiting list. Transpl Int. 2017;30(8):807-816.

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PORTFOLIO

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PhD Training	**Year**	**Workload (hours)**
Courses		
Introduction to data-analysis (NIHES)	2015	28
Introduction to SPSS (MolMed)	2015	28
Workshop EndNote (Eramus MC)	2015	6
Good clinical practice – 'BROK' (NFU)	2015	42
Survival Analysis Course (MolMed)	2015	17
Weekly research group meeting 'Journal Club'	2015-2018	60
Biomedical Writing Course (MolMed)	2016	56
Indesign CS6 Workshop (MolMed)	2016	6
Writing Grant Proposals Workshop (MolMed)	2016	14
Scientific Integrity course (Erasmus MC)	2017	8
Attendance at conferences and seminars		
Digestive Disease Days (NVGE/NVH)	2015-2019	72
International Liver Congress (EASL)	2016-2019	80
Diner pensant hepatologie	2015-2019	24
Erasmus Liver day	2015-2019	32
Liver meeting (AASLD)	2016	28
Transplantation Congress (NTV-BTS)	2018	8
Digestive Disease Week (AGA)	2018	20
Lagerhuisdebat hepatologie	2018	4
Presentations		
Changes in microbiological flora causing spontaneous bacterial (poster) – International Liver Congress EASL.	2016	12
Bacterascites is associated with poor clinical outcome in decompensated cirrhosis (oral) – Digestive Disease Days.	2016	36

PhD Training	Year	Workload (hours)
Bacterascites is associated with poor clinical outcome in decompensated cirrhosis (poster) – Annual meeting AASLD.	2016	12
Infection with a multi-drug resistant organism is associated with increased mortality in patients listed for liver transplantation (poster) – Annual meeting AASLD.	2016	12
Bacterascites, is it spontaneous bacterial peritonitis? (oral) – Diner pensant hepatologie.	2016	24
Ectopische varicesbloedingen (oral) – Diner pensant hepatologie.	2017	36
New Insights to Hepatic Encephalopathy (oral)– Onderwijsavond MDL Erasmus MC.	2017	36
Get involved in education (oral) – PhD day MDL.	2017	36
Risks and benefits of colonoscopy in pre-liver transplantation screening (oral) – Transplantation Congress NTV-BTS.	2018	36
The efficacy and clinical outcome of transjugular intrahepatic portosystemic shunts in the management of ectopic variceal bleeding: a multicenter retrospective study (poster) – International Liver Congress EASL.	2018	12
Risks and benefits of colonoscopy in pre-liver transplantation screening (oral) - Digestive Disease Week.	2018	36
Yield and safety of colonoscopy in patients evaluated for liver transplantation (oral) - Digestive Disease Days.	2018	24
The efficacy and safety of rifaximin: a 2-year observational study of overt hepatic encephalopathy (poster) – International Liver Congress EASL.	2019	12
The efficacy and safety of rifaximin: a 2-year observational study of overt hepatic encephalopathy (oral) – Digestive Disease Days.	2019	36
Ectopic varices in patients with portal hypertension (oral) – Erasmus Liver Day.	2019	36

PhD Training	Year	Workload (hours)
Teaching		
Tutor first year Bachelor student group	2016-2017	84
Coach Bachelor students	2016-2018	56
Supervising part-time science project David de Jong	2016	56
Supervising masterthesis of Pim Aarts	2017	112
Supervising masterthesis of Laurèlle van Tilburg	2017	112
Supervising part-time science project of Tugce Atalik	2017	56
Supervising masterthesis of Lennart Buck	2018	112

ABOUT THE AUTHOR

Rosalie Christine Oey was born on October 11, 1989. Although she started her academic studies in dentistry at the Academisch Centrum Tandheelkunde Amsterdam in 2007, she found her true calling in medical school a year later at the University of Amsterdam.

She completed her master's degree cum laude in 2013 after finishing a research internship at Addenbrooke's Hospital, Cambridge, United Kingdom, where she focused on the isolation and cultivation of induced pluripotent stem cells from peripheral blood. She further broadened her horizon with an internship Oncology at the Antoni van Leeuwenhoek Hospital in Amsterdam and an internship Hepatology at the Queen Elizabeth Hospital in Birmingham. This sparked her interest in Hepatology, an interest she continued to pursue after passing her doctors exam in 2015.

From the ancient Greek belief that the liver can predict the future to the discovery of effective hepatitis C treatment only 8 years ago, the liver is known for its captivating history. Rosalie shares this old fascination with the mysteries of this regenerative organ. Less a soothsayer and more a researcher, she has dedicated her post-doctoral studies to the clinical complications of patients in the final stages of liver disease under the guidance of prof. dr. R.A. de Man and dr. H.R. van Buuren.

In 2018 Rosalie started her residency with a two-year training in Internal Medicine (program director: dr. H. Boom) in the Reinier de Graaf Gasthuis as part of the specialist training in Gastroenterology and Hepatology (cluster Erasmus MC - program director: prof. dr. C.J. van der Woude; Reinier de Graaf Gasthuis - program directors dr. J.T Brouwer, dr. B.J. Veldt). Here, she aspires to improve and provide excellent health care for all future patients.

Paranymphs Elsbeth Dekker & Koen Janssen

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