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The role of bacterial infections in liver cirrhosis

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2016

Document Version:

Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

SARGENTI, KONSTANTINA. (2016). *The role of bacterial infections in liver cirrhosis*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Lund]. Lund University: Faculty of Medicine.

Total number of authors:

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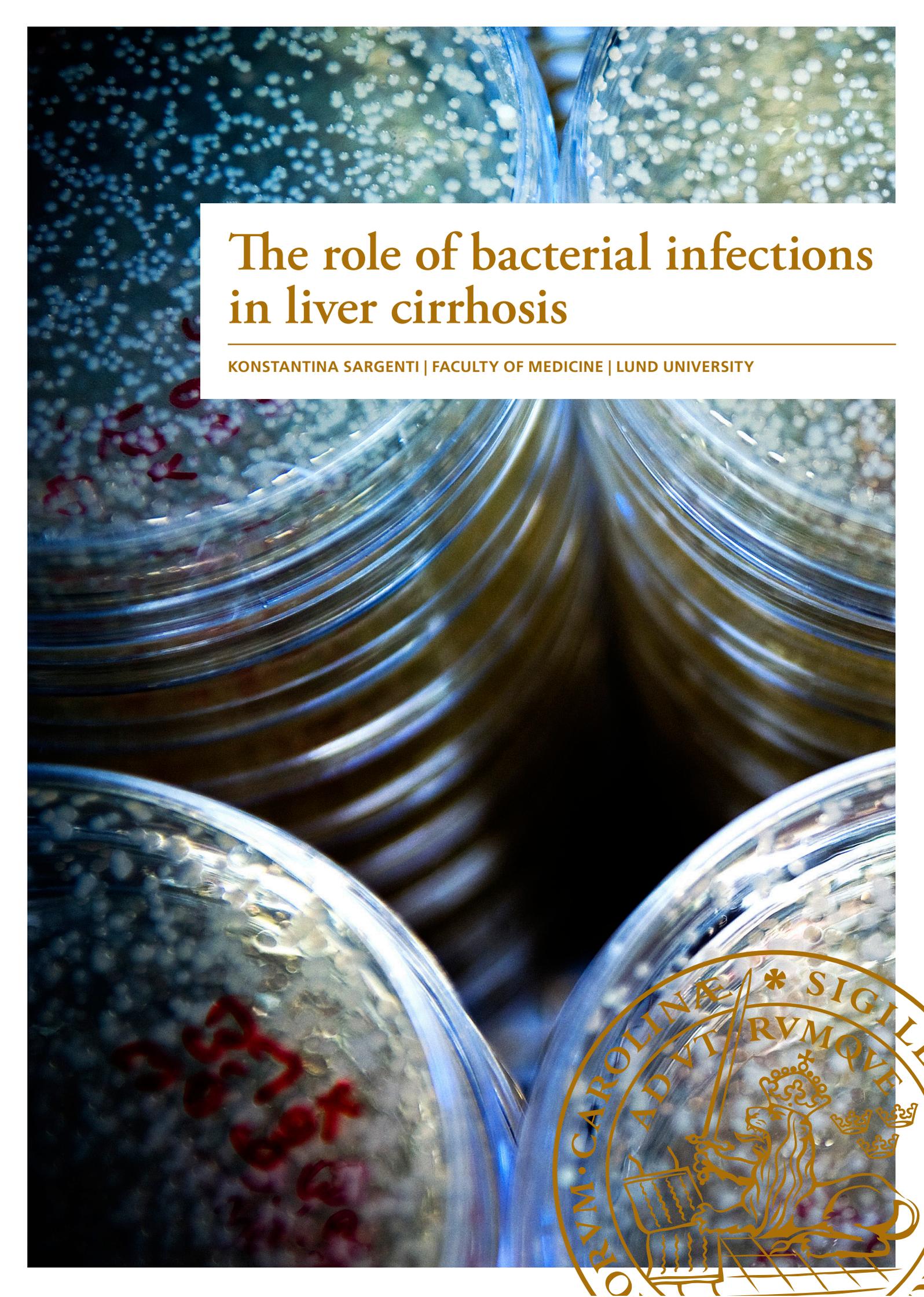
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A close-up photograph of several glass petri dishes containing bacterial cultures. The cultures are visible as white, fuzzy, and sometimes red streaks on the agar surface. The lighting is dramatic, with strong highlights and deep shadows, creating a sense of depth and focus on the microbial growth.

The role of bacterial infections in liver cirrhosis

KONSTANTINA SARGENTI | FACULTY OF MEDICINE | LUND UNIVERSITY



The role of bacterial infections in liver cirrhosis

Konstantina Sargenti, MD



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DOCTORAL DISSERTATION

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To be defended at Aulan, KK, Skåne University Hospital, Malmö. October 13,
2016 at 13.00

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Copenhagen, Danmark

Organization LUND UNIVERSITY Department of Clinical Sciences, Lund	Document name Doctoral dissertation
Author Konstantina Sargenti	Date of issue October 13, 2016
Sponsoring organization	
Title and subtitle: The role of bacterial infections in liver cirrhosis	
<p>Abstract</p> <p>General aim: To obtain longitudinal population-based data on the role of bacterial infections in liver cirrhosis and to investigate the polymorphonuclear (PMN) leukocyte and monocyte function in ambulatory cirrhotic patients</p> <p>Methods: All patients with incident cirrhosis in 2001-2010 residing in an area of 600,000 inhabitants were included in a retrospective study. All serious bacterial infections (resulting in or occurring during an inpatient hospital episode) during this period were registered. Cirrhosis etiology, acquisition type of infection, infection severity features, and bacterial resistance were analyzed. Patients were followed longitudinally until death, transplant, or end of 2011. Consecutive ambulatory cirrhotics without acute decompensation were enrolled in a prospective study in 2013 and followed until death, transplant or end of 2014. Oxidative burst and phagocytosis of circulating PMNs and monocytes were investigated after in vitro <i>E. coli</i> stimulation. Seventeen healthy blood donors served as controls. Baseline clinical and laboratory data as well as follow-up data on the development of cirrhosis complications and bacterial infections were collected.</p> <p>Results: In the retrospective part, 398 serious bacterial infections occurred in 241/633 (38%) patients diagnosed with cirrhosis in the study period (2276 patient-years). Among patients diagnosed with cirrhosis each year from 2001 to 2010 rising trends were noted in infection occurrence ($p < 0.001$). Alcoholic etiology was not an independent predictor of infection occurrence ($p = 0.056$) but it was related to pneumonia and infections caused by gram-positive bacteria in multivariate analysis ($p < 0.05$ for both). Forty-seven percent of all infections were healthcare-associated (HCA) and 21% hospital acquired (HA). Antibiotic-resistant infections were more frequent among HA than HCA or community-acquired infections ($p < 0.05$). Decompensated status, use of antibiotics and proton pump inhibitors (PPIs) at infection diagnosis were independent predictors of HCA/HA infections ($p < 0.05$). HCA/HA infections were significantly related to infection-related acute-on-chronic liver failure (ACLF) ($p < 0.05$). In Cox regression analysis, infection-related ACLF was an independent negative predictor of transplant-free survival in decompensated patients ($p = 0.049$). The prospective study comprised 60 ambulatory patients. Compared to controls, cirrhotics showed increased resting and stimulated oxidative burst as well as reduced phagocytosis of PMNs, and increased stimulated monocyte burst ($p < 0.05$ for all). Increased stimulated monocyte and PMN burst were independent predictors of sepsis, severe sepsis and ACLF occurrence. Also, increased stimulated monocyte burst was associated with worse transplant-free survival ($p < 0.05$ for all).</p> <p>Conclusions: In a population-based cirrhotic cohort, bacterial infections increased over time. Alcoholic etiology was not independently related to the occurrence of bacterial infections. Infection-related ACLF was a negative predictor of survival in decompensated disease. Decompensated liver disease, antibiotics, and use of PPIs were predictors of serious HCA/HA infections, which were associated with the development of ACLF. Stimulated PMN and monocyte oxidative burst are increased in ambulatory cirrhotics without acute decompensation. In turn, these changes are associated to sepsis and ACLF occurrence.</p>	
Key words: Liver cirrhosis; bacterial infection; healthcare-associated infection; hospital acquired infection; proton pump inhibitors; outcome; alcoholic liver disease; bacterial resistance; compensated cirrhosis; decompensated cirrhosis; acute-on-chronic liver failure; polymorphonuclear leukocytes; monocytes; oxidative burst; phagocytosis	
Classification system and/or index terms (if any)	
Supplementary bibliographical information	Language English
ISSN and key title 1652-8220	ISBN 978-91-7619-302-0
Recipient's notes	Number of pages 113
	Price
	Security classification

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The role of bacterial infections in liver cirrhosis

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ISBN 978-91-7619-302-0

ISSN 1652-8220

Lund University, Faculty of Medicine Doctoral Dissertation Series 2016:75

Printed in Sweden by Media-Tryck, Lund University
Lund 2016



"The only good is knowledge and the only evil is ignorance"
Socrates (469 BC-399 BC)

To Andreas who left us too soon

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Abbreviations

ICU	Intensive Care Unit
HCA	Healthcare-Associated
HA	Hospital-Acquired
CA	Community-Acquired
IBO	Intestinal Bacterial Overgrowth
SBP	Spontaneous Bacterial Peritonitis
BT	Bacterial Translocation
LPS	Lipopolysaccharides
MLN	Mesenteric Lymph Nodes
SIRS	Systemic Inflammatory Response Syndrome
TNF α	Tumor Necrosis Factor alpha
ALD	Alcoholic Liver Disease
PPI	Proton Pump Inhibitors
ACLF	Acute on Chronic Liver Failure
CLIF	Chronic Liver Failure
SOFA	Sequential Organ Failure Assessment score
AKI	Acute Kidney Injury
PMN	Polymorphonuclear leukocytes
ROS	Reactive oxygen species
PEth	Phosphatidyl-Ethanol
AUDIT	Alcohol Use Disorders Identification Test
MRSA	Methicillin-Resistant Staphylococcus Aureus
VRE	Vancomycin-Resistant Enterococcus

ESBL	Extended-Spectrum β -lactamase- producing Enterobacteriaceae
QRGMR	Quinolone-Resistant Gram-Negative Rods
DDD	Defined Daily Dose
PMA	Phorbol 12-Myristate 13-Acetate
MFI	Median Fluorescence Intensity
ROC	Receiver Operating Characteristics
HR	Hazard Ratio
CI	Confidence Interval
HCC	Hepatocellular Carcinoma
OR	Odds Ratio
AUROC	Area Under the ROC curve

List of papers

This thesis is based on four studies presented in four papers, which are referred to in the text by their corresponding Roman numerals

- I. Sargenti K, Prytz H, Strand A, Nilsson E, Kalaitzakis E. Healthcare-associated and nosocomial bacterial infections in cirrhosis: predictors and impact on outcome. *Liver Int* 2015 Feb;35(2):391-400
- II. Sargenti K, Prytz H, Nilsson E, Kalaitzakis E. Predictors of mortality among patients with compensated and decompensated liver cirrhosis: the role of bacterial infections and infection-related acute-on-chronic liver failure. *Scand J Gastroenterol* 2015 Jul;50(7):875-83
- III. Sargenti K, Prytz H, Nilsson E, Bertilsson S, Kalaitzakis E. Bacterial infections in alcoholic and nonalcoholic liver cirrhosis. *Eur J Gastroenterol Hepatol* 2015 Sep;27(9):1080-6
- IV. Sargenti K, Johansson Å, Bertilsson S, Mattsby-Baltzer I, Klintman D, Kalaitzakis E. Dysfunction of circulating polymorphonuclear leukocytes and monocytes in ambulatory cirrhotics predicts patient outcome. *Dig Dis Sci* 2016 Aug;61(8):2294-302

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Introduction

The occurrence of bacterial infections in liver cirrhosis

Bacterial infections: a frequent complication of liver cirrhosis

Infection induces a systemic host response with three stages of severity called sepsis, severe sepsis (when an acute organ failure occurs), and septic shock (when hypotension does not respond to adequate fluid resuscitation) [1]. Patients with liver cirrhosis have increased risk to develop bacterial infection, sepsis, sepsis-induced organ failure and death [1]. Bacterial infection is present at admission or develops during hospitalization in about 30% of patients with cirrhosis [2-5], an incidence that is 4-5 fold higher than that observed in the general population. Critically ill cirrhotic patients, in particular, are especially at high risk of developing bacterial infection. Infection is more common in cirrhotic than in non-cirrhotic intensive care unit (ICU) patients with higher in-hospital mortality rates [6]. Importantly, according to recent data from US, the prevalence of infections among hospitalized cirrhotics appears to be increasing [7]. However, there are few longitudinal, population-based data on the occurrence of bacterial infections in cirrhotic patients with different cirrhosis etiologies.

Healthcare-associated and hospital-acquired bacterial infections

Occurrence and predictors

Healthcare-associated (HCA) and hospital-acquired (HA) infections, in particular, are of concern in cirrhosis [5, 8-10]. Previous reports have shown that approximately 30% of bacterial infections occurring in cirrhotic patients are community-acquired (CA), 30% are HCA, and 35% to 40% are HA [5, 11]. Although the incidence of HCA/HA infections is thought to be increasing [3, 8, 11, 12], there are no data on their occurrence in longitudinal population-based cirrhotic cohorts. In a previous prospective study, HA infections were associated with invasive procedures during hospitalization and with hospitalization in rooms in which there was a need to place additional beds [5]. However, there are few data on other potential predictors of HCA and HA infections in cirrhosis.

Bacterial resistance

Recently published data suggest that there has been an increase in the number of Gram-positive infections [2, 3, 8], as opposed to the predominantly Gram-negative bacteria causing infections in patients with cirrhosis in older reports [13]. This has been attributed mainly to invasive procedures increasingly performed in these patients in the last two decades [3, 5] as well as to the fact that several cirrhotics often receive long-term quinolone prophylaxis [3, 14]. As mentioned above, HA and HCA infections occurring in cirrhotic patients are increasingly reported in recent years [5, 15] and bacterial resistance as well as Gram-positive pathogens are more frequent in these patients [5, 8, 14-16]. However, published reports, showing that up to 32% of infections are due to multidrug resistant pathogens and up to 37% of E.coli infections due to quinolone-resistant bacteria, originate mainly from the South of Europe [3, 5, 8, 14-16] which has disproportionately high rates of antibiotic resistance compared to Scandinavia. There are only few data on the relation of antibiotic-resistant infections with infection acquisition type in cirrhotic cohorts from areas of low prevalence of bacterial resistance like Sweden. Furthermore, it seems reasonable that, following cirrhosis diagnosis, bacterial resistance may develop as patients are increasingly exposed to invasive procedures and to various antibiotic treatments. However, no study has investigated the development of bacterial resistance in HCA/HA infections following diagnosis of cirrhosis in a longitudinal fashion and in population-based cohorts.

Pathogenesis and risk factors of bacterial infections in liver cirrhosis

Increased intestinal overgrowth and bacterial translocation

Intestinal bacterial overgrowth (IBO) is a common feature in patients with liver cirrhosis and occurs predominantly in the small intestine. IBO is multifactorial, and contributing factors include modulation of gastric acid secretion, lack of bile constituents and antimicrobial peptides as well as portal hypertension [17]. Also, features of gut dysfunction in patients with cirrhosis such as small bowel manometry disturbances and delayed gut transit may be associated with the development of IBO [18, 19]. Patients with cirrhosis and IBO develop spontaneous bacterial peritonitis (SBP) more frequently than patients without bacterial overgrowth [17]. Experimental IBO itself can result in microbial translocation and liver inflammation [20].

Bacterial translocation (BT) refers to the migration of bacteria or bacterial products (lipopolysaccharides [LPS], endotoxins) from the lumen of the intestine

to extraintestinal sites such as the mesenteric lymph nodes (MLN). Apart from IBO and intestinal motility disturbances, impairment of the intestinal barrier function is also responsible for BT [21]. Several mechanisms have been proposed to explain intestinal barrier dysfunction in cirrhosis including disruption of tight junction by alcohol and its metabolites [22], structural gut alterations (vascular congestion, edema, widened intracellular spaces) by portal hypertension *per se* [23], as well as altered expression of enterocyte tight junction proteins which is more evident in decompensated disease [24]. In a prospective study on cirrhotic patients, BT of enteric organisms to MLN occurred more frequently in patients with advanced liver disease, being approximately five times more prevalent in Child Pugh class C patients than in the remaining cirrhotic patients [25]. Bacterial derived toxins bind to Toll-like receptors and initiate a cascade of cell signaling, leading to release of NO and pro-inflammatory cytokines. In systemic inflammatory response syndrome (SIRS), anti-inflammatory cytokines are unable to balance this "cytokine storm" resulting in excessive inflammation. Consequently, this increased permeation of bacteria and bacterial products such as endotoxin from the gut in advanced cirrhosis leads to worsening of the hyperdynamic circulation and plays an important role in the pathogenesis of infections (particularly from gram-negative pathogens) [4]. BT of enteric organisms to MLN has been involved in the pathogenesis of bacterial infections in experimental cirrhosis. The prevalence of BT to MLN has been found to be 40% in cirrhotic rats with ascites and 80% in cirrhotic rats with SBP [25]. The release of large amounts of cytokines in cirrhotic patients with sepsis [26] intensifies the hyperdynamic state. Altered hemodynamics and widespread inflammation lead to impaired tissue oxygenation, cell necrosis, apoptosis and organ failure [27].

Immune dysfunction

Liver cirrhosis presently may be considered an immunodeficiency-acquired condition. Cirrhosis associated immune dysfunction is a complication of cirrhosis of any etiology and includes two main syndromic alterations: (i) immunodeficiency, due to an impaired response to pathogens at different levels of the immune system, and (ii) systemic inflammation, as a consequence of persistent and inadequate stimulation of cells of the immune system.

Immunodeficiency is the result of abnormalities provoked by cirrhosis that affect cellular and soluble components of the immune response both in the liver and systemically. Cirrhosis results in reticuloendothelial dysfunction, due to reduced number of liver reticuloendothelial mononuclear cells in the liver and portosystemic shunting, as well as decreased hepatic synthesis of molecules of the innate immune response [28]. Depressed reticuloendothelial system phagocytic activity has been shown to be associated with the occurrence of bacterial

infections in liver cirrhosis [29]. Besides, cirrhosis affects the functions of circulating and intestinal populations of immune cells. The functional impairment of neutrophils and monocytes in liver cirrhosis is described below. Liver cirrhosis is characterized by memory B cell dysfunction, T cell depletion, and poor response of NK cells to cytokine stimulation [30]. In addition, cirrhotic patients have low levels of immunoglobulins IgM, IgG, and IgA in ascitic fluid, as well as low C3, C4 concentrations in both serum and ascitic fluid, thus leading to diminished bactericidal activity [31, 32]. Systemic inflammation accompanies immunodeficiency and is reflected by an increased production of pro-inflammatory cytokines and the upregulated expression of cell activation markers. Systemic inflammation is attributed to persistent immune cell stimulation [33-35]. Specifically, monocytes are major contributors to increased tumor necrosis factor alpha (TNF α) levels in cirrhosis [34]. The intensity of the systemic inflammation correlates with the severity of liver disease [36, 37]. Finally, it has been demonstrated that the grade of cirrhosis-associated immune dysfunction is related to the risk of severe bacterial infection development [38, 39].

Alcoholism

As mentioned above, bacterial infections develop partly as a consequence of immune dysfunction that is more evident in advanced liver disease [27]. Liver cirrhosis is frequently associated with alcoholism, which has also been shown to be associated with defects in innate and adaptive immunity [40]. Alcohol misuse in patients with liver disease is associated with increased intestinal permeability and endotoxemia [41]. An increased frequency of bacterial infections among cirrhotics with alcoholic liver disease (ALD) [42], in particular bacteremia [43, 44] and meningitis [45], has been reported. However, the potential role of alcoholic cirrhosis as a risk factor for the development of bacterial infections has not been confirmed in other studies [2, 5, 46-48]. Although alcohol misuse in non-cirrhotic individuals has been reported to be associated with infections caused by bacteria resistant to common antibiotics [49-51], it is unclear whether this holds true for patients with ALD-cirrhosis.

Use of proton pump inhibitors

Proton pump inhibitors (PPIs) are commonly used in patients with cirrhosis for gastric acid suppression. In previous reports, the use of acid suppressive therapy in hospitalized cirrhotic patients was found to be inadequate in 42–81% of cases [52, 53], based on current guidelines and recommendations. PPIs undergo extensive hepatic metabolism. In patients with liver impairment, the half-life of PPIs

becomes 4 to 8 hours longer with increasing risk of accumulation and toxicity [54].

The effect of PPIs has been studied in case series in cirrhotic patients showing that their use was associated with a significantly higher risk of gut-based infections such as SBP and *Clostridium difficile* [53, 55, 56]. The mechanism cited is related to IBO and a direct immunosuppressive effect making patients prone to bacterial translocation. In a large study from the US, veterans with decompensated cirrhosis who were started on PPI therapy after decompensation had a significantly higher risk of developing serious infections compared with those who were not initiated on gastric acid suppression [57]. More recent data have also shown that the use of PPIs may affect the rate of bacterial infections in cirrhosis [58].

Previous studies have shown an association between HCA and HA bacterial infections and antacid administration in non-cirrhotic individuals [59, 60]. However, a potential relationship between infection acquisition type and PPI use is unexplored in liver cirrhosis.

Genetic and other risk factors

Previous studies indicate that common gene variants linked to impaired mucosal barrier function and bacterial translocation, represent genetic risk factors for bacterial infections in patients with liver cirrhosis. Specifically carriers of Nod-like receptors 2 and Toll-like receptors 2 risk variants have been shown to have a higher risk for SBP [61]. Additional aspects of immunodeficiency are complicated by malnutrition, immunosuppressive medications, autoimmunity, and virus infection [27, 62]. Finally, diabetes has been reported to be an independent predictor of bacterial infections in cirrhotic patients [63, 64], including those undergoing liver transplantation [65].

Prognostic significance of bacterial infections in liver cirrhosis

The magnitude of the problem of bacterial infections during the course of cirrhosis

The consequences of bacterial infection include prolonged hospitalization, organ failures, de-listing from liver transplant, susceptibility to further infections and death. Bacterial infections increase mortality four-fold in cirrhosis and they are a very common cause of admissions and increased healthcare costs in these patients

[66]. The hospital mortality rate of cirrhotic patients requiring ICU admission is estimated to be greater than 50% [67] and has remained unchanged over 50 years [68]. Up to 80% of deaths in cirrhotics hospitalized in the ICU have been shown to be related to infection and the hospital mortality of these patients is estimated to be between 30% and 70% when septic shock occurs [6].

The natural history of liver cirrhosis is characterized by a relatively asymptomatic phase, termed "compensated" cirrhosis followed by a rapidly progressive phase with complications of portal hypertension and/or liver dysfunction, termed "decompensated" cirrhosis. Compensated and decompensated cirrhosis have markedly different survival as well as different predictors of mortality and, therefore, prognostically, they are considered to be separate entities [69, 70]. Under constant challenge of molecular patterns released from a leaky gut or necrotic liver cells, the immune response pattern in cirrhosis switches from a predominantly "pro-inflammatory" phenotype in patients with stable cirrhosis to a predominantly "immunodeficient" one in patients with severely decompensated cirrhosis and organ failures. A recent systematic review proposes that sepsis has prognostic importance mostly in decompensated patients thus representing a separate cirrhosis stage, following decompensation [71]. However, longitudinal population-based data on the impact of bacterial infection development and its severity on survival in both compensated and decompensated cirrhosis are scarce.

Acute-on-chronic liver failure

Organ failures associated with bacterial infections include renal failure, liver failure, cerebral failure (hepatic encephalopathy), as well as respiratory failure which may develop in cirrhotic patients in the absence of pneumonia or septic shock. Relative adrenal insufficiency is the most recently identified organ failure in infected cirrhotic patients. It develops in 60% of patients with severe infections and further impairs circulatory function [61]. Recently published data have shown that acute-on-chronic liver failure (ACLF) is a specific syndrome characterized by acute decompensation, organ failure and high short-term mortality [72-74]. Bacterial infections are well-recognized precipitating factors of this syndrome [72-76].

Proposed mechanisms for organ damage in infection-related ACLF

Mechanisms for organ damage in infection-related ACLF are poorly understood. There is evidence of an excessive immune response of the host with increased production of pro-inflammatory molecules, resulting in tissue damage (a process called immunopathology) and to the development of organ failures in cirrhotic patients. In addition, a role for direct tissue damage caused by bacterial toxins and virulence factors cannot be excluded. Tissue damage may also be related to failed

tolerance; i.e. failure of endurance mechanisms that normally protect tissues against direct tissue damage by bacteria and immunopathology [77, 78].

Existing ACLF definitions and recent prospectively collected data on the syndrome

As a means of identifying cirrhotic patients at high risk for multiple organ failures and high short-term mortality both the Asia-Pacific Association for the study of Liver [79] and 2 recent prospective studies using large cohorts of patients in Europe (CANONIC study) [72] and in North America (NACSELD study) [80] attempted to define ACLF.

The CANONIC study included patients hospitalized with acute decompensation of cirrhosis and used the Chronic Liver Failure (CLIF)-Sequential Organ Failure Assessment (SOFA) score to recognize organ failures. It provided a robust definition of ACLF into three ACLF grades, with increasing risk of short-term death from grade 1 (22%) to grade 3 (77%). The prevalence of ACLF in patients hospitalized with acute decompensation was 30% [72]. The syndrome was particularly prevalent in alcoholic and hepatitis B associated cirrhosis [72]. The development of ACLF occurred in the setting of systemic inflammation, the severity of which correlated with the number of organ failures and mortality [72]. Bacterial infection was the most common precipitating event of ACLF (33%). Among patients with bacterial infection, ACLF was more common in patients with SBP or pneumonia than in those with infections at other sites. Acute kidney injury (AKI) was the most common organ failure and even mild renal dysfunction was important for defining ACLF [72].

The NACSELD study focused specifically on ACLF precipitated by infections and attempted to develop a simple definition of infection-related ACLF composed of user-friendly definitions of extra-hepatic organ failure to predict survival. Infection-related ACLF was shown to be independently related to HA infections and severity of liver disease as well as infections other than SBP [80]. The same study demonstrated that the presence of ≥ 2 extrahepatic organ failures was associated with increased mortality in infected cirrhotic patients; the increase in mortality with only a single organ failure was low [80].

Both these studies included exclusively hospitalized patients with cirrhosis. Population-based data, however, on the occurrence, predictors, and outcome of infection-related ACLF are lacking.

Infection acquisition type as prognostic indicator

Previous studies have reported conflicting results regarding the prognosis of HCA and HA infections in cirrhosis. Some showed that infection acquisition type (CA vs. HCA/HA) is related to infection-related morbidity and mortality in cirrhosis [5,

8-11, 81], while others reported similar mortality rates in HCA, HA and CA infections [12, 82, 83]. Recently, second infections, which would be HA in origin, have been reported to be major predictors of mortality independent of liver disease severity [83]. Also, it remains unclear whether the occurrence of HCA and HA infections is associated with the occurrence of AKI or ACLF.

Polymorphonuclear leukocyte and monocyte function in ambulatory cirrhotic patients and their prognostic significance

Polymorphonuclear leukocyte function

Polymorphonuclear leukocytes (PMNs) are recruited to inflammatory sites, and upon activation by pro-inflammatory mediators, they produce reactive oxygen species (ROS) to eradicate pathogens. Whilst derangements in migration and phagocytosis of PMNs have been demonstrated in decompensated patients admitted due to cirrhosis complications [84-86], particularly in patients with alcoholic hepatitis [85], existing data on defects in innate immunity in ambulatory cirrhotics without acute decompensation are not unanimous. Some studies report high resting oxidative burst and/or reduced phagocytic capacity of PMNs [86-89] whereas others show normal PMN function [90]. Impaired phagocytosis and high resting burst of PMNs have been demonstrated to be associated with increased risk of infection and mortality in hospitalized cirrhotics with alcoholic hepatitis [85], as well as in a cohort comprising of both patients with stable cirrhosis and ACLF [86]. However, the potential relationship between patient outcome and deranged PMN function is poorly investigated in ambulatory cirrhotic patients without acute decompensation.

Monocyte function

Monocytes are increased in cirrhosis and show impaired function of the circulating monocyte Fc- γ receptor, which is needed for clearance of IgG coated bacteria [91]. Monocytes have been shown to be spontaneously activated in hospitalized Child-Pugh C cirrhotics with ascites, producing TNF α [92]. In patients with ACLF, monocytes have been reported to be functionally deactivated which may be associated with adverse outcome [93]. However, data on potential abnormalities in monocyte function in ambulatory cirrhotic patients without acute decompensation

are very limited and derive mainly from old reports [94], while their prognostic significance in this group remains unknown.

Aims of the present studies

The limited amount of data on the role of bacterial infections in liver cirrhosis deriving from population-based cirrhotic cohorts raised the following questions:

- Which is the occurrence of HCA/HA infections and their predictors in a population-based cohort of cirrhotic patients (I)?
- Are HCA/HA infections related to use of PPIs (I)?
- Is there a potential association between infection acquisition type and patient outcome (I)?
- Is there a relation between infection acquisition type and bacterial resistance patterns in an area of low background prevalence of bacterial resistance to antibiotics such as Sweden (I)?
- Which are the independent predictors of survival in compensated and decompensated cirrhosis in a longitudinal population-based incident cohort of cirrhotic patients (II)? Is bacterial infection and its severity among these predictors (II)?
- How frequent is infection-related ACLF in cirrhosis (II)?
- Which are the predictors of infection-related ACLF and its fatal outcome in cirrhosis (II)?
- Are there any time-trends in the incidence of bacterial infections in ALD and non-ALD cirrhosis (III)?
- Is alcoholic etiology of cirrhosis related to the occurrence, localization, bacterial resistance patterns and outcome of bacterial infections (III)?

Furthermore, specific aims of paper IV were to address following questions:

- Is there an impairment of PMN and monocyte function in ambulatory cirrhotic patients without acute decompensation compared to healthy controls?
- Is there a relation between PMN/monocyte function and cirrhosis etiology or pro-inflammatory cytokine levels?
- Which is the potential relation of PMN and monocyte dysfunction with the occurrence of bacterial infections and mortality in ambulatory cirrhotic patients?

Subjects and methods

The studies were performed according to the Declaration of Helsinki and were approved by the regional Ethics Committee. The methods used are discussed in this chapter. For further details, see separate papers (I-IV).

Subjects

Papers I-III

All adult patients (≥ 18 yr) diagnosed with cirrhosis in the period 2001-2010 at Skåne University Hospital in Sweden were identified by means of a search of the computerized discharge diagnosis register of the hospital. Patients with chronic liver disease are in effect exclusively diagnosed and followed-up at their local public healthcare institutions in Sweden. Private or public community-based hepatology practice is extremely rare in the country and this is also the case for outpatient/day hospital activities regarding these patients. By means of the initial computerized search in the discharge diagnosis register, all patients with at least one outpatient or inpatient episode with the diagnosis of cirrhosis were identified (using ICD-10 codes K70.3, K74.3, K74.6, B18.1G, B18.2G, K76.0, K83.0, K73.2, K75.4, I85.0, I85.9, C22.0 or C22.9), but the final cohort included in the study comprised only patients with first-time cirrhosis diagnosis during the study period. Although our institution accepts referrals of patients with cirrhosis, no inpatients are transferred to other hospitals. Patients with a previous liver transplant and those residing outside the primary catchment area of the hospital were excluded. Thus, all patients with incident cirrhosis in the primary catchment area of our institution (population of 600,000) were included. Medical records were systematically reviewed and the diagnosis of cirrhosis was re-evaluated and confirmed. In Sweden, all health care contacts of patients are registered in the electronic patient record system and notes of clinicians and, in the event of emergency or inpatient care, of nurses regarding the care patients receive are also stored electronically. Thus medical records, including clinician and nurse notes for both outpatient and inpatient episodes, laboratory tests, imaging and endoscopy

exams, and histopathology results, are computerized. It is obligatory for clinicians and nurses to register any patient contact, outpatient or inpatient (including diagnosis codes but also mandatory structured test), in medical records. The discharge diagnosis register was used merely for identification of patients who had received the diagnosis of liver cirrhosis, but, subsequently medical records scrutinization included all the components of medical records for both inpatient and outpatient patient episodes. Patients were followed longitudinally until death, liver transplantation, or the end of 2011. At the time of cirrhosis diagnosis and at diagnosis of each infection, data were extracted with regard to patient demographics and etiology, severity and complications of liver disease.

Paper IV

Consecutive, ambulatory patients with cirrhosis recruited from the liver outpatient clinic at Skåne University Hospital in Sweden between April and October 2013 were included in a prospective longitudinal cohort study. Only patients without acute decompensation in the last 30 days were included. Thus, exclusion criteria were age <18 yr or >75 yr, severe complications of cirrhosis such as gastrointestinal bleeding, hepatorenal syndrome or hepatic encephalopathy (Westhaven grade >1) in ≤ 30 days, bacterial infection or surgery in ≤ 30 days, severe trauma or blood transfusion in ≤ 10 days. Patients treated with systemic antibiotics (including those on prophylactic antibiotic therapy), steroids or any other immunosuppressive medications or those with hepatic or extrahepatic malignancy, or severe systemic diseases (such as severe chronic heart failure) were also excluded. Ongoing infection was excluded upon enrollment by means of clinical examination, laboratory investigations and, if necessary, by imaging tests (such as chest x-ray). All patients were examined with abdominal ultrasound and if ascites was detected, diagnostic paracentesis was performed to rule out spontaneous bacterial peritonitis. On the day of inclusion in the study, data on demographics, comorbid illness, etiology and history of complications of cirrhosis were collected. Patients were followed prospectively until transplant, death or the end of 2014.

Definitions

Cirrhosis diagnosis (I-IV)

The diagnosis of cirrhosis was established histologically or based on a combination of at least 2 of the following: clinical, biochemical, and imaging data.

Patient classification

Papers I- III

Patients were classified into a compensated (absence of variceal bleeding and ascites) and a decompensated group (variceal bleeding and/or ascites) at cirrhosis diagnosis, as agreed upon in the Baveno IV consensus conference [95].

Patients were classified into two groups according to cirrhosis etiology: ALD and non-ALD cirrhosis. All patients who had a long history of heavy alcohol consumption (≥ 80 g ethanol per day for male patients and ≥ 60 g for female patients) for a duration of ≥ 10 years were considered having ALD cirrhosis. Patients were considered having ongoing alcohol misuse if they (or their relatives) had reported alcohol ingestion following cirrhosis diagnosis and/or if alcohol consumption was suspected/registered in the medical records (eg admissions to the accident and emergency department for alcohol-related reasons, positive relevant blood test results, such as phosphatidyl-ethanol (PEth), etc).

Paper IV

If there were a history of ascites, encephalopathy, variceal bleeding or jaundice, patients were considered to have decompensated disease.

Drinking habits were assessed by means of the Alcohol Use Disorders Identification Test (AUDIT) [96]. AUDIT is a well validated 10-item screening tool to assess alcohol consumption, drinking behaviors, and alcohol related problems. An AUDIT score ≥ 8 was considered to be in keeping with ALD cirrhosis [96, 97]. All patients with ALD-cirrhosis in our cohort had had a long history of heavy alcohol consumption.

Alcohol consumption among patients with ALD cirrhosis was quantified by means of measurement of PEth in whole blood, a direct ethanol metabolite which has been established as a sensitive and specific marker of prolonged alcohol overconsumption and a measure of prolonged (last 2 weeks) alcohol intake [98, 99].

Alcohol consumption in the month prior to enrollment was also assessed using a timeline follow-back method [100, 101], which is a well-established

method to gather detailed drinking data in alcohol dependent individuals [102]. In short, participants completed a retrospective log based on a monthly calendar to indicate daily alcohol drinking to provide the total number of alcoholic drinks consumed in the previous 30 days prior to enrollment. This quantity was then divided by the total number of drinking days (within the previous 30 days) to calculate drinks per drinking day. Patients with ALD cirrhosis were further classified into current drinkers if they reported alcohol consumption at timeline follow-back and/or confirmed by a raised blood PEth. In cases that quantifiable blood PEth in self-reported abstainers was found, patients were classified as current drinkers.

Infection diagnosis (I-IV)

Mixed bacterial infections (bacterial infections at different sites diagnosed simultaneously) were considered as a single infection episode. Further infections diagnosed following a first infection during the same hospitalization were considered separate, as they may have a different clinical course [83].

We defined infections as follows:

SBP based on ascitic fluid polymorphonuclear cells $>250/\text{mm}^3$ [11] and spontaneous bacteremia based on positive blood cultures in the absence of any other possible cause of bacteremia. If a bacteremia was detected in a patient with urinary tract infection, pneumonia, spontaneous bacterial peritonitis, or other bacterial infection, it was interpreted as secondary to these infections and defined by the primary infection [11].

Pneumonia, urinary tract infection, skin and soft-tissue infection and other infections were defined according to conventional criteria [103].

Secondary peritonitis was defined as previously described [104].

Definition of infection acquisition type (I-III)

Infections were defined as HCA if they were diagnosed within 48 hours of admission in i. patients hospitalized for at least 2 days, ii. those who had an inpatient hospital episode or surgery in the previous 6 months, or iii. those who resided in a nursing home or a long-term care facility, or were on chronic hemodialysis. Infections were classified as HA if they were diagnosed ≥ 48 hours of hospital admission, and CA if the infection diagnosis was made <48 hours of hospital admission and the HCA infection criteria were not fulfilled [59].

Clinical outcomes (I-IV)

In papers I-III, the electronic medical records of patients identified in our institution as well as any record these patients had in any public hospital in the whole of the health-care region (population 1.3 million) were scrutinized (the same electronic system of medical records is used by all hospitals in the health-care region). There are no private hospitals in the region. All serious bacterial infections (i.e. those resulting in or occurring during an inpatient hospital episode) were identified by means of a detailed review of all the components of medical records (and not by means of a search of the computerized discharge diagnosis register in order to avoid misclassification) and registered. The primary endpoint was death or liver transplantation. Outcomes recorded for each serious infection episode were sepsis, severe sepsis, AKI, ACLF, and in-hospital mortality. Infection-related mortality was defined as death that could be attributed, at least partly, to a serious infection. Data on date and cause of death were obtained from medical records and were confirmed through linkage to the Cause of Death Register by means of the unique national registration number assigned to all Swedish residents.

In paper IV, the primary endpoint was death or liver transplantation and first-time serious bacterial infection (i.e. infection resulting in or occurring during an inpatient hospital episode), first-time sepsis, severe sepsis, and ACLF precipitated by any factor during follow-up were also recorded.

Definition of outcome variables

Sepsis and severe sepsis (I-IV)

Sepsis was defined as ≥ 2 SIRS criteria associated with a confirmed bacterial infection, and severe sepsis as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion [105]. Diagnostic criteria for sepsis-induced organ dysfunction are shown in table 1.

Table 1. Diagnostic criteria for sepsis-induced organ dysfunction

Sepsis induced hypotension
Lactate >upper limits laboratory normal
Urine output <0.5 ml/kg/hr for more than 2hrs despite adequate fluid resuscitation
PaO ₂ /FiO ₂ <250 in the absence of pneumonia as infection source
PaO ₂ /FiO ₂ <200 in the presence of pneumonia as infection source
Creatinine >176µmol/L
Bilirubin >34µmol/L
Platelet count <100.000µL
International normalized ratio >1.5

PaO₂, partial pressure of arterial oxygen; FiO₂, fraction of inspired oxygen

AKI (I-III)

AKI was diagnosed as a 50% increase in serum creatinine from a stable baseline. AKI was classified according to the Acute Kidney Injury Network criteria as follows: i. AKI stage 1: increase of ≥ 150 -200% (1.5-2-fold) from baseline, ii. AKI stage 2: increase in serum creatinine to 200-299% (>2-3-fold) from baseline, and iii. AKI stage 3: increase in serum creatinine to ≥ 300 % (>3-fold) from baseline or serum creatinine of ≥ 354 $\mu\text{mol/l}$ with an acute increase of ≥ 44 $\mu\text{mol/l}$ or initiation of renal replacement therapy [106].

AKI was diagnosed and staged using admission serum creatinine or serum creatinine 6 months prior to infection episode as baseline.

ACLF (I-IV)

ACLF diagnosis was based on CLIF-SOFA scale (table 2) and the criteria provided by the CANONIC study (table 3) [72]. The classification of ACLF into grades is shown in table 3 [72].

Table 2. Definition of organ failures according to Chronic Liver Failure (CLIF)-Sequential Assessment of Organ Failure (SOFA) scale.

Organ/system	Definition of failure
Liver	Bilirubin ≥ 206 $\mu\text{mol/L}$
Kidney	Creatinine ≥ 176 $\mu\text{mol/L}$ or renal replacement
Cerebral	HE grade 3 or 4
Coagulation	INR > 2.5 or platelets $\leq 20 \times 10^3/\mu\text{L}$
Circulation	Use of vasopressors
Lungs	$\text{PaO}_2/\text{FiO}_2 \leq 200$ or $\text{SpO}_2/\text{FiO}_2 \leq 214$

HE, hepatic encephalopathy; INR, international normalized ratio; PaO_2 , partial pressure of arterial oxygen; FiO_2 , fraction of inspired oxygen; SpO_2 , pulse oximetric saturation

Table 3. Definition of absence and grades of acute-on-chronic liver failure according to the CANONIC study.

No acute-on-chronic liver failure No organ failure OR single organ failure + creatinine $< 133 \mu\text{mol/L}$ + no hepatic encephalopathy OR single cerebral failure + creatinine $< 133 \mu\text{mol/L}$
Grade 1 Single organ failure + creatinine $133 \leq 175 \mu\text{mol/L}$ and/or hepatic encephalopathy grad 1-2 OR single cerebral failure + creatinine $133 \leq 175 \mu\text{mol/L}$
Grade 2 2 organ failures
Grade 3 3 organ failures or more

Single organ failure refers to failure of liver, coagulation, circulation or lungs

Microbiology (I, III)

In the case of culture-positive infections, all bacteria and their antibiotic susceptibility patterns were registered. The following bacteria were considered to cause antibiotic-resistant infections: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), ampicillin-resistant *Enterococcus*, extended-spectrum β -lactamase-producing *Enterobacteriaceae* (ESBL), quinolone-resistant gram-negative rods (QRGNR), or isolates resistant to 3 or more classes of antibiotics [11, 107].

Comorbidity (I-III)

At diagnosis of each bacterial infection episode occurring during follow-up, the Charlson comorbidity index was calculated as a measure of the burden of comorbid illness [108]. Charlson comorbidity index is a valid method of classifying comorbidity and estimating risk of death from comorbid disease for use in longitudinal studies. Each condition is assigned a score of 1, 2, 3, or 6, depending on the risk of dying associated with each one (table 4). Scores are summed to provide a total score to predict 10-year mortality. Liver disease was not taken into account at calculation of Charlson comorbidity index since the patient cohort used had this condition in common.

Table 4. Weighted index of Charlson comorbidity

Conditions with weight 1	Conditions with weight 2	Conditions with weight 6
✓ Myocardial infarct	✓ Hemiplegia	✓ Metastatic solid tumor
✓ Congestive heart failure	✓ Moderate or severe renal disease	✓ Acquired immunodeficiency syndrome
✓ Peripheral vascular disease	✓ Diabetes with end-organ damage	
✓ Cerebrovascular disease	✓ Any tumor	
✓ Dementia	✓ Leukemia	
✓ Chronic pulmonary disease	✓ Lymphoma	
✓ Connective tissue disease		
✓ Ulcer disease		
✓ Mild liver disease		
✓ Diabetes without end-organ damage		

Medications (I)

Data on PPI use and dose at cirrhosis diagnosis and upon each serious infection episode were registered. PPI use was defined as regular use of PPIs prior to cirrhosis and infection diagnosis respectively, independent of the treatment duration. Commencement of PPIs during the same hospital episode as the infection but prior to infection diagnosis was registered as new PPI therapy. Patients receiving PPIs were classified into those receiving less than, equal to, or more than one defined daily dose (DDD). One DDD was 20 mg for omeprazole, esomeprazole, rabeprazole and pantoprazole and 15 mg for lansoprazole [109].

PMN and monocyte function (IV)

At the time of study inclusion, peripheral blood was collected in vacutainer tubes containing sodium heparin (Becton Dickinson, BD, New York, USA), for immediate assessment of phagocytosis and ROS production by peripheral blood PMNs and monocytes, using the Phagotest and Phagoburst assays respectively (Glycotope Biotechnology, GmbH, Germany) [85, 89]. The tests were performed on whole blood and evaluated using flow cytometry (Navios, with the CXP Software for acquisition, and the Kaluza software for analysis, Beckman Coulter, Brea, CA). ROS production was investigated without stimulus at 37°C (resting oxidative burst) and after activation with phorbol 12-myristate 13-acetate (PMA) or opsonised *Escherichia coli* (*E. coli*). In the last step, red blood cells were lysed and the remaining cells were fixed. Finally, propidium iodide was added to facilitate single cell analysis. The collection gate was set to 15.000 cells. No patients were found to have ROS deficiency. Oxidative burst was determined by the percentage of cells producing ROS. Median values of fluorescence intensity (MFI) of the ROS producing cells were used to quantify oxidative burst activity. Phagocytic activity was calculated from the percentage of cells undergoing phagocytosis. Phagocytic capacity was assessed by quantifying the number of *E.coli* bacteria engulfed per individual cell, expressed as the MFI. The collection gate was set to 10.000 cells. Seventeen healthy blood donors served as controls. They had no liver disease nor any other acute or chronic condition or acute alcohol intake, as per blood donation requirements in Sweden.

Cytokine analyses (IV)

At the time of inclusion in the study, peripheral blood was collected in 5 ml EDTA or SST tubes to obtain plasma or serum respectively. Before storage at -80°C the samples were centrifuged at 2200 g for 10 min. Levels of the pro-inflammatory

cytokines TNF α , IL-6, IL-8 were determined in serum (TNF α and IL-6) or plasma (IL-8) at an accredited laboratory (Clinical Immunology and Transfusion Medicine, Lund, Sweden) using the IMMULITE[®] 1000 system, Siemens, Germany. Limits of normal (based on analysis of 50 healthy blood donors, 25 females and 25 males) were set as follows; IL-6 <8 ng/L; IL-8 < 60 ng/L and TNF α <15ng/L.

Statistics

Statistical analyses in the current thesis were performed by using SPSS statistics versions 20-22. Data were expressed as mean and standard deviation (I-III) and as median and interquartile range (IV), or as n and percentage as appropriate. When comparing groups, the Fisher's exact test was used for categorical variables, while the ANOVA test (I), the student's t test (II,III) and the Mann-Whitney *U* test (IV) were used for continuous variables. All tests were 2-tailed and were conducted at a 5% significance level.

Paper I

In an attempt to identify independent predictors of HCA/HA infections and to investigate whether infection acquisition type was independently related to infection episode outcome (severe sepsis, infection-related ACLF and infection-related AKI stage 3), all parameters related to the development of HCA/HA infections and infection episode outcome respectively with a p value ≤ 0.1 in univariate analysis, were entered into multivariate logistic regression analyses. Predictors of infection-related mortality were assessed with survival analysis (Kaplan-Meier) and groups were compared with the log-rank test. Cox regression analysis was used to identify independent predictors of infection-related mortality.

Paper II

Negative predictors of transplant-free survival in compensated and decompensated patients at cirrhosis diagnosis were assessed with survival analysis (Kaplan-Meier) and groups were compared with the log-rank test. Cox regression analysis was subsequently used to identify independent predictors of survival in the two groups. In an attempt to identify independent predictors of infection-related ACLF and its short-term mortality, all parameters related to them with a p value ≤ 0.1 in

univariate analysis, were entered into multivariate logistic regression analyses (performed among infection episodes).

Paper III

Density incidence rates for various outcome variables were computed by dividing the total number of infectious events occurring in patients diagnosed with incident cirrhosis in each year from 2001 to 2010 during follow-up by the number of person-years for which each patient group was followed. The rates were multiplied by 100 and time trends were assessed with the Pearson's correlation coefficient. In order to adjust for confounders in the relation between on the one hand cirrhosis etiology (ALD vs. non-ALD) and on the other hand infection severity features, pneumonia, length of stay, or microbiological patterns/bacterial resistance, all parameters related to each one of these variables with a p value ≤ 0.1 in univariate analysis, were entered into multivariate logistic regression analyses. Length of hospital stay was assessed as a dichotomous variable using its mean value (days) in the study as a cut-off. Infection occurrence and infection-related mortality in patients with ALD vs. non-ALD cirrhosis were assessed with survival analysis (Kaplan-Meier) and groups were compared with the log-rank test. The relationship between ALD cirrhosis and occurrence of serious infections was evaluated further with Cox regression analysis. In this analysis, decompensation (at cirrhosis diagnosis or during follow-up) was entered as a time dependent variable along with other potential confounders.

Paper IV

The Spearman's rank correlation coefficient rho (r) was calculated for correlation analysis. The relation between PMN and monocyte function, on one hand, and patient outcome, on the other hand, was assessed with Cox regression analysis. Adjustment for potential confounders (i.e. age, gender and MELD score) was performed by further Cox regression analysis in the cases of variables of cell function that were univariately related to patient outcome at $p < 0.05$. The utility of *E.coli* stimulated burst in predicting the occurrence of outcome variables within the first year after recruitment was assessed by means of receiver operating characteristics (ROC) analysis. Survival analysis was performed using the Kaplan-Meier method and groups were compared with the log-rank test.

Results

The cohort used in papers I-III comprised 633 patients with a first-time diagnosis of cirrhosis during the study period, followed under 2276 patient-years (table 5). Twelve patients were lost to follow-up.

Table 5. Patient characteristics at cirrhosis diagnosis (papers I-III) (n=633)

Age (y)	59 (12)
Sex (male)	412 (65)
MELD score	10.4 (7)
Etiology	
ALD-cirrhosis (or mixed)	363 (57)
Viral	98 (16)
Cryptogenic/NASH	84 (13)
Cholestatic	39 (6)
Other	49 (8)

Data presented as mean (SD) or n (%) as appropriate

ALD, alcoholic liver disease; NASH, non-alcoholic steatohepatitis

The occurrence and impact of bacterial infections during the course of liver cirrhosis (II)

The occurrence and severity of bacterial infections in compensated and decompensated liver cirrhosis

During a median follow-up of 36 months (range: 0.1-134 months), 241/633 patients (38%) experienced a total of 398 serious bacterial infection episodes (median 1, range 1-8) (853 patient-years). The flow of patients from cirrhosis diagnosis through serious bacterial infections to mortality, liver transplant, or end of follow-up are shown in figure 1.

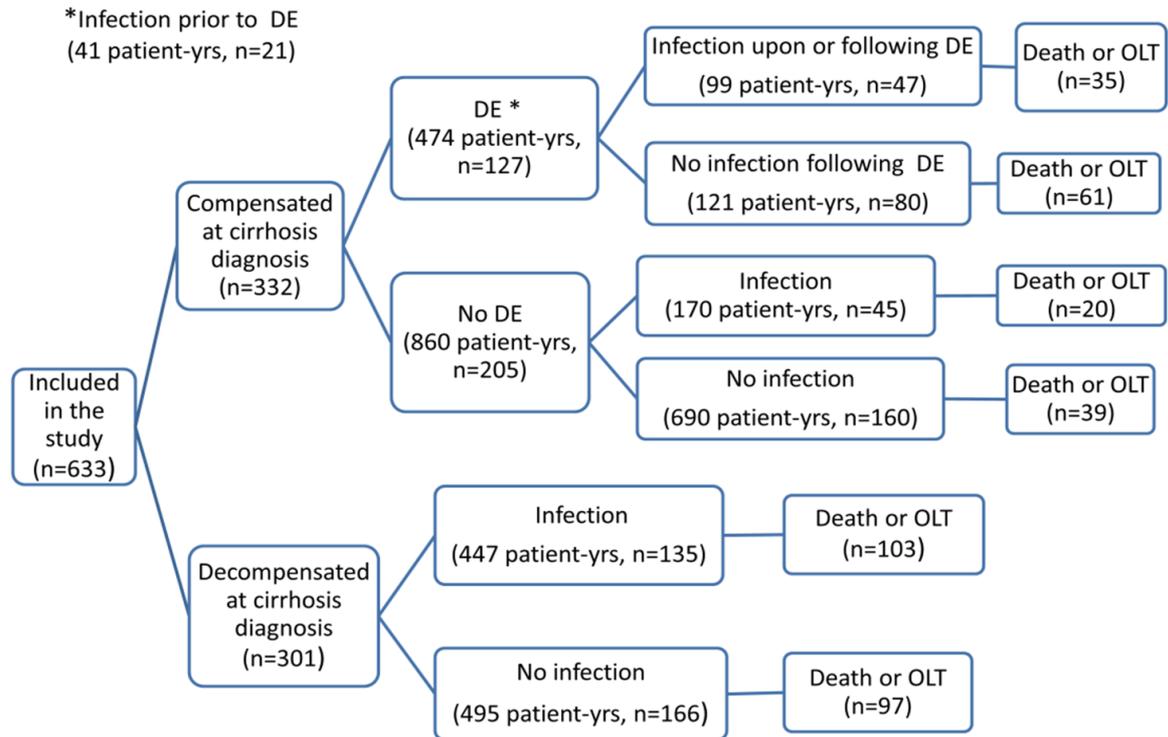


Figure 1. Flow of patients from cirrhosis diagnosis through serious bacterial infection episodes to mortality or liver transplantation

DE, decompensation; infection, serious bacterial infection (i.e. bacterial infection episode resulting in or occurring upon an inpatient hospital episode); OLT, orthotopic liver transplantation

Seven of 21 initially compensated patients with infection prior to decompensation experienced even infection upon or following decompensation.

Infection-related morbidity and mortality were significantly more frequent in the decompensated compared to the compensated group (table 6).

Table 6. Baseline characteristics and infection-related features in patients with compensated and decompensated disease at cirrhosis diagnosis

Parameter	Compensated at cirrhosis diagnosis (n=332)	Decompensated at cirrhosis diagnosis (n=301)	p value
Age (yr)	58 (12)	61 (11)	<0.001
Male gender	213 (64)	199 (66)	0.617
MELD at cirrhosis diagnosis	7.8 (5.7)	13.1 (7.2)	<0.001
Etiology			
Alcoholic cirrhosis (or mixed)	136 (41)	227 (75)	<0.001
Viral	81 (24)	17 (6)	<0.001
Cryptogenic/NASH	47 (14)	37 (13)	0.558
Cholestatic	29 (9)	10 (3)	0.005
Other	40 (12)	10 (3)	<0.001
HCA/HA infection (incidence rate)	39 (3.5)	100 (10.6)	<0.001
Sepsis (incidence rate)	90 (6.7)	108 (11.4)	<0.001
Severe sepsis (incidence rate)	51 (3.8)	68 (7.2)	<0.001
Infection-related AKI (incidence rate)	59 (4.4)	85 (9.0)	<0.001
Infection-related mortality (incidence rate)	28 (2.1)	42 (4.4)	0.002
Overall mortality/OLT (incidence rate)	155 (11.6)	200 (21.2)	<0.001

Data are expressed as means (SD) or number of patients (%) or number of patients (incidence rate per 100 patient-years) as appropriate

Patients with variceal bleeding or/and ascites at cirrhosis diagnosis were classified as decompensated and patients without these features as compensated [95]

NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; HCA/HA, health-care associated/hospital-acquired; AKI, acute kidney injury; OLT, orthotopic liver transplantation

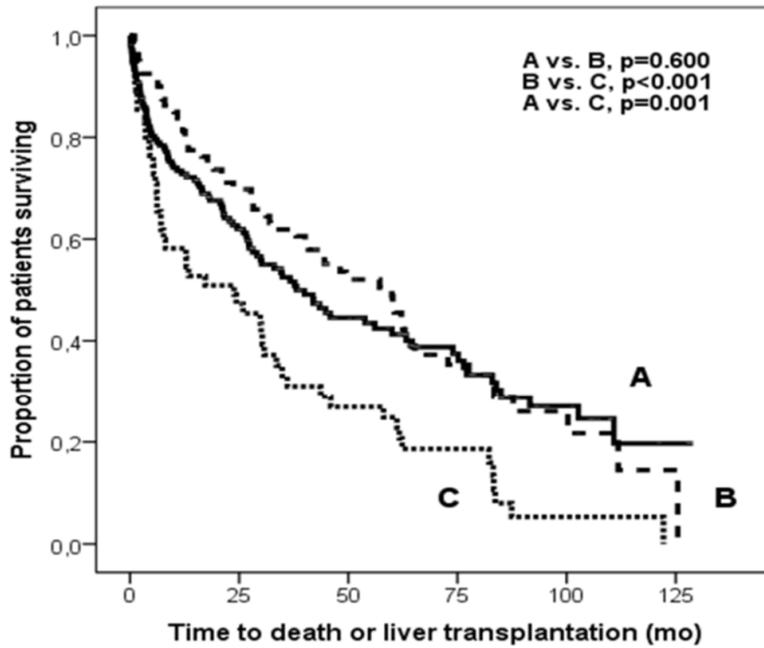
Bacterial infection is not a predictor of survival in patients with compensated disease at cirrhosis diagnosis

A total of 106 (32%) patients with compensated disease at cirrhosis diagnosis experienced at least one serious bacterial infection during the follow-up period (figure 1). Independent predictors of mortality or transplantation (combined outcome) in compensated patients at cirrhosis diagnosis (with or without decompensation during follow-up) selected by Cox regression analysis were: MELD score (hazard ratio (HR) 1.05, 95% confidence interval (CI) 1.02-1.08) upon cirrhosis diagnosis and decompensation during follow-up (HR 2.10, 95% CI 1.44-3.06) but not age, diabetes mellitus upon cirrhosis diagnosis, varices, gender, alcoholic cirrhosis, occurrence of serious bacterial infection, HCA/HA infection,

sepsis, severe sepsis, infection-related AKI, infection-related ACLF, hepatocellular carcinoma (HCC) or encephalopathy (data not shown). Similar results were also obtained when compensated patients with HCC were excluded from the analysis (data not shown).

Infection-related ACLF is a predictor of survival in patients with decompensated disease at cirrhosis diagnosis

A total of 135/301 (45%) of patients presenting with decompensated cirrhosis had at least one serious bacterial infection episode (figure 1). Forty-two out of 301 (14%) were diagnosed with a serious bacterial infection during their hospitalization upon first presentation with cirrhosis (12/42 with infection-related ACLF). Decompensated patients without serious bacterial infections during follow-up had similar transplant-free survival to those with serious bacterial infections without associated ACLF (figure 2). However, compared to both groups, decompensated patients with infection-related ACLF during follow-up had significantly worse transplant-free survival (figure 2). In Cox regression analysis, male gender (HR 1.40, 95% CI 1.01-1.94), age (HR 1.04 per year, 95% CI 1.03-1.06), MELD score (HR 1.05, 95% CI 1.03-1.08) and diabetes mellitus (HR 1.48, 95% CI 1.04-2.09) upon cirrhosis diagnosis, hepatic encephalopathy during follow-up (HR 1.47, 95% CI 1.08-2.00), and infection-related ACLF (HR 1.14, 95% CI 1.01-1.20) were found to be independent predictors of mortality or transplantation in decompensated patients, but not alcoholic etiology of cirrhosis, serious bacterial infection, HCA/HA infection, sepsis, severe sepsis, infection-related AKI, or HCC (data not shown) (figure 3). After exclusion of patients with HCC, transplant-free survival was still worse among decompensated patients with vs. those without infection-related ACLF (log-rank test $p < 0.001$; data not shown). The majority of deaths or transplants (65%) occurred during the first 3 months following the infection episode complicated by ACLF.



Patients at risk						
A	166	84	45	27	12	3
B	80	54	34	18	6	1
C	55	26	13	7	1	0

Figure 2. The impact of infection-related acute-on-chronic liver failure on survival in decompensated cirrhosis. Transplant-free survival in decompensated patients without serious bacterial infection (A; n=166), with serious bacterial infection not complicated by acute-on-chronic liver failure (B; n=80), or with infection-related acute-on-chronic liver failure (C; n=55) during follow-up
 Death or liver transplantation were considered events.
 Patients were censored at the end of follow-up.

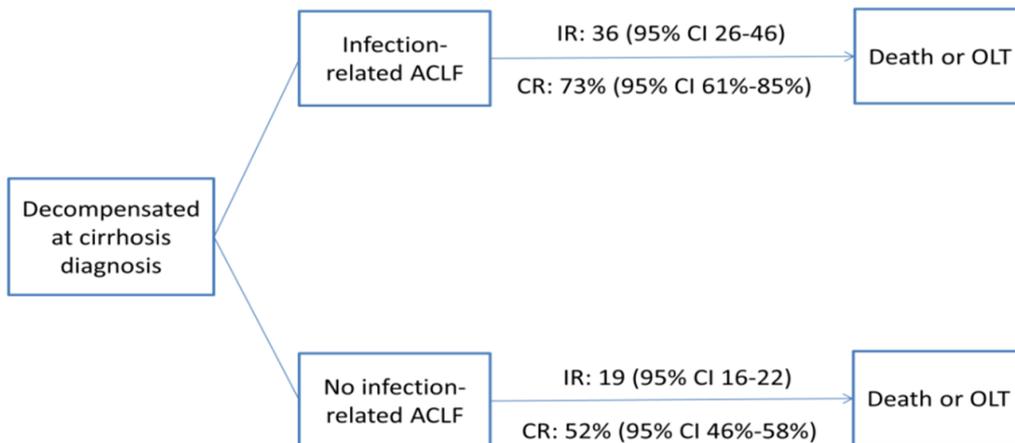


Figure 3. Overall mortality/transplant incidence rates and 3-year overall mortality/transplant cumulative risk in patients presenting with decompensated cirrhosis according to the occurrence of infection-related acute-on-chronic liver failure
 ACLF, acute-on-chronic liver failure; IR, incidence rate per 100-person-years; CI, confidence interval; CR, 3-year cumulative risk; OLT, orthotopic liver transplantation

Bacterial infection in alcoholic and non-alcoholic liver cirrhosis (III)

Time trends in the occurrence and outcome of serious bacterial infections experienced by annual incident ALD and non-ALD cohorts in 2001-2010

The frequency of serious infections increased significantly in patients diagnosed with incident cirrhosis later during the study period in both ALD and non-ALD cirrhosis (figure 4, 5, 6). This appeared to be related to an increase in HCA and HA infections, which was more evident in the ALD group (figure 5). Similarly, an increased frequency of sepsis, severe sepsis, infection-related ACLF and infection-related in-hospital mortality was observed in both etiology groups, but reached statistical significance only in the group of ALD cirrhosis (figure 4, 5, 6). A trend towards decreasing MELD score values in patients diagnosed in study years 2001-2010 was observed (from mean score 11 in 2001 to 10 in 2010, $p=0.061$), suggesting that severity of cirrhosis at diagnosis is probably not a major predictor of the observed changes in the frequency of infections during the study period.

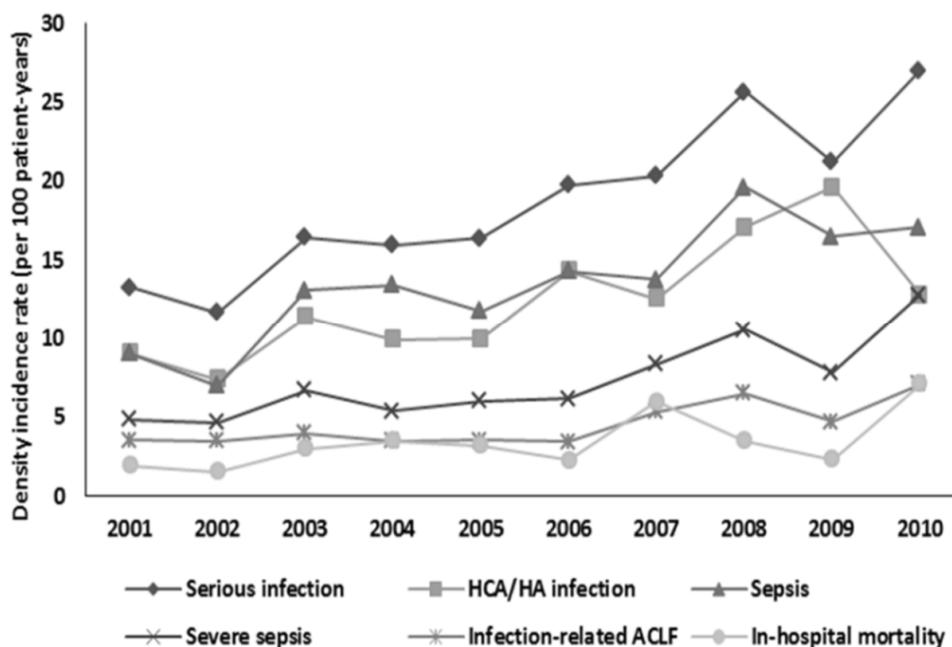


Figure 4. Density incidence rates of serious bacterial infection episodes occurring in all patients diagnosed with incident cirrhosis (n=633) in the study period 2001-2010.

Density incidence rates increased for serious infection, HCA/HA infection, sepsis, severe sepsis, infection-related ACLF and in-hospital mortality ($p<0.05$ for all).

p-values are provided by Pearson correlation test

HCA/HA, healthcare-associated/hospital-acquired; ACLF, acute-on-chronic liver failure

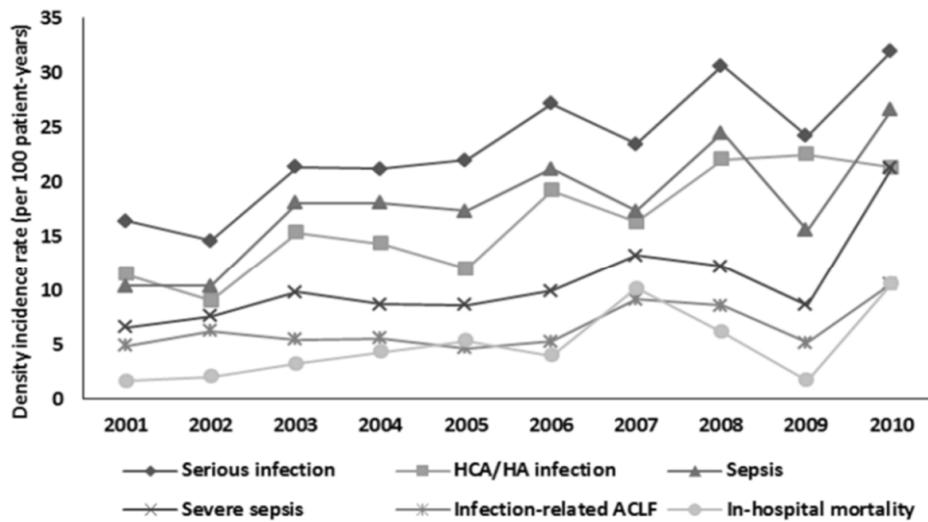


Figure 5. Density incidence rates of serious bacterial infection episodes occurring in patients diagnosed with incident ALD cirrhosis (n=363) in the study period 2001-2010.

Density incidence rates increased for serious infection, HCA/HA infection, sepsis, severe sepsis, infection-related ACLF and in-hospital mortality (p=0.001, p=0.001, p=0.012, p=0.018, p=0.055, p=0.058 respectively)

p-values are provided by Pearson correlation test

ALD, alcoholic liver disease; HCA/HA, healthcare-associated/hospital-acquired ; ACLF, acute-on-chronic liver failure

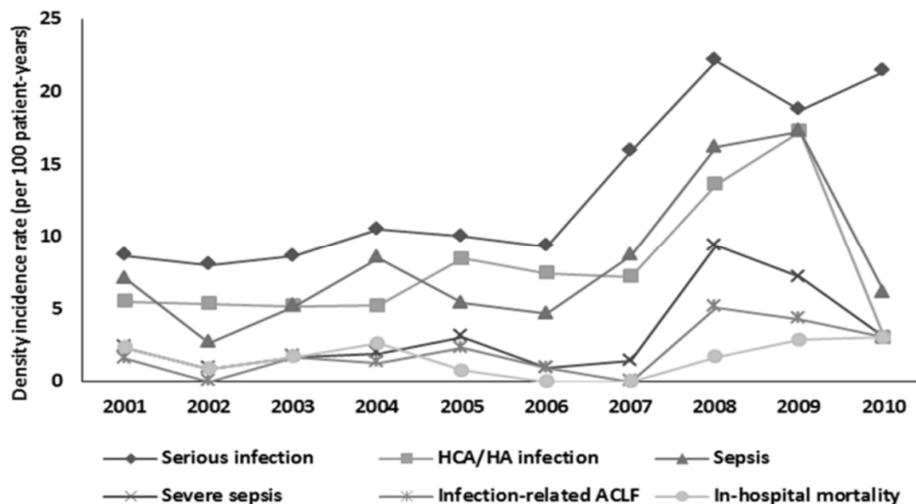


Figure 6. Density incidence rates of serious bacterial infection episodes occurring in patients diagnosed with incident non-ALD cirrhosis (n=270), in the study period 2001-2010.

Density incidence rates increased for serious infection (p=0.001), but not for HCA/HA infection, sepsis, severe sepsis, infection-related ACLF and in-hospital mortality (p>0.05 for all)

p-values are provided by Pearson correlation test

ALD, alcoholic liver disease; HCA/HA, healthcare-associated/hospital-acquired; ACLF, acute-on-chronic liver failure

Occurrence and localization of serious bacterial infections in ALD and non-ALD cirrhosis

During a median follow-up of 36 months (range: 0.1-134 months), 164/363 (45%) patients with ALD and 77/270 (28%) patients with non-ALD cirrhosis experienced at least one serious infection episode. Overall, a total of 272 (median 1, range 1-8) and 126 (median 1, range 1-5) serious bacterial infection episodes occurred in each group, respectively. Serious infections occurred more frequently in ALD compared to non-ALD cirrhotic patients during follow-up (figure 7).

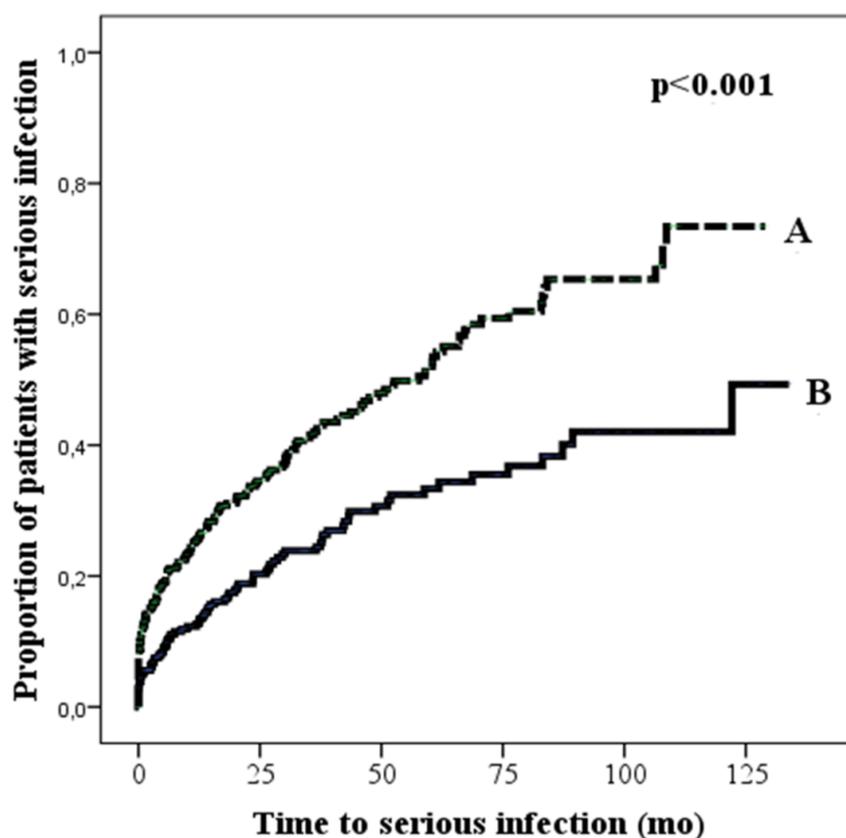


Figure 7. Relationship between cirrhosis etiology and infection occurrence. Proportion of patients with ALD cirrhosis (A) and non-ALD cirrhosis (B) with at least one serious bacterial infection during follow-up
The development of a serious bacterial infection was considered to be an event
Patients were censored at death, transplantation, or end of follow-up
ALD, alcoholic liver disease

ALD patients with compared to those without continuous alcohol misuse following diagnosis of cirrhosis in the medical records experienced serious infections more frequently (51% vs. 38%, $p=0.025$). However, after adjusting for confounders (age and MELD score at cirrhosis diagnosis, decompensation at cirrhosis diagnosis or during follow-up [95]) by means of Cox regression analysis, alcoholic etiology was not found to be independently related to the occurrence of

serious infections (HR 1.36, 95% CI 0.99-1.87), irrespective of whether there was evidence of continuous alcohol misuse (HR 1.34, 95% CI 0.95-1.89) or not (HR 1.35, 95% CI 0.93-1.96) following cirrhosis diagnosis. Among patients with decompensated disease upon cirrhosis diagnosis, patients with vs. those without ALD cirrhosis did not differ significantly in the occurrence of serious bacterial infections ($p=0.142$; data not shown).

Pneumonia occurred more often in ALD vs. non-ALD patients (table 7) which was confirmed in survival analysis (figure 8). No significant differences were observed between the two etiology groups with regard to other infection sites (table 7). After adjustment for confounders (C-reactive protein, age, MELD score and encephalopathy at infection diagnosis, comorbidity, length of hospital stay, intensive care unit care) by means of logistic regression analysis, ALD cirrhosis was still independently related to the occurrence of pneumonia (odds ratio (OR) 2.63, 95% CI 1.16-5.93). Serious infection episodes due to pneumonia occurred more often in patients with vs. without active alcohol misuse at infection diagnosis (19% vs. 10%, $p=0.027$). Among patients with compensated disease upon infection diagnosis, those with ALD experienced pneumonia more frequently compared with those with non-ALD cirrhosis (19% vs. 10%, $p=0.189$). Similarly, among serious infections occurring in patients with decompensated status, pneumonia was more frequently experienced by patients with ALD vs. non-ALD cirrhosis (16% vs. 6%, $p=0.041$).

Table 7. Characteristics of serious bacterial infection episodes occurring in patients with ALD and non-ALD cirrhosis

	ALD cirrhosis (n=272)	Non-ALD cirrhosis (n=126)	p value
Age at infection diagnosis (yr)	62 (10)	65 (12)	0.002
Male gender	85 (31)	47 (37)	0.253
MELD score at infection diagnosis	18 (8)	14 (8)	<0.001
Decompensated status at infection diagnosis^a	220 (81)	67 (53)	<0.001
Hepatic encephalopathy at infection diagnosis	86 (32)	29 (23)	0.096
HCC	11 (4)	4 (3)	0.784
Active alcohol misuse at infection diagnosis	172 (63)		
Charlson comorbidity index>1^b	65 (24)	48 (38)	0.004
HCA/HA infection	188 (69)	81 (64)	0.358
Mean arterial pressure (mm/Hg)	72 (33)	83 (28)	0.002
White cell count (x10⁹/L)	11 (9)	11 (6)	0.299
CRP (mg/L)	80 (80)	78 (75)	0.816
Site of infection			
Urinary tract infection	50 (18)	26 (21)	0.587
Pneumonia	45 (17)	10 (8)	0.020
Skin/soft-tissue infection	34 (13)	17 (13)	0.872
Spontaneous bacterial peritonitis	43 (16)	18 (14)	0.766
Spontaneous bacteremia	36 (13)	12 (9)	0.325
Mixed	8 (3)	11 (9)	0.020
Other	55 (20)	33 (26)	0.195

Data are expressed as means (SD) or number of patients (%) as appropriate

ALD, alcoholic liver disease; HCC, hepatocellular carcinoma; HCA/HA, healthcare-associated/hospital-acquired; CRP, C-reactive protein

^a Decompensated status was defined as the occurrence of ascites and/or variceal bleeding [95]

^b A score of 1 was the median value of Charlson comorbidity index (excluding liver disease)

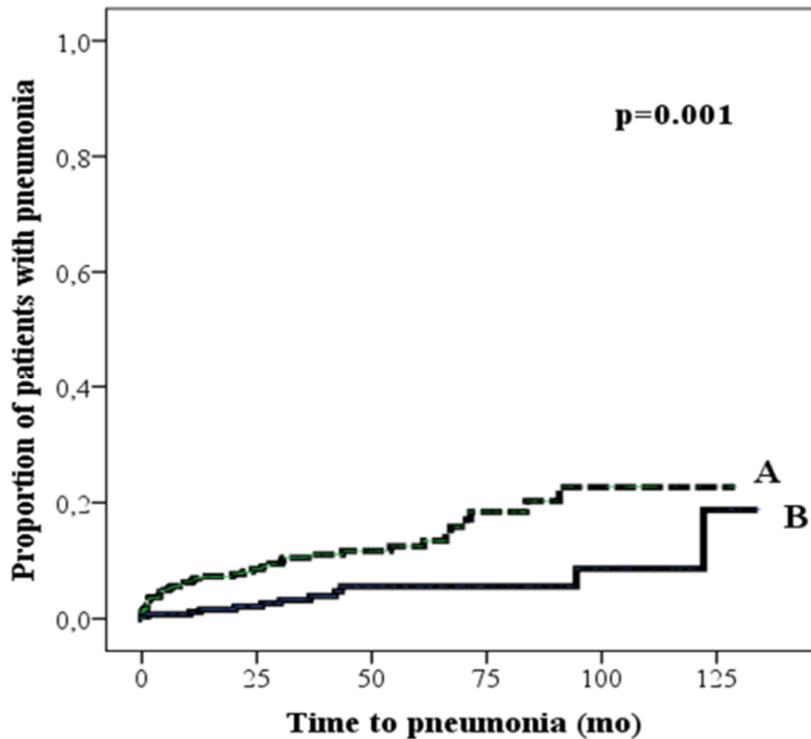


Figure 8. Proportion of patients with ALD cirrhosis (A) or non-ALD cirrhosis (B) and hospitalization with pneumonia during follow-up
 Patients were censored at death, liver transplantation or end of follow-up
 ALD, alcoholic liver disease

Bacterial resistance patterns in culture positive serious bacterial infections in ALD and non-ALD cirrhosis

In all, 261/398 of all serious bacterial infections had a positive bacterial culture (169/272 (62%) in ALD patients vs. 92/126 (73%) in non-ALD patients, $p=0.041$). The proportion of infections caused by gram-positive strains was significantly higher in ALD compared to non-ALD patients (table 8). Resistance to piperacillin-tazobactam, carbapenems and third-generation cephalosporins was more common in serious infections occurring in ALD vs non-ALD patients (table 8).

Table 8. Microbiologic findings in culture positive serious bacterial infection episodes occurring in patients with ALD and non-ALD cirrhosis

	ALD cirrhosis (n=168)	Non-ALD cirrhosis (n=92)	p value
Gram-negative bacilli	63 (37)	51 (55)	0.006
Enterobacteriaceae resistant to at least one β-lactam	8 (5)	3 (3)	0.752
QRGNR	1 (1)	10 (11)	<0.001
Gram-positive cocci	94 (56)	36 (39)	0.014
Enterococcus	36 (21)	8 (9)	0.009
Ampicillin-resistant Enterococcus	17 (10)	2 (2)	0.023
Gram-negative and gram-positive strains	11 (6)	5 (5)	0.794
Third-generation cephalosporin resistant strains	60 (36)	25 (27)	0.167
Carbapenem-resistant strains	20 (12)	2 (2)	0.009
Piperacillin-tazobactam resistant strains	22 (13)	5 (5)	0.057
Quinolone resistant strains	44 (33)	27 (33)	1.000
Bacteria causing antibiotic-resistant infections^a	22 (13)	14 (15)	0.708

Data presented as n (%)

QRGNR, quinolone resistant gram negative rods

^asee Subjects and Methods for definition

After adjustment for confounders (infection site, comorbidity, HCA/HA infection, second infection, ongoing antibiotic use, MELD score at infection diagnosis, performance of invasive procedure within 24 hours before infection diagnosis) by means of logistic regression analysis, alcoholic cirrhosis was an independent predictor of the occurrence of infections caused by gram-positive bacteria (OR 2.20, 95% CI 1.24-3.93), and Enterococcus (OR 2.66, 95% CI 1.11-6.40) in particular, but not resistance to piperacillin-tazobactam (OR 1.66, 95% CI 0.41-6.76) or carbapenems (OR 6.04, 95% CI 0.88-41.45). Among patients with decompensated status upon infection diagnosis, serious infections caused by gram positive bacteria (68% vs. 37%, p=0.007) or Enterococcus (19% vs. 5%, p=0.077) occurred more frequently in those with ALD vs. non-ALD cirrhosis. Similarly, among patients with decompensated disease upon infection diagnosis, those with ALD experienced more often serious infections caused by gram positive bacteria (53% vs. 41%, p=0.188) or Enterococcus (22% vs. 12%, p=0.143) compared to those with non-ALD cirrhosis, although these differences did not reach statistical significance.

Bacterial infection outcome in ALD and non-ALD cirrhosis

Serious bacterial infection episodes in patients with ALD vs. non-ALD cirrhosis were more often complicated by sepsis, severe sepsis, AKI, and ACLF (table 9). After adjustment for confounders (gender, infection site, HCA/HA infection, diabetes, MELD score, albumin, sodium, leukocyte count, C-reactive protein, and mean arterial pressure at infection diagnosis), by means of logistic regression analysis, ALD cirrhosis (with or without active alcohol misuse) was not found to be an independent predictor of either sepsis, severe sepsis or infection-related AKI ($p > 0.05$ for all).

Prolonged length of hospital stay (>18 days) was significantly more frequent in hospital episodes due to or with bacterial infections in patients with ALD vs. non-ALD cirrhosis, and this relationship persisted after adjustment for confounders (infection site, ongoing antibiotic use at infection diagnosis, HCA/HA infection, gastrointestinal bleeding during the same hospitalization with infection, sepsis, severe sepsis, infection-related ACLF, infection-related AKI, albumin, sodium, MELD score, leukocyte count, mean arterial pressure at infection diagnosis) by means of logistic regression analysis (OR 2.11, 95% CI 1.09-4.09).

Table 9. Infection-related morbidity and outcome in serious bacterial infection episodes occurring in patients with ALD and non-ALD cirrhosis

	ALD cirrhosis (n=272)	Non-ALD cirrhosis (n=126)	p value
Sepsis	213 (78)	83 (66)	0.010
Severe sepsis	119 (44)	32 (25)	0.001
Infection-related AKI	150 (57)	49 (40)	0.002
Infection-related ACLF	75 (28)	20 (16)	0.011
Length of stay >18 days^a	98 (36)	24 (19)	0.001
ICU-care	15 (5)	9 (7)	0.506
In-hospital mortality	58 (21)	18 (14)	0.102

Data are expressed as number of patients (%)

AKI, acute kidney injury; ACLF, acute-on-chronic liver failure; ICU, intensive care unit

^a 18 days was the mean value of length of hospital stay in all infections during the study period

When survival analysis was performed among patients with at least one serious infection during follow-up, no significant difference between ALD and non-ALD cirrhotics was observed with regard to infection-related mortality (figure 9). When ALD patients with active alcohol misuse following cirrhosis diagnosis and at least one serious bacterial infection during follow-up were compared to abstinent ALD

patients with regard to infection-related in-hospital mortality, no significant difference was observed (38% vs. 25%, $p=0.120$).

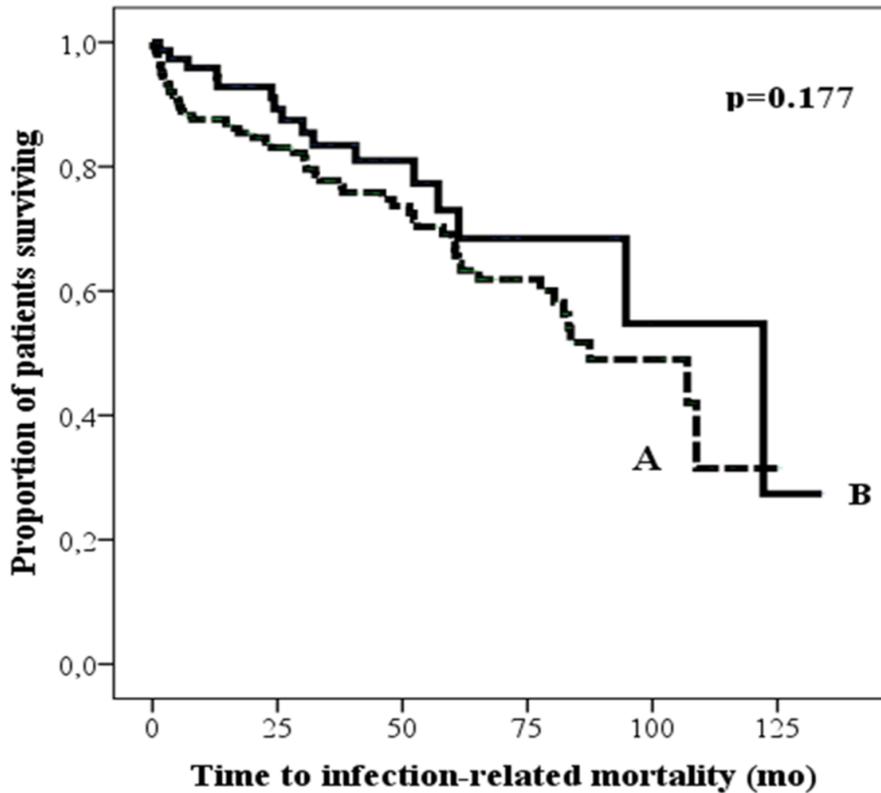


Figure 9. Relationship between cirrhosis etiology and infection-related mortality. Survival of patients with ALD (A) and non-ALD cirrhosis (B) with at least one serious bacterial infection during follow-up
Infection-related death was considered to be an event
Patients were censored at non-infection-related death, transplantation or end of follow-up
ALD, alcoholic liver disease

The role of HCA/HA bacterial infections in liver cirrhosis (I)

Occurrence of CA, HCA, HA infections

Most infection episodes were HCA (47%), followed by CA (32%) and HA (21%) (table 10). The occurrence of HCA/HA serious infections increased with time from cirrhosis diagnosis with 58%, 71%, and 92% of first, second and third serious infection episodes experienced by the patients in our cohort being HCA or HA infections ($p < 0.001$). In all, 174/633 (27%) developed at least one HCA (n=186 infections in 133 patients; median 1, range: 1-5) and/or HA infection (n=83 infections in 61 patients; median 1, range: 1-7).

Demographics and infection site

Liver impairment was more severe in HCA/HA infections compared to CA (table 10). HA infections were more frequently related to recent invasive procedures or gastrointestinal bleeding and were less commonly due to SBP or mixed infections in comparison to the other groups. Neither patient age or gender, nor cirrhosis etiology or infection occurrence in other sites differed significantly among the three infection acquisition groups (table 10).

Table 10. Characteristics of community-acquired, health-care associated and hospital-acquired serious bacterial infection episodes (n=398)

	Community-acquired (n=129)	Healthcare-associated (n=186)	Hospital-acquired ^a (n=83)	p value
Age (y)	63 (9)	62 (11)	60 (11)	0.616
Sex (male)	81 (63)	130 (70)	55 (66)	0.417
Decompensated status^b	74 (57)	146 (78)	67 (81)	<0.001
ALD-cirrhosis	84 (65)	126 (68)	62 (75)	0.333
MELD score at infection diagnosis	15 (8)	17 (7)	18 (9)	0.005
Charlson comorbidity index>1^c	31 (24)	60 (32)	22 (26)	0.082
Gastrointestinal bleeding^d	17 (13)	9 (5)	15 (18)	0.002
Invasive procedures ≤24 h before infection diagnosis^e	2 (2)	9 (5)	17 (20)	<0.001
Site of infection				
Urinary tract Infection	25 (19)	30 (16)	21 (25)	0.209
Spontaneous bacterial peritonitis	22 (17)	34 (18)	5 (6)	0.029
Pneumonia	19 (15)	29 (16)	7 (8)	0.272
Skin/soft-tissue infection	20 (15)	22 (12)	9 (11)	0.526
Spontaneous bacteremia	16 (12)	17 (9)	15 (18)	0.114
Mixed	5 (4)	14 (7)	0 (0)	0.024
Other	22 (17)	40 (21)	26 (31)	0.049

Data presented as mean (SD) or n (%) as appropriate.

ALD, alcoholic liver disease

^aTwenty-four out of 83 (29%) of HA infections occurred as subsequent (new) infections following a first infection episode during the same hospitalization

^bDecompensated status was defined as the occurrence of ascites and/or variceal bleeding [95]

^cA score of 1 was the median value of Charlson comorbidity index (excluding liver disease)

^dGastrointestinal bleeding occurring during the same hospital episode as the infection

^eInvasive procedures ≤24h before infection diagnosis in the current study were as follows: paracentesis, urinary or central venous catheter, endoscopic variceal ligation, sclerotherapy, placement of a transjugular intrahepatic portosystemic shunt, open surgery, dental extraction, laser lithotripsy, fine needle aspiration biopsy of liver mass.

Medications

Therapy with steroids and antibiotics was more frequent in HCA/HA than CA infections (table 11). Steroids were given mainly for autoimmune hepatitis (n=7/26) or co-existing medical conditions (n=17/26) and less commonly for alcoholic hepatitis (n=2/26). The most frequent indication for antibiotic therapy at infection diagnosis was another ongoing infection (commonly another serious infection treated prior to the diagnosis of a subsequent HA infection, n=16/49; but also skin/soft-tissue infections diagnosed and treated in an outpatient setting prior to the serious infection considered in the current study, n=12/49) followed by prophylactic therapy (n=21/49) mainly for hepatic encephalopathy or recurrent cholangitis.

Patients with HCA/HA infections used PPIs regularly more often and at a higher daily dose compared to those with CA infections (table 11). These results did not change after exclusion of patients on steroids (44% vs. 64% vs. 78% respectively, $p < 0.001$ for PPI use and 25% vs. 36% vs. 41%, respectively $p = 0.039$ for PPI > 1 DDD). PPI use was still more common in HCA/HA vs. CA infections when compensated (55% vs. 34%, $p = 0.036$) and decompensated patients (73% vs. 51%, $p = 0.001$) were analyzed separately. PPI use increased from the first to subsequent serious HCA/HA infections experienced by patients in our cohort (64% vs. 79%, $p = 0.011$) which was also true for their dose (33% vs. 45%, $p = 0.05$ of patients treated with >1 DDD).

Table 11. Medications in community-acquired, health-care associated and hospital-acquired serious bacterial infection episodes (n=398)

	Community-acquired (n=129)	Healthcare-associated (n=186)	Hospital-acquired ^a (n=83)	p value
Steroids	4 (3)	12 (6)	10 (12)	0.036
Immunosuppressive drugs other than steroids	1 (1)	4 (2)	0 (0)	0.284
Antibiotics	7 (5)	20 (11)	22 (26)	<0.001
PPI user at infection diagnosis^b	57 (44)	120 (64)	66 (80)	<0.001
New PPI therapy^c	6 (5)	2 (1)	15 (18)	<0.001
>1 DDD of PPIs	32 (25)	66 (35)	35 (42)	0.023

Data presented as n (%).

PPI, proton pumps inhibitors; DDD, defined daily dose

^aTwenty-four out of 83 (29%) of HA infections occurred as subsequent (new) infections following a first infection episode during the same hospitalization

^bRegular PPI use prior to infection diagnosis

^cCommencement of PPI therapy during the same hospital episode as the infection but prior to infection diagnosis

Outcome

Serious HCA and HA infection episodes were more frequently complicated by severe sepsis, ACLF, stage 3 AKI, and in-hospital mortality (table 12).

Table 12. Infection outcome in community-acquired, health-care associated and hospital-acquired serious bacterial infections (n=398)

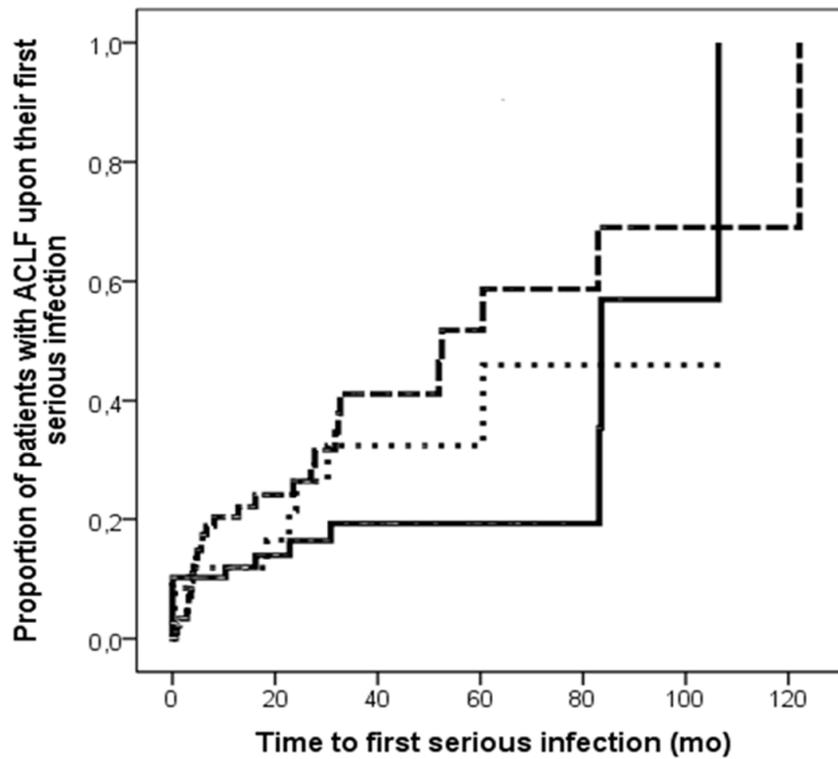
	Community-acquired (n=129)	Healthcare-associated (n=186)	Hospital-acquired ^a (n=83)	p value
Sepsis	94 (72)	140 (75)	62 (75)	0.889
Severe sepsis	39 (30)	75 (40)	37 (45)	0.072
Infection-related ACLF	19 (15)	51 (27)	25 (30)	0.011
Infection-related AKI stage 1	36 (28)	46 (25)	12 (15)	0.106
Infection-related AKI stage 2	13 (10)	32 (18)	9 (11)	0.141
Infection-related AKI stage 3	10 (8)	22 (12)	20 (26)	0.001
In-hospital mortality	13 (10)	44 (24)	19 (23)	0.007
Length of stay (days)	15 (13)	15 (16)	33 (27)	<0.001

Data presented as mean (SD) or n (%) as appropriate.

ACLF, acute-on-chronic liver failure; AKI, acute kidney injury

^aTwenty-four out of 83 (29%) of HA infections occurred as subsequent (new) infections following a first infection episode during the same hospitalization

After adjustment for confounders by means of logistic regression analysis, HCA/HA infections were found to be independent predictors of infection-related ACLF (OR 2.78, 95% CI 1.31-5.86), but not severe sepsis (OR 1.39, 95% CI 0.75-2.56) or stage 3 infection-related AKI (OR 1.68, 95% CI 0.63-4.49). Among patients experiencing serious bacterial infections in our cohort, infection-related ACLF developed more commonly among those with HCA and HA infections (figure 10 and 11).



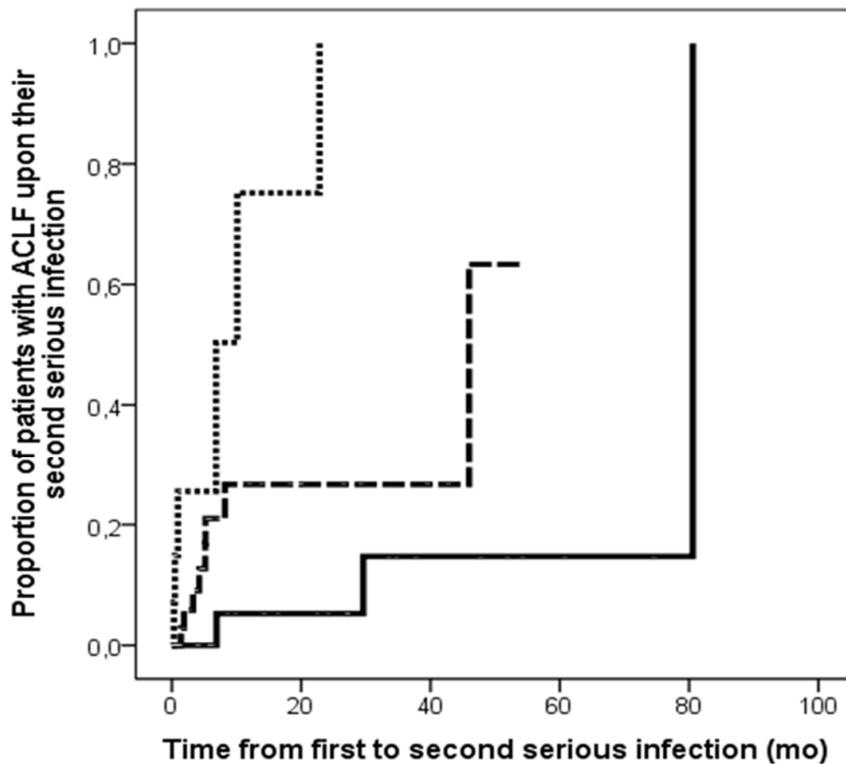
Number at risk		0	20	40	60	80	100	120
CA	101	40	23	12	5	1	0	
HCA	99	34	15	7	4	1	1	
HA	41	18	8	5	2	2	0	

Figure 10. Proportion of patients with at least one serious infection developing infection-related ACLF upon their first serious bacterial infection

ACLF; acute-on-chronic liver failure

community-acquired (continuous line), healthcare-associated (dashed line) or hospital-acquired (dotted line)

Patients were censored at development of a first serious infection (log-rank test: HCA vs. CA $p=0.077$; HA vs. CA $p=0.792$; HCA vs. HA $p=0.416$)



Number at risk	0	20	40	60	80	100
CA	25	14	6	3	1	0
HCA	43	7	3	0	0	0
HA	18	1	0	0	0	0

Figure 11. Proportion of patients with a second serious infection developing infection-related ACLF upon their second serious bacterial infection

ACLF; acute-on-chronic liver failure

community-acquired (continuous line), healthcare-associated (dashed line) or hospital-acquired (dotted line)

Patients were censored at development of a second serious infection (log-rank test: HCA vs. CA $p=0.029$; HA vs. CA $p<0.001$; HCA vs. HA $p=0.001$)

Re-defining infection-related ACLF according to NACSELD study [110], yielded very low numbers of infection-related ACLF in our cohort (only 13/398 (3%) of all serious infections in our study were complicated by ACLF; 5 or 4% of CA vs. 5 or 3% of HCA vs. 3 or 4% of HA, $p=0.827$) and thus no further analysis was attempted.

Among patients with at least one bacterial infection episode during follow-up, patients who developed HCA/HA infections had significantly higher infection-related mortality (Kaplan Meier, $p=0.001$; data not shown). After adjustment for confounders with Cox regression analysis, HCA/HA infections were not independently related to infection-related mortality ($p>0.05$).

Bacterial resistance

In all, 261/398 of bacterial infections (66% of CA, 66% of HCA and 65% of HA infections, $p=0.992$) had a positive bacterial culture. The proportion of infections caused by gram-positive or gram-negative bacteria did not differ significantly between the three infection acquisition groups (table 13). However, resistance to commonly used antibiotics was more common in serious HA vs. HCA vs. CA infections. This was true for third-generation cephalosporins, piperacillin-tazobactam, and quinolones, although it did not reach statistical significance in the latter (table 13). Bacteria causing antibiotic-resistant infections were more frequently isolated in serious HA infections (14/83, 17%), as compared with HCA (12/186, 6%) and CA (10/129, 8%) episodes ($p<0.05$). In logistic regression analysis, HCA/HA antibiotic-resistant infection was found to be related to the occurrence of severe sepsis (OR 3.58, 95% CI 1.09-11.80) but not AKI, ACLF, or in-hospital mortality ($p>0.05$ for all).

Table 13. Microbiologic findings in culture-positive serious bacterial infections (n=261)

	Community - acquired (n=85)	Healthcare- associated (n=122)	Hospital- acquired (n=54)	p value
Gram-negative bacilli	39 (46)	50 (41)	25 (46)	0.745
Enterobacteriaceae resistant to at least one β-lactam	1 (1)	4 (3)	6 (11)	0.014
QRGNR	3 (3)	6 (5)	2 (4)	0.868
Gram-positive cocci	42 (49)	66 (54)	23 (43)	0.393
Ampicillin-resistant Enterococcus	6 (7)	5 (4)	8 (15)	0.041
Gram-negative and gram-positive strains	4 (5)	6 (5)	6 (11)	0.234
Third-generation cephalosporin resistant strains	21 (25)	39 (32)	25 (46)	0.034
Piperacillin- tazobactam resistant strains	8 (9)	7 (6)	12 (23)	0.004
Quinolone resistant strains	18 (25)	34 (34)	19 (41)	0.183
Bacteria causing antibiotic-resistant infections^a	10 (12)	12 (10)	14 (26)	0.014

Data presented as n (%)

QRGNR, quinolone resistant gram negative rods

^aSee Subjects and Methods for definition

Predictors of HCA/HA infections

In regression analysis, decompensated status (OR 3.84, 95% CI 2.35-6.27), use of antibiotics (OR 3.11, 95% CI 1.29-7.48) and PPIs (OR 2.06, 95% CI 1.27-3.33) at infection diagnosis but not infection site, recent invasive procedures, gastrointestinal bleeding or steroid use were independent predictors of HCA/HA infections. PPI use was still predictive of serious HCA/HA infections when analysis was repeated after exclusion of patients with concomitant therapy with steroids (OR 1.92, 95% CI 1.17-3.15) or new PPI users (OR 2.19, 95% CI 1.34-3.58).

Subsequent HCA/HA infections: relation with PPI use and increasing bacterial resistance

Use of PPIs was not significantly related to the occurrence of all types of subsequent infections (ie, HCA, HA and CA), in our cohort (figure 12). However, in survival analysis, the independent relation between PPI use and occurrence of HCA/HA infection was demonstrated in subsequent HCA/HA infection. PPI users, in particular those with > 1 DDD of PPIs, suffered second serious HCA/HA infections more commonly following a first serious HCA/HA infection episode (figures 13, 14). These results did not change when sensitivity analysis was performed excluding new PPI users (figures 13, 14; footnote).

Small numbers in the new PPI users group (i.e. those who started PPIs during the same hospital episode as the infection but prior to infection diagnosis; table 11, n=17) did not allow a direct comparison of this group with the non-PPI user group or that of patients who used PPIs prior to hospitalization.

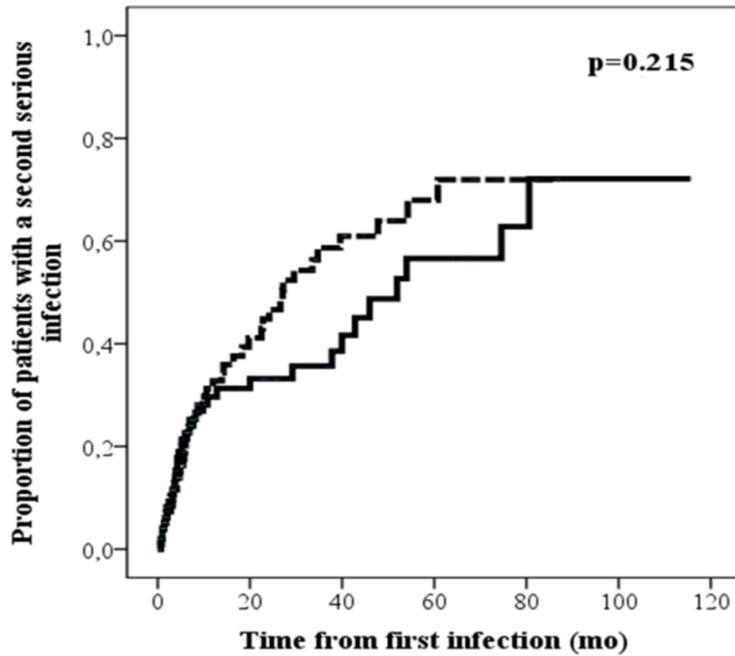
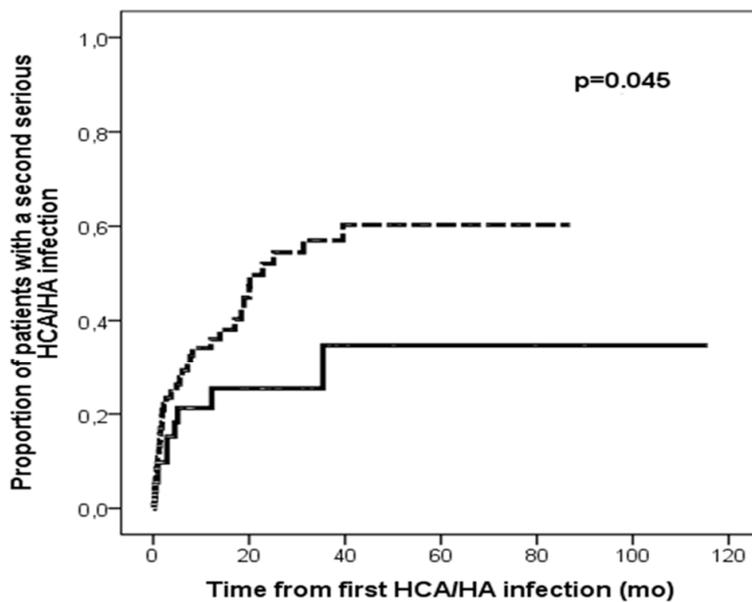


Figure 12. Development of a subsequent bacterial infection after hospitalization with a first bacterial infection in PPI users (dashed line) vs non-PPI users (continuous line)

PPI, proton pump inhibitors

Patients were censored at last follow-up until end of 2011, liver transplantation, or death



Number at risk

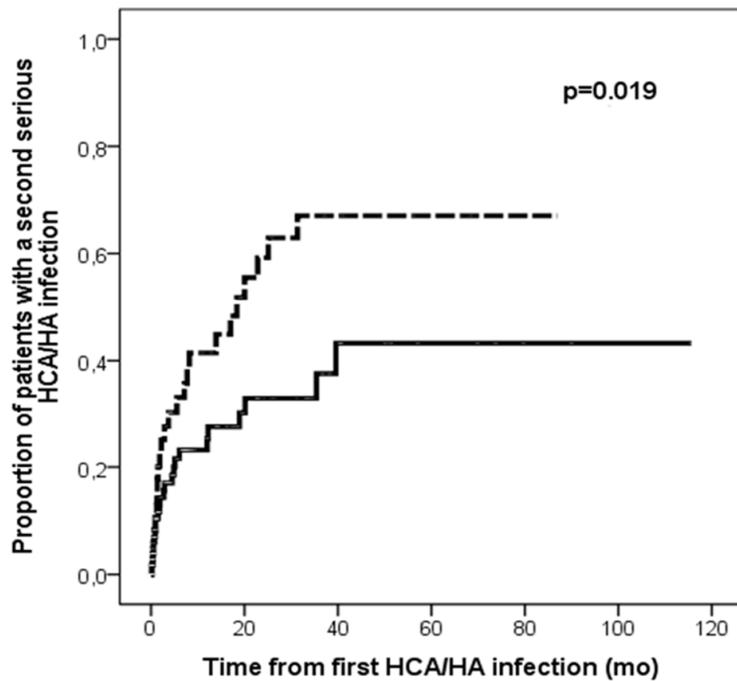
PPI users	115	23	12	6	1	0	0
Non-PPI users	59	16	5	3	2	1	0

Figure 13. Proportion of PPI-users (dashed line) vs. non-PPI users (continuous line) developing a second serious HCA/HA infection during follow-up following a first serious HCA/HA infection

HCA, healthcare-associated; HA, hospital-acquired

Patients were censored at last follow-up until end of 2011, liver transplantation, or death.

When new PPI users (those starting PPI therapy during the same hospital episode as the infection prior to infection diagnosis, n=14) were excluded from analysis: p=0.015



Number at risk	0	20	40	60	80	100	120
DDD>1	61	13	7	4	1	0	0
DDD≤1	113	26	10	5	2	1	0

Figure 14. Proportion of PPI-users with >1 DDD (dashed line) vs. non-PPI users/PPI users with ≤1 DDD (continuous line) developing a second serious HCA/HA infection during follow-up following a first serious HCA/HA infection

HCA, healthcare-associated; HA, hospital-acquired; DDD, defined daily dose

Patients were censored at last follow-up until end of 2011, liver transplantation, or death. When new PPI users (those starting PPI therapy during the same hospital episode as the infection prior to infection diagnosis, n=14) were excluded from analysis: p=0.011

Subsequent serious HCA/HA infections following a first serious HCA/HA bacterial infection episode were more frequently caused by third-generation cephalosporin resistant strains (49% vs. 29%, p=0.010), piperacillin-tazobactam resistant strains (17% vs. 7%, p=0.075), ampicillin-resistant enterococcus (12% vs. 4%, p=0.074) or enterobacteriaceae resistant to at least one β -lactam (11% vs. 3%, p=0.04). However, they did not differ significantly in the occurrence of gram-positive bacteria (54% vs. 48%, p=0.532) nor were they significantly more often antibiotic-resistant infections (18% vs. 13%, p=0.379).

The role of infection-related ACLF in liver cirrhosis (II)

Predictors of infection-related ACLF occurrence

In all, 95/398 (24%) of all serious bacterial infection episodes occurring in the study period were complicated by ACLF. Patient characteristics in serious bacterial infections with and without ACLF are summarized in table 14.

In logistic regression analysis, only the MELD score at infection diagnosis (OR 1.27, 95% CI 1.20-1.35), mixed infection (OR 6.41, 95% CI 1.97-20.85), active alcohol misuse at infection diagnosis (OR 2.73, 95% CI 1.42-5.26) and serious HCA or HA infection (OR 2.12, 95% CI 1.02-4.40) were shown to be independent predictors of infection-related ACLF. The independent relation between the occurrence of HCA/HA infection and infection-related ACLF is also outlined above under the subheading "The role of HCA/HA infections in liver cirrhosis".

Predictors of in-hospital mortality in serious bacterial infection episodes with ACLF

In-hospital mortality was significantly higher in serious bacterial infection episodes with vs. without ACLF and increased with increasing number of organ failures (table 14).

In logistic regression analysis, at least one comorbidity in the Charlson comorbidity index (OR 3.29, 95% CI 1.049-10.37), albumin levels at infection diagnosis (OR 0.90, 95% CI 0.82-0.98) and ACLF grade >1 (OR 3.81, 95% CI 1.45-10.05), but not C-reactive protein, age at infection diagnosis, respiratory, renal or circulatory failure ($p > 0.05$ for all; data not shown), were shown to be independent predictors of fatal infection-related ACLF.

Table 14. Patient characteristics in serious bacterial infection episodes with or without ACLF and in those complicated by infection-related ACLF with or without in-hospital mortality

Parameter	No ACLF (n=303)	ACLF (n=95)	p value	ACLF without in-hospital mortality (n=48)	ACLF with in-hospital mortality (n=47)	p value
Age (yr)	63 (11)	63 (10)	0.573	61 (10)	66 (9)	0.011
Male sex	190 (63)	76 (80)	0.002	40 (83)	36 (77)	0.452
Alcoholic cirrhosis	197 (65)	75 (79)	0.011	40 (83)	35 (74)	0.324
Active alcohol misuse at infection diagnosis	119 (39)	54 (58)	0.003	29 (60)	25 (54)	0.677
Charlson index>1 ^a	90 (30)	23 (25)	0.361	6 (12)	17 (36)	0.009
MELD at infection diagnosis	14.1 (6.9)	24.5 (6.6)	<0.001	24.9 (6.5)	23.9 (6.8)	0.506
HCA/HA infection	193 (64)	76 (79)	0.004	35 (73)	41 (87)	0.123
Second infection	15 (5)	9 (9)	0.136	7 (15)	2 (4)	0.159
Laboratory data at infection diagnosis						
Leukocyte count (x10 ⁹ /L)	10.8 (8.5)	13.5 (7.6)	0.007	13.6 (7)	13.4 (8.2)	0.990
CRP (mg/L)	76 (75)	90 (89)	0.127	73 (72)	108 (101)	0.059
Sodium (mmol/L)	134 (5)	132 (6)	0.002	131 (6)	132 (6)	0.656
Albumin (g/L)	25 (6)	23 (6)	0.004	24 (7)	21 (4)	0.029
Site of infection						
Urinary tract infection	63 (21)	13 (13)	0.137	9 (19)	4 (8)	0.232
Spontaneous bacterial peritonitis	47 (15)	14 (15)	1.000	9 (19)	5 (10)	0.386
Pneumonia	43 (14)	12 (13)	0.865	2 (4)	10 (21)	0.014
Skin/soft-tissue infection	44 (15)	7 (7)	0.079	3 (6)	4 (8)	0.714
Spontaneous bacteremia	33 (11)	15 (16)	0.209	6 (13)	9 (19)	0.412
Mixed infection ^b	9 (3)	10 (10)	0.005	5 (10)	5 (11)	1.000
Other	63 (21)	25 (26)	0.260	14 (29)	11 (23)	0.642
Mean arterial pressure(mm/Hg)	76 (34)	75 (26)	0.935	79 (27)	72 (25)	0.239
ICU-care	5 (2)	19 (20)	<0.001	6 (12)	13 (28)	0.077
In-hospital mortality	29 (10)	47 (49)	<0.001			
Organ failures						
Liver				13 (27)	8 (17)	0.324
Kidney				34 (71)	42 (89)	0.039

Cerebral	10 (21)	16 (34)	0.173
Coagulation	9 (19)	7 (15)	0.785
Circulation	3 (6)	10 (21)	0.040
Lungs	4 (8)	11 (23)	0.053
ACLF grade >1	12 (25)	27 (57)	0.002

Data are expressed as means (SD) or number of patients (%) as appropriate

ACLF, acute-on-chronic liver failure; HCA/HA, health-care associated/hospital-acquired; CRP, C-reactive protein; ICU, intensive care unit

^aA score of 1 was the median value of Charlson comorbidity index (excluding liver disease)

^bThe following sites of infection were involved in mixed infections: urinary tract infection, spontaneous bacterial peritonitis, skin/soft-tissue infection, pneumonia, spontaneous bacteremia, gastroenteritis

PMN and monocyte function in ambulatory cirrhotic patients and their prognostic role (IV)

Patient characteristics at study inclusion are shown in table 15. Data on phagocytosis were available in 46/60 (76%) patients.

Table 15. Baseline characteristics of cirrhotic patients included in paper IV (n=60)

Male gender	42 (70)
Age, years	63 (57-69)
Diabetes	21 (35)
Other comorbidity^a	35 (58)
<i>Etiology of cirrhosis</i>	
ALD (or mixed)	31 (52)
Viral	7 (12)
Cryptogenic/NASH	14 (23)
Cholestatic	4 (7)
Other	4 (7)
<i>Decompensation</i>	
Prior to study inclusion	10 (17)
Current	13 (21)
MELD score	6 (6-8)
Child Pugh class A/B/C	50 (83) / 8 (13) / 2 (4)

Data are expressed as n (%) or median (interquartile range) as appropriate

ALD, alcoholic liver disease; NASH, non-alcoholic steatohepatitis; MELD, model for end-stage liver disease

^aThe most common comorbid conditions other than diabetes were: arterial hypertension (n=19), cardiovascular disease (n=9), and chronic obstructive pulmonary disease (n=4)

Increased oxidative burst and reduced phagocytosis in cirrhotic patients compared to controls

Compared to healthy controls, cirrhotics showed increased resting and *E.coli* stimulated PMN burst activity (figure 15) as well as reduced phagocytosis of opsonized *E.coli* (table 16). A similar pattern was observed in monocytes, with an increased *E.coli* stimulated burst (figure 16) and a tendency towards decreased phagocytic capacity (table 16).

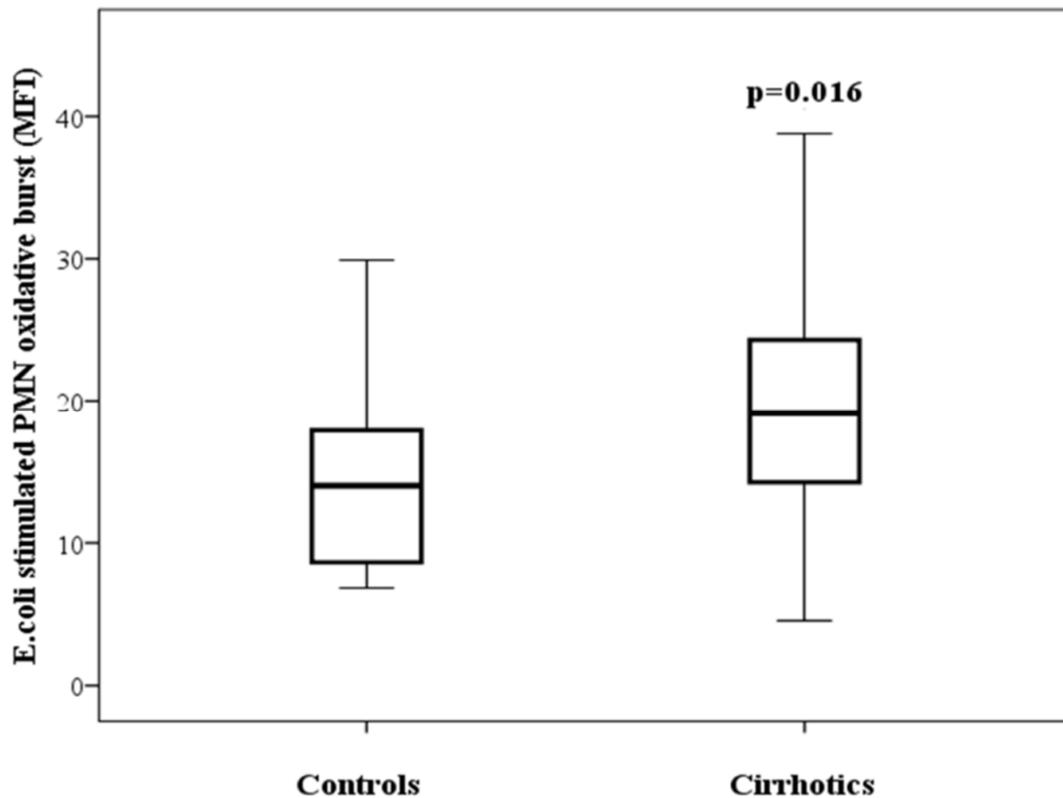


Figure 15. *E.coli* stimulated PMN oxidative burst in cirrhotics (n=60) and controls (n=17)

Cirrhotics showed increased *E.coli* stimulated PMN oxidative burst, as measured by MFI, compared to controls (19.1 vs. 14.0).

PMN, polymorphonuclear leukocyte; MFI, median fluorescence intensity

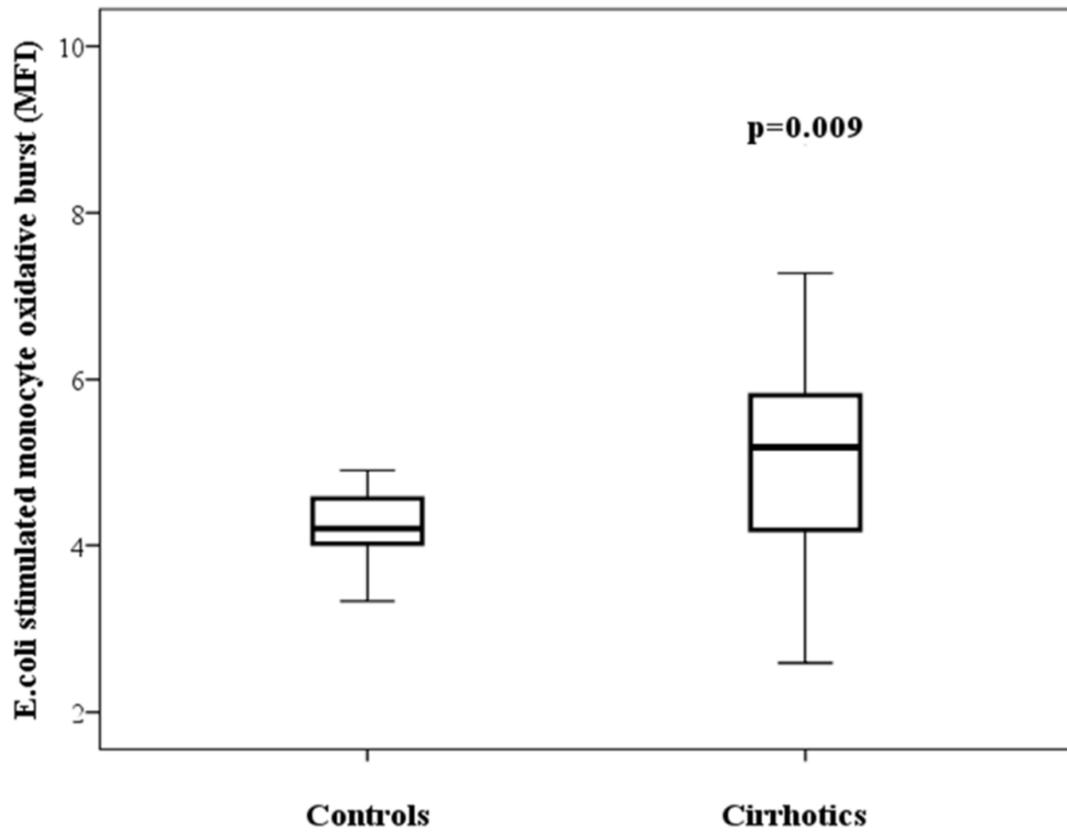


Figure 16. *E.coli* stimulated monocyte oxidative burst in cirrhotics (n=60) and controls (n=17)
Cirrhotics showed increased *E.coli* stimulated monocyte oxidative burst, as measured by MFI, compared to controls (5.2 vs. 4.2).
MFI, median fluorescence intensity

Table 16. Polymorphonuclear leukocyte and monocyte function in patients with cirrhosis, with or without decompensation, and in healthy controls

	Cirrhotics (n=60)	No decompensation (n=37)	Prior or current decompensation (n=23)	Controls (n=17)
MELD score		6 (6-7)	7 (6-12)	
ALD cirrhosis		14 (38)	17 (74) ^d	
PMN function				
Resting oxidative burst, %*	0.5 (0.3-0.9)	0.4 (0.2-0.6)	0.6 (0.4-1.1)	0.2 (0.1-0.5) ^{a,c}
Resting oxidative burst, MFI	3.4 (3.0-3.8)	3.3 (3.0-3.8)	3.4 (3.0-3.9)	3.3 (3.0-3.7)
E.coli oxidative burst, MFI	19.1 (14.0-24.3)	18.4 (13.5-26.3)	19.6 (15.9-24.3)	14.0 (8.3-19.3) ^{a,b,c}
PMA oxidative burst, MFI	16.5 (16.8-30.2)	16.5 (8.9-16.5)	19.4 (8.9-19.4)	19.9 (11.1-31.2)
Phagocytic activity, %**	95.9 (87.6-97.8)	95.9 (88.9-98.1)	95.6 (86.7-97.7)	98.0 (97.5-99.0) ^{a,b,c}
Phagocytic capacity, MFI	51.9 (39.6-51.0)	48.3 (42.4-69.6)	64.1 (35.1-71.5)	69.6 (61.2-83.9) ^{a,b,c}
Monocyte function				
Resting oxidative burst, %*	0.7 (0.3-1.9)	0.6 (0.1-1.4)	1.2 (0.4-3.9) ^d	1.0 (0.4-2.6)
Resting oxidative burst, MFI	2.9 (2.5-3.2)	2.8 (2.5-3.1)	3.1 (2.8-3.1) ^d	2.5 (1.9-3.3)
E.coli oxidative burst, MFI	5.2 (4.1-5.7)	5.0 (3.9-5.5)	5.2 (4.6-5.8)	4.2 (3.9-4.6) ^{a,b,c}
PMA oxidative burst, MFI	4.3 (3.3-6.2)	3.8 (3.3-5.4)	4.9 (3.6-6.9) ^d	4.0 (3.5-4.6) ^c
Phagocytic activity, %**	89.3 (74.1-94.9)	89.2 (76.6-94.2)	88.8 (72.3-95.4)	90.0 (81.0-93.0)
Phagocytic capacity, MFI	30.7 (17.1-39.7)	31.1 (19.7-31.5)	29.1 (15.2-29.1)	34.8 (31.1-40.2)
Cytokines				
S-IL-6, ng/L		5 (3-11)	9 (7-12) ^d	
P-IL-8, ng/L		6 (5-16)	7 (5-17)	
S-TNFα, ng/L		13 (11-16)	15 (12-16)	

Data are expressed as n (%) or median (interquartile range) as appropriate

ALD, alcoholic liver disease; MELD, model for end-stage liver disease; PMN, polymorphonuclear leukocyte; MFI, median fluorescence intensity; PMA, phorbol-12-myristate-13-acetate; IL, interleukin; TNF, tumor necrosis factor

Cytokine levels were not measured in controls and data on phagocytosis were available in 23/31 patients with ALD or mixed, 6/7 with viral, 11/14 with cryptogenic/NASH, 4/4 with cholestatic and 2/4 with other cirrhosis (46/60, 76% of all cirrhotics)

p<0.05 in cirrhotics vs. controls^a; in patients without decompensation vs. controls^b; in patients with prior or current decompensation vs. controls^c; in patients with vs. those without prior or current decompensation^d

*Percent of bursting cells

**Percent of cells undergoing phagocytosis

Relation of PMN and monocyte function with severity and alcoholic etiology of cirrhosis

Both resting and PMA stimulated monocyte burst was higher in patients with prior or current decompensation vs. those without (table 16). Phagocytic capacity of PMNs and monocytes did not differ significantly between the two groups (table 16). Since the group of patients with prior or current decompensation was heterogeneous (MELD scores ranging from 6 to 17), analyses were repeated between patients with MELD score ≤ 6 vs. MELD score >6 (MELD score=6 was the median MELD value at study inclusion) showing lower phagocytic capacity of both PMNs and monocytes in the latter group although the results did not reach statistical significance (MFI 58.2 (39.7-67.5) vs. 49.9 (40.3-79.8), $p=0.816$ and 31.7 (18.3-39.1) vs. 27.7 (16.2-43.2), $p=0.965$, respectively). Furthermore, the MELD and Child-Pugh scores correlated positively with the percentage of resting monocytes producing ROS ($r=0.220$, $p=0.097$ and $r=0.390$, $p=0.002$ respectively), and the latter correlated also with the percentage of resting PMNs producing ROS ($r=0.391$, $p=0.002$). No differences in PMN function were observed in patients with vs. without prior or current decompensation (table 16). Alcoholic etiology was not significantly related to PMN or monocyte cell function (table 17).

Table 17. Polymorphonuclear leukocyte and monocyte function in patients with ALD vs. non-ALD cirrhosis

	Non-ALD cirrhosis (n=29)	ALD cirrhosis (n=31)
Male gender	20 (69)	22 (71)
Age (years)	64 (56-70)	63 (57-67)
MELD score	6 (6-8)	6 (6-8)
Prior or current decompensation	6 (21) ^a	17 (55)
Abstainers		8 (26)
Current drinkers		23 (74)
PMN function		
Resting oxidative burst, %*	0.4 (0.2-0.8)	0.5 (0.3-0.9)
Resting oxidative burst, MFI	3.6 (3.1-3.9)	3.1 (2.9-3.6)
E.coli oxidative burst, MFI	20.5 (15.2-26.3)	18.2 (13.5-22.8)
PMA oxidative burst, MFI	19.9 (10.5-34.2)	13.3 (7.7-24.1)
Phagocytic activity, %**	96.3 (87.6-98.1)	94.7 (87.9-97.8)
Phagocytic capacity, MFI	52.2 (44.4-74.7)	51.7 (35.1-69.6)
Monocyte function		
Resting oxidative burst, %*	0.7 (0.3-2.4)	0.7 (0.3-1.8)
Resting oxidative burst, MFI	2.8 (2.2-3.3)	3.0 (2.7-3.2)
E.coli oxidative burst, MFI	5.3 (4.0-5.5)	5.2 (4.2-5.9)
PMA oxidative burst, MFI	4.6 (3.5-6.7)	4.1 (3.3-6.1)
Phagocytic activity, %**	90.2 (75.4-94.2)	87.1 (74.1-95.4)
Phagocytic capacity, MFI	33.3 (20.5-40.2)	29.1 (15.2-39.1)
Cytokines		
S-IL-6, ng/L	4 (3-8) ^a	9 (6-12)
P-IL-8, ng/L	5 (3-9)	9 (5-22)
S-TNFα, ng/L	14 (5-14)	13 (11-16)

Data are expressed as n (%) or median (interquartile range) as appropriate

Data on phagocytosis were available in 23/29 (79%) patients with non-ALD and in 23/31 (74%) patients with ALD cirrhosis (in 6/8 (75%) abstainers and 17/23(74%) current drinkers)

The median alcohol intake per drinking day during the last 30 days prior to study inclusion reported in timeline follow-back method by ALD patients was 12 gr ethanol/day (range 0-96). 7 (30%) ALD patients had ≥ 1 points scored on AUDIT questions 4-6 (range 1-9) (a score >0 in these questions of AUDIT imply the potential presence or incipience of alcohol dependence)

^a $p < 0.05$

ALD, alcoholic liver disease; MELD, model for end-stage liver disease; PMN, polymorphonuclear leukocyte; MFI, median fluorescence intensity; PMA, phorbol-12-myristate-13-acetate; IL, interleukin; TNF, tumor necrosis factor

*Percent of bursting cells

**Percent of cells undergoing phagocytosis

Relation of PMN and monocyte function with pro-inflammatory cytokine levels

The percentage of resting PMNs producing ROS were correlated with IL-6 ($r=0.289$, $p=0.032$) and TNF α levels ($r=0.346$, $p=0.009$). Moreover, impaired phagocytic capacity of PMNs correlated with increased levels of TNF α ($r=-0.333$, $p=0.024$).

The percentage of resting monocytes producing ROS were correlated with IL-6 ($r=0.236$, $p=0.085$) and TNF α levels ($r=0.277$, $p=0.041$). *E.coli* stimulated monocyte burst tended to be related to IL-6 ($r=0.229$, $p=0.089$) and TNF α levels ($r=0.233$, $p=0.081$). Moreover, impaired phagocytic capacity of monocytes tended to correlate with increased levels of TNF α ($r=-0.261$, $p=0.080$).

Correlation between percentage of bursting cells and burst intensity (MFI)

As expected, the percentage of bursting cells correlated positively with burst intensity (MFI). This correlation was observed for both PMNs and monocytes after stimulation with *E.coli* ($r=0.370$, $p=0.004$ and $r=1.000$, $p=0.002$, respectively) and stimulation with PMA ($r=0.679$, $p<0.001$ and $r=1.000$, $p<0.001$, respectively).

Correlation between oxidative burst and phagocytosis

A correlation between resting oxidative burst (MFI) and phagocytic capacity (MFI) for both PMNs ($r=0.392$, $p=0.008$) and monocytes ($r=0.351$, $p=0.019$) was observed. Apart from this, there was no significant correlation between oxidative burst and phagocytosis for PMNs or monocytes ($p>0.05$ for all, data not shown).

Correlation between CRP levels and PMN/monocyte function

Plasma CRP levels were available in 59/60 (98%) patients at inclusion in the study. Plasma CRP levels at inclusion were low (median CRP: 2mg/L, interquartile range: 4.4, range: 0.6-45). No significant correlations between CRP levels and PMN or monocyte function were observed ($r= 0.202-0.961$, $p>0.05$ for all; data not shown).

Relation of PMN and monocyte function with patient outcome

During a median follow-up of 20 months (range 3-21), 15 (25%) patients experienced at least one serious bacterial infection (pneumonia (n=2), urinary tract infection (n=3), spontaneous bacterial peritonitis (n=3), skin/soft-tissue infection (n=3), cholangitis (n=2) and secondary peritonitis (n=2)), 9 (15%) sepsis, 5 (8%) severe sepsis, 7 (12%) ACLF (n=4 infection-related ACLF; n=3 ACLF due to gastrointestinal bleeding) and 8 (13%) died or received a liver transplant. Neither PMN nor monocyte resting burst could predict the occurrence of serious bacterial infection during follow-up in univariate Cox regression analysis ($p>0.05$; data not shown).

PMN function and outcome

In Cox regression analysis, however, *E.coli* stimulated PMN burst (MFI) was related to the occurrence of ACLF and severe sepsis. These associations persisted after adjustment for confounders (HR 1.15, 95% CI 1.04-1.28 and HR 1.12, 95% CI 1.03-1.22, respectively). Similarly, in ROC analysis *E.coli* stimulated PMN burst ≥ 21.5 *E.coli* (MFI) could predict the occurrence of ACLF (area under the ROC (AUROC) curve 0.87, 95% CI 0.72-1.00) and severe sepsis (AUROC 0.89, 95% CI 0.77-1.00) within the first year following inclusion in the study with a sensitivity of 100% and a specificity of 69% (figures 17, 18, 19, 20).

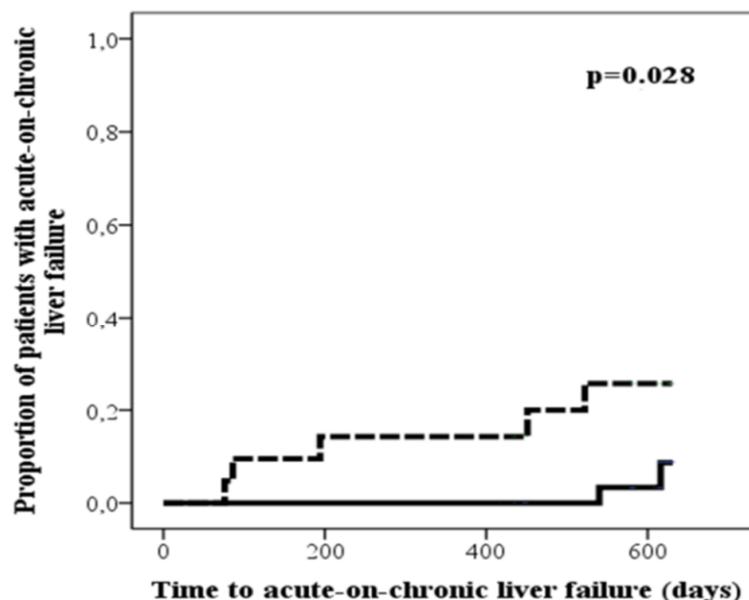


Figure 17. Development of acute-on-chronic liver failure during follow-up in patients with *E.coli* stimulated PMN burst (MFI) ≥ 21.5 (dashed line) or < 21.5 (continuous line) at inclusion in the study.

Patients were censored at death, transplant or end of follow-up
PMN, polymorphonuclear leukocyte; MFI, median fluorescence intensity

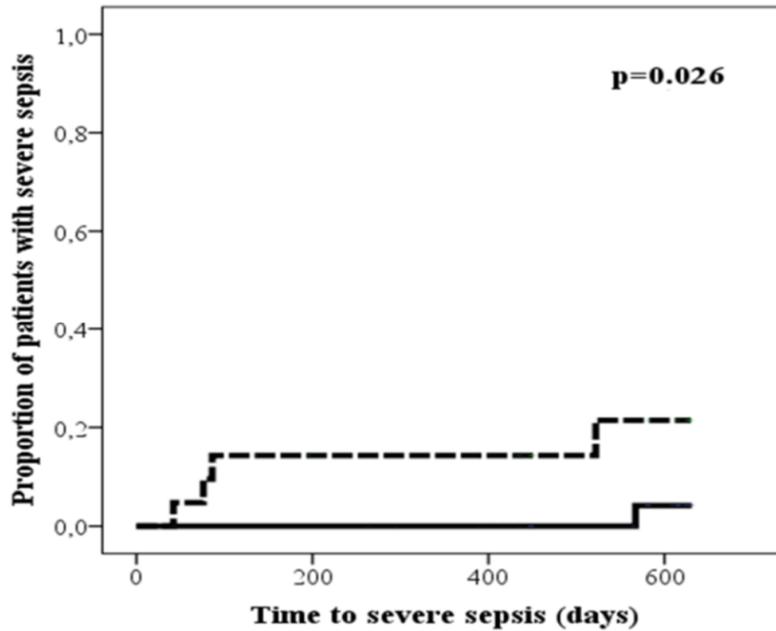


Figure 18. Development of severe sepsis during follow-up in patients with *E.coli* stimulated PMN burst (MFI) ≥ 21.5 (dashed line) or < 21.5 (continuous line) at inclusion in the study.
 Patients were censored at death, transplant or end of follow-up
 PMN, polymorphonuclear leukocyte; MFI, median fluorescence intensity

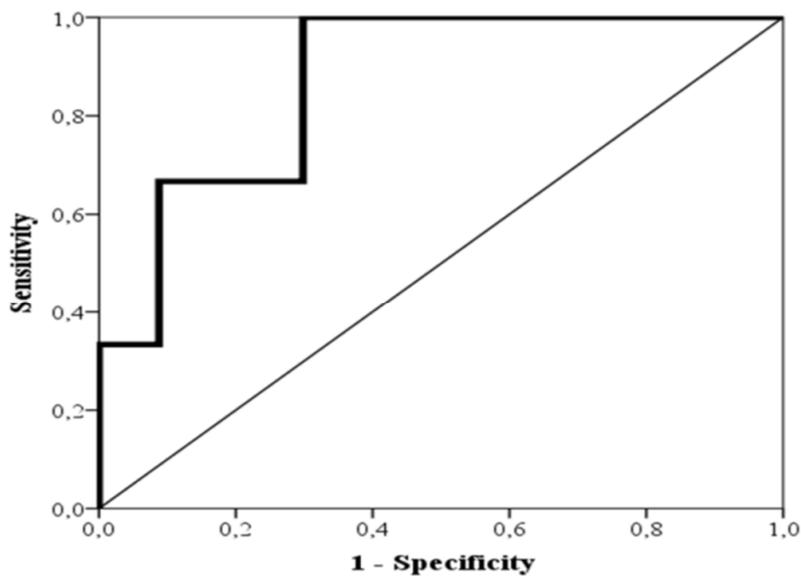


Figure 19. Receiver operating characteristics curves for the utility of *E.coli* stimulated PMN oxidative burst (MFI) in predicting the occurrence of acute-on-chronic liver failure within the first year following inclusion in the study
 PMN, polymorphonuclear leukocytes; MFI, median fluorescence intensity

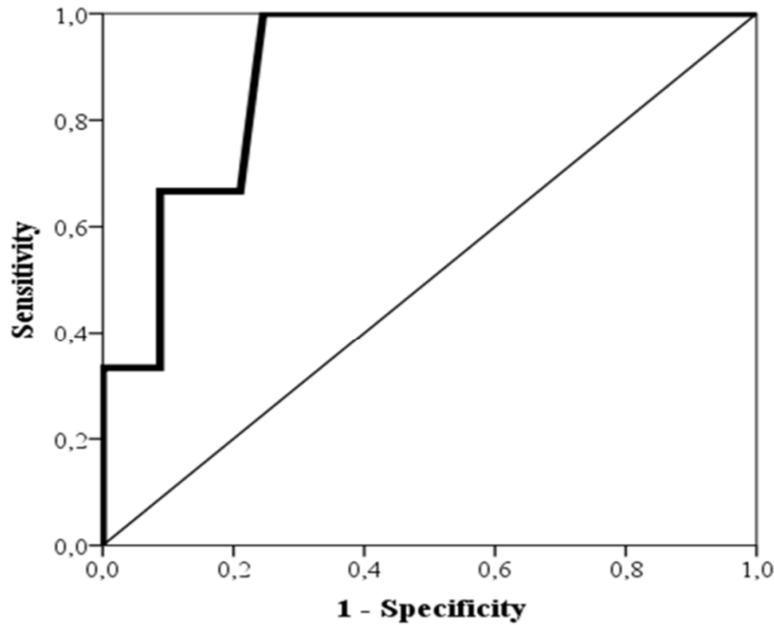


Figure 20. Receiver operating characteristics curves for the utility of *E.coli* stimulated PMN oxidative burst (MFI) in predicting the occurrence of severe sepsis within the first year following inclusion in the study
 PMN, polymorphonuclear leukocytes; MFI, median fluorescence intensity

Monocyte function and outcome

In adjusted Cox regression analysis, *E.coli* stimulated monocyte burst (MFI) was found to be related to the occurrence of sepsis (HR 1.23, 95% CI 1.04-1.45), severe sepsis (HR 1.32, 95% CI 1.08-1.62), and ACLF during follow-up (HR 1.49, 95% CI 1.16-1.89). Similarly, increasing *E.coli* stimulated monocyte burst and lower monocyte phagocytic activity were related to worse transplant-free survival even after adjustment for confounders (HR 1.35, 95% CI 1.08-1.68 and HR 0.92, 95% CI 0.84-0.99, respectively). In ROC analysis, *E.coli* stimulated monocyte burst (MFI) could predict the occurrence of severe sepsis (AUROC 0.89, 95% CI 0.76-1.00), ACLF (AUROC 0.88, 95% CI 0.74-1.00) and transplant or death (AUROC 0.88, 95% CI 0.74-1.00) within a year following inclusion in the study (figures 21, 22, 23).

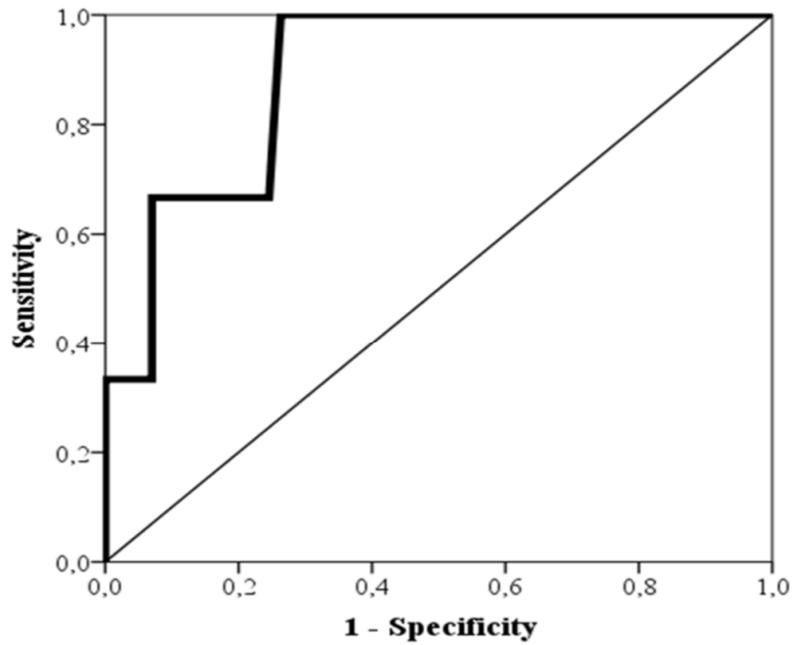


Figure 21. Receiver operating characteristics curves for the utility of *E.coli* stimulated monocyte oxidative burst (MFI) in predicting the occurrence of severe sepsis within the first year following inclusion in the study
MFI, median fluorescence intensity

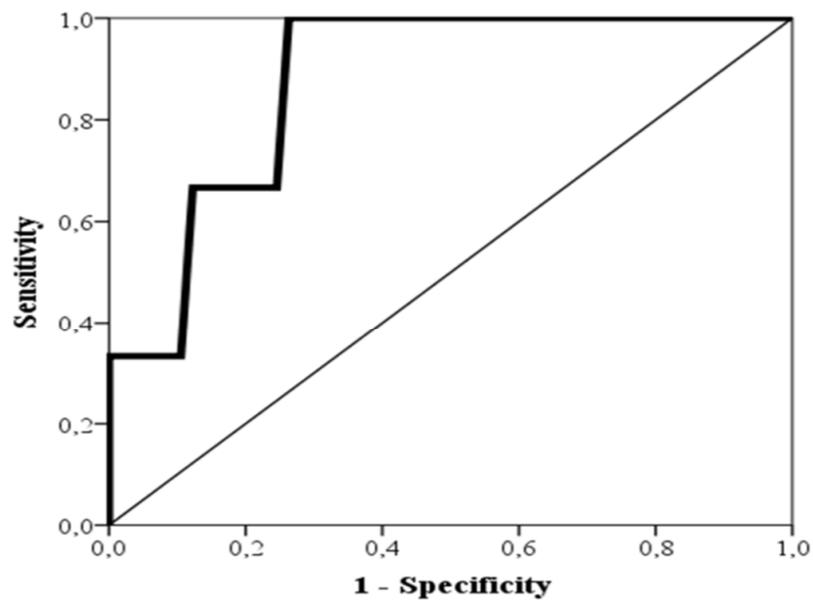


Figure 22. Receiver operating characteristics curves for the utility of *E.coli* stimulated monocyte oxidative burst (MFI) in predicting the occurrence of acute-on-chronic liver within the first year following inclusion in the study
MFI, median fluorescence intensity

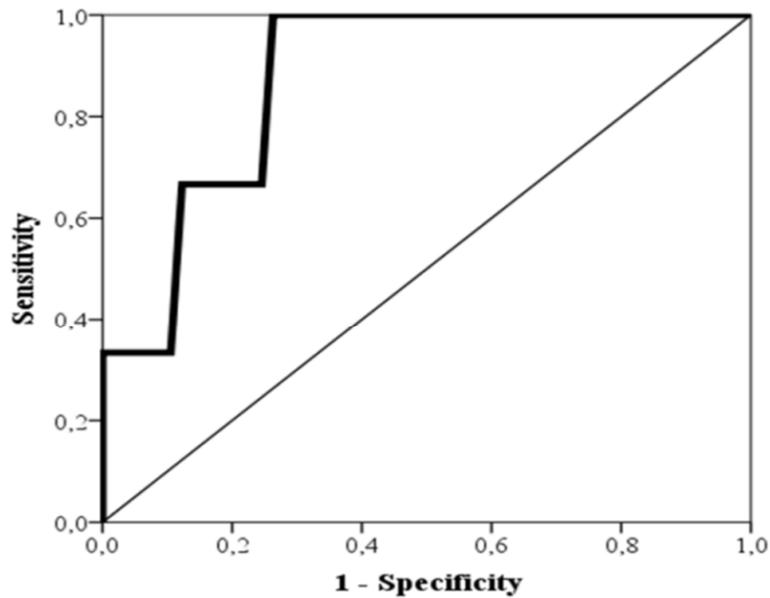


Figure 23. Receiver operating characteristics curves for the utility of *E.coli* stimulated monocyte oxidative burst (MFI) in predicting the occurrence of death or transplant within the first year following inclusion in the study

MFI, median fluorescence intensity

A cut-off of ≥ 5.58 in *E.coli* stimulated monocyte burst (MFI) demonstrated the best sensitivity 100% and specificity 74% to predict 1-year mortality or transplant and the occurrence of ACLF. However, in survival analysis (extending over one year) the differences between groups did not reach statistical significance (figures 24, 25). The same cut-off was used to predict the occurrence of severe sepsis (sensitivity 100% and specificity 74%) within a year (figure 26).

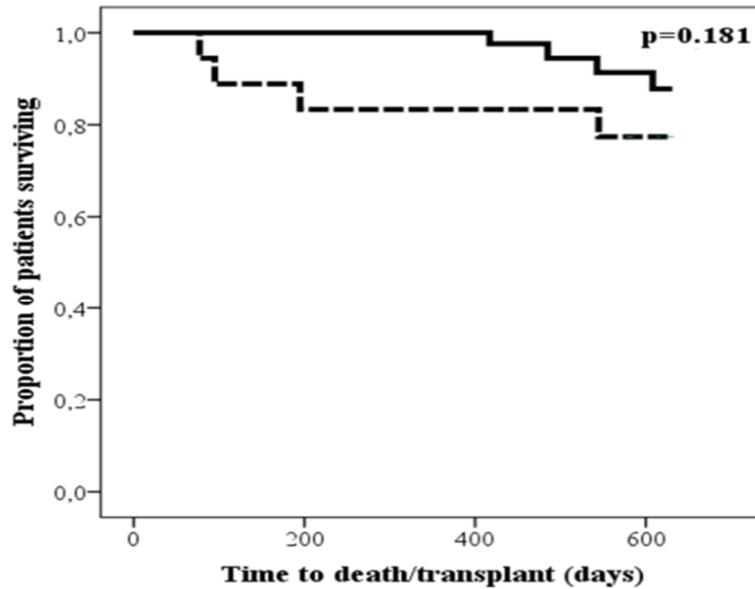


Figure 24. Transplant-free survival during follow-up in patients with *E.coli* stimulated monocyte burst (MFI) ≥ 5.58 (dashed line) vs. < 5.58 (continuous line) at inclusion in the study. Death or transplant was considered event and patients were censored at the end of follow-up. MFI, median fluorescence intensity

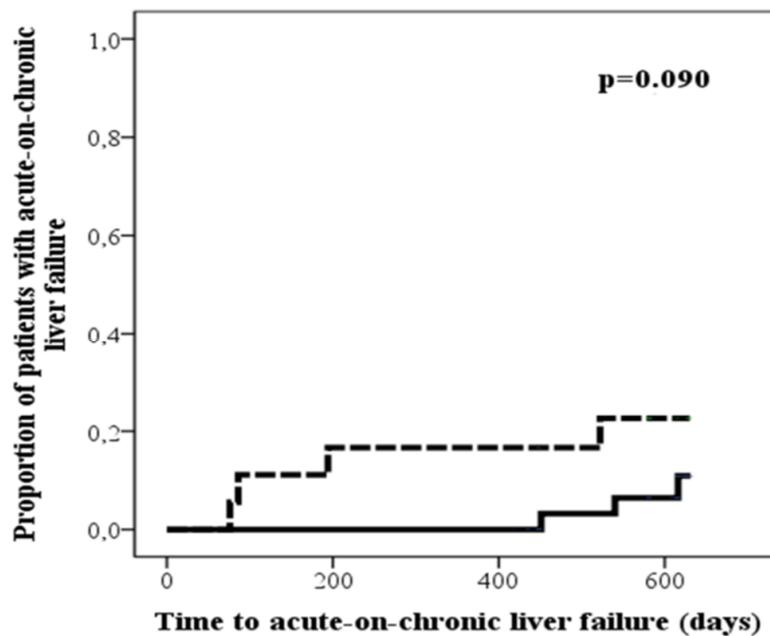


Figure 25. Development of acute-on-chronic liver failure during follow-up in patients with *E.coli* stimulated monocyte burst (MFI) ≥ 5.58 (dashed line) vs. < 5.58 (continuous line) at inclusion in the study. Patients were censored at death, transplant or end of follow-up. MFI, median fluorescence intensity

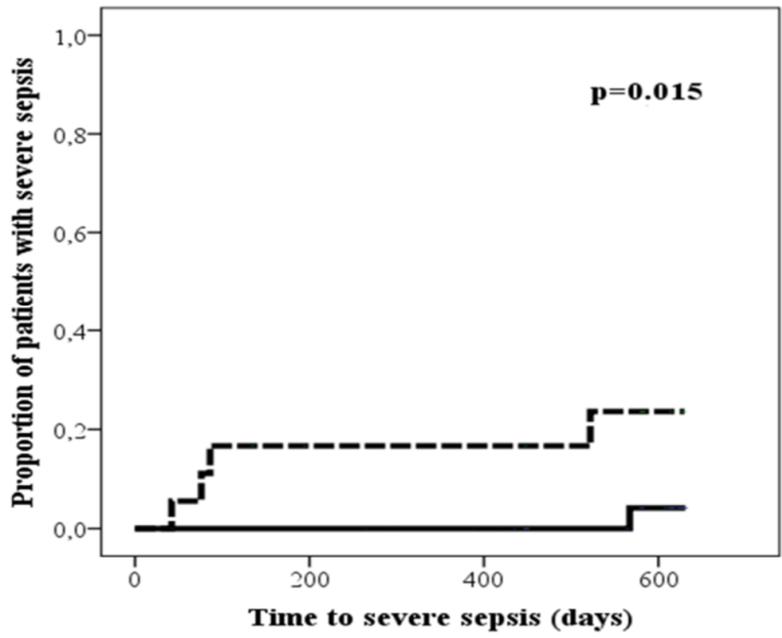


Figure 26. Development of severe sepsis during follow-up in patients with *E.coli* stimulated monocyte burst (MFI) ≥ 5.58 (dashed line) vs. < 5.58 (continuous line) at inclusion in the study.
 Patients were censored at death, transplant or end of follow-up
 MFI, median fluorescence intensity

Discussion

The impact of bacterial infection in compensated and decompensated liver cirrhosis (II)

Interestingly, no significant impact of bacterial infections, regardless of severity, was found on survival of patients diagnosed with compensated cirrhosis. It seems that it is rather the severity of liver disease at cirrhosis diagnosis and the occurrence of decompensation that have major prognostic importance in this group of patients. A possible explanation for this finding could be provided by recently published data showing a more severe functional failure of neutrophils in patients with advanced cirrhosis [85, 86]. On the other hand, the occurrence of bacterial infections was high and impaired 5-year survival in a recent prospective study on initially compensated patients with biopsy-proven viral cirrhosis [111]. The discrepancy between the findings of this study and those presented in paper II could possibly be explained by differences between the two cohorts used. Specifically paper II presents a population-based study of cirrhosis in a well-defined geographical area, while in the aforementioned study viral cirrhosis patients from various French institutions who had undergone liver biopsy and who, in their vast majority, underwent antiviral therapy, were included. Up to 45% of patients with compensated cirrhosis due to hepatitis C may not be candidates for therapy [112]. The findings of paper II suggest that although bacterial infections frequently develop in patients diagnosed with compensated cirrhosis they seem to independently affect survival predominantly in those diagnosed with decompensated disease in keeping with previous studies [1-3, 5, 13, 71, 113]. Further studies investigating the burden of bacterial infections in compensated cirrhosis are warranted.

In patients with decompensated cirrhosis, infection-related ACLF, rather than sepsis *per se* as previously suggested [71], was found to be a major predictor of survival. This finding indicates that the occurrence of organ failures secondary to infection alters the natural history of decompensated cirrhosis and is in keeping with previously published data on ACLF showing that nearly all patients die within three years following ACLF occurrence [75]. The prognostic impact of infection-related ACLF in paper II was dramatic during the first three months following the infection episode, in which the majority of deaths or transplants

occurred. This is also in accordance with previous observations in the cohort in which the definition of ACLF used in paper II was developed [72]. However, as various definitions of ACLF exist [72, 79, 110] further comparative studies are warranted in order to explore the usefulness of different definitions of ACLF in the prediction of outcome of cirrhotic patients with bacterial infections.

Time trends in occurrence of bacterial infection in cirrhosis (III)

The findings showing increasing occurrence of bacterial infections in patients with cirrhosis in later years are consistent with those of a recent study from the US on hospitalized cirrhotics [7]. One explanation for this may be the observed rising frequency of HCA/HA infections in cirrhotic patients. An increase in this type of infections, possibly as a result of frequent performance of invasive procedures, has also been suggested in previous reports [5, 11]. The results of paper III further indicate that this increase is more evident in ALD compared to non-ALD cirrhosis. In contrast with published data from the US [7], we found an increase in infection-related in-hospital mortality in patients diagnosed with cirrhosis during the later years, particularly in patients with ALD-cirrhosis. In turn, this could be related to the increasing rates of sepsis, severe sepsis and, particularly, infection-related ACLF, which is known to be associated with high mortality rates [72]. The increasing rates of these infection severity features could possibly also be attributed to the increasing rates of HCA/HA infections, which have been shown to be associated with adverse patient outcomes [5, 11]. The relation between HCA and HA infections and infection-related ACLF in particular, has been shown in paper II, as well as in a recent study by NACSELD consortium [80]. Interestingly, this increase in infection morbidity and mortality occurred in our cohort despite a trend for decreasing disease severity at cirrhosis diagnosis during the study period.

The impact of alcoholic etiology of cirrhosis on the occurrence, resistance patterns and outcome of bacterial infections (III)

Alcohol-induced alterations in both innate and adaptive immune cell functions result in impaired host defense to invading pathogens [40]. Furthermore, acquired C3 deficiency, impaired function of macrophage receptors and neutrophil dysfunction have been shown to predispose ALD cirrhotics to infections [85, 91,

114]. Alcohol consumption was shown to be a significant risk factor for bacterial infection in a recent study which, however, included only cirrhotic patients with acute variceal bleeding [115]. In paper III, although serious infections were experienced more frequently by ALD compared to non-ALD patients, no independent relation between cirrhosis etiology and occurrence of bacterial infections was found following adjustment for cirrhosis severity. These findings indicate that susceptibility to bacterial infections is associated more with liver disease severity than etiology, in keeping with previously published data [2, 5, 27, 47, 48]. They are also in accordance with the data presented in paper IV as well as those from a recent study showing similar function of neutrophils in ALD and non-ALD cirrhotic patients [89].

The finding that ALD cirrhosis is an independent predictor of pneumonia is novel and in line with data on the impact of alcohol on pulmonary immunity and its role in predisposing non-cirrhotic individuals to bacterial pulmonary infections [116]. These results suggest that physicians should maintain a high level of clinical suspicion of pneumonia in hospitalized cirrhotics with ALD and they underline the need for vaccination of these patients against pneumococcus. However, data on smoking history of the patients in our cohort were not available. There is a high rate of co-occurrence between smoking and alcohol use [117] and smoking is a well-recognized risk factor for the development of pneumonia [118]. Thus, it is conceivable that this may have confounded our findings.

Previous studies in non-cirrhotic individuals with pneumonia have reported a more severe course in patients abusing alcohol [116]. Although two studies in cirrhosis have shown poor outcome in patients with ALD and bacteremia [44] or meningitis [45], most existing data show no difference with regard to infection-related morbidity and mortality between infected patients with ALD and non-ALD cirrhosis [10, 42, 43, 119, 120]. Similarly, no relation between bacterial infection severity features and cirrhosis etiology was found in paper III. Nevertheless, length of hospital stay was more often prolonged in bacterial infections occurring in patients with ALD cirrhosis, which indicates that bacterial infections in these patients may cause increased utilization of hospital resources [7].

Interestingly, infections caused by gram positive bacteria were more common in patients with ALD cirrhosis, which can probably be explained by the higher occurrence of pneumonia and enterococcal infections in these patients. In turn, the more frequent occurrence of enterococcus in culture-positive infections in ALD cirrhosis could explain the higher rates of bacterial resistance to commonly used antibiotics in these infections. Previous studies have established an association between alcoholism and bacterial resistance to antibiotics in non-cirrhotic individuals [49, 50] and alcohol misuse has been defined as a risk factor for drug-resistant *Streptococcus pneumoniae* infection [51]. Previous data deriving from small cirrhotic cohorts have reported higher rates of resistant bacteria and *Enterococcus* in spontaneous bacterial peritonitis occurring in ALD compared to

non-ALD patients [121, 122]. Taken together, the results of paper III and previous findings suggest the need for a heightened suspicion of gram positive and potentially resistant organisms in patients with ALD cirrhosis hospitalized with bacterial infections.

The role of HCA and HA bacterial infections in liver cirrhosis (I)

The observed relation between PPI use and the occurrence of HCA or HA infections in cirrhosis in paper I, is novel. Several studies have reported an increased risk of bacterial infections, in particular gastroenteritis and pneumonia, in non-cirrhotic PPI users [59, 60, 123]. Also, the risk of SBP and *Clostridium difficile* disease is reported to be increased in cirrhotic patients using PPIs [53, 56, 57, 124]. PPI treatment facilitates intestinal bacterial proliferation by acid suppression and may impair gastrointestinal motility, predisposing to bacterial translocation, and neutrophil function by decreasing the production of reactive oxygen species [125-127]. In paper I, the association between PPI use and the occurrence of serious HCA and HA infections could not be explained by concomitant immunosuppressive drug therapy, comorbid conditions, liver disease severity, or older age. The finding that more intense PPI treatment was associated with an increased risk for serious HCA and HA infection in cirrhosis, is in line with that of a study in non-cirrhotic patients with HA *Clostridium difficile* disease showing a dose-response relationship between PPI use and infection [128]. In a recent prospective study including cirrhotic patients who survived an initial infection, the risk of subsequent infections within 6 months was increased in older patients, as well as in those taking PPIs or SBP prophylaxis [129]. It is unclear what the proportions of the different acquisition types of the index infections in this cohort were, as these were not presented in the article. In any case, subsequent infections occurring within 6 months following discharge after the index hospitalization (ie, all infections registered during follow-up evaluation in this study [129]), would be considered HCA (or HA if they occurred >48 h after hospital admission [5]), and thus these recent data, which suggest a relationship between PPI use and subsequent HCA/HA infections, seem to be consistent with the findings of paper I [130]. PPIs are vastly overprescribed in cirrhosis, with an adequate indication for PPI use being present in fewer than half of cirrhotic PPI users [52, 53]. Thus, clinicians should routinely review the indications of PPI treatment in patients with cirrhosis under their care, as PPI discontinuation, if appropriate, might potentially improve the infection risk profile of these patients.

Patients with serious HCA and HA infections had an increased risk for in-hospital mortality, in line with previous studies [5, 8, 10, 11]. Although infection acquisition type *per se* did not have an impact on infection-related mortality in multivariate analysis, it was found to be independently related to infection-related ACLF which, in turn, is known to be a predictor of mortality [72, 73]. These data are in line with the results of NACSELD study, demonstrating an independent relation between infection-related ACLF and HA infections [110]. A possible explanation for this observation could be that patients developing HCA or HA infections have more severe liver disease, with an excessive imbalanced cytokine response leading to sepsis-related organ failures [1]. Thus, not surprisingly, our patients with HCA or HA infections also had a higher frequency of severe sepsis and AKI compared to those with CA infections.

Another potential explanation of the impact of HCA and HA infections on patient outcome is that they are more often caused by bacteria resistant to commonly used antibiotics [5, 11]. Severe sepsis rates were significantly higher in HCA/HA antibiotic-resistant infections in paper I indicating a more severe clinical course. Studies from Italy and Spain have recently reported a high prevalence of antibiotic-resistant bacteria in cirrhosis, especially in infections acquired in the healthcare environment: 23-35% in HA and 14-41% in HCA infections [5, 11]. Sweden is a country with sustained low antibiotic use and bacterial resistance [131-133]. Despite this, 17% of HA infections were caused by antibiotic-resistant bacteria, a proportion lower than previously reported in studies from Southern Europe [5, 11] but undoubtedly worrying. Nearly half of culture-positive HA infections were caused by strains resistant to third-generation cephalosporins which is half to that described in a recent study from Spain [11] but similar to data from Denmark [134], a country with low rates of bacterial resistance like Sweden [135]. Resistance rates to quinolones in HA infections in paper I were as high as those to third-generation cephalosporins, and similar to resistance rates reported for HCA/HA SBP in Korea, Germany, or Spain [9, 12, 81]. Resistance to third-generation cephalosporins and to piperacillin-tazobactam was more frequent in subsequent serious HCA/HA infections following a first HCA/HA infection episode, suggesting that bacterial resistance may develop as patients are increasingly exposed to invasive procedures and to various antibiotic treatments. These findings indicate that neither quinolones nor cephalosporins should be considered clinically effective in infections of nosocomial origin, particularly in those occurring in patients with previous HCA or HA infections, and also that piperacillin-tazobactam could be an alternative as empiric therapy.

Infection-related ACLF in liver cirrhosis (II)

Infection-related ACLF defined according to the criteria established by the CANONIC study [72] was frequent complicating about a quarter of serious bacterial infections. Only 3% of all serious infections were complicated by ACLF according to the definition derived from NACSELD study [80], in our cohort. This reflects probably differences among the two patient-cohorts with regard to baseline patient characteristics and patient selection as well as potential differences in management of cirrhotic patients between the two studies (for example regarding the decision to initiate dialysis or not). To our knowledge, paper II presents the first population-based study assessing the occurrence of infection-related ACLF in a cohort of patients with incident cirrhosis.

The findings showing that liver disease severity and HCA/HA infections predict ACLF development are in line with those from the recent study from NACSELD consortium [110]. The observation that mixed infections (i.e. infections at different sites diagnosed simultaneously) are related to infection-related ACLF development is novel. A possible explanation for this could be that patients developing mixed infections had more severe immunological disturbances leading to both increased infection susceptibility and more pronounced inflammatory response with more severe tissue injury [136].

Inpatient mortality in infection-related ACLF was about 50%, in line with previous studies on patients with ACLF due to various causes (34-65%) [72-76, 110]. Also, these findings are in keeping with published data from unselected cirrhotic cohorts on the prognostic importance of extrahepatic organ failures upon acute decompensation both long-term [75, 137] and short-term [72, 74, 110]. Our finding that multiple extrahepatic organ failures are related to mortality are also in accordance with recently published data from the NACSELD consortium showing that hospitalized infected patients with cirrhosis with >2 extrahepatic organ failures are at higher risk of delisting or death before liver transplantation [138]. Significant comorbid illness was related to increased mortality in serious infections with ACLF, which is also in accordance with previous reports demonstrating increased overall mortality in cirrhotics with multiple chronic diseases [139]. At least 40% of cirrhotic patients have comorbidities that increase mortality [140]. Our findings suggest that cirrhotic patients with comorbidities and bacterial infections should be treated rigorously as risk for poor outcome is high in this group.

PMN and monocyte functional impairment in ambulatory cirrhotic patients and their impact on outcome (IV)

Resting and stimulated oxidative burst of circulating PMNs has previously been shown to be increased in hospitalized cirrhotics with complications of cirrhosis [85, 141]. In ambulatory cirrhotic patients without acute decompensation, however, published data are not unanimous with some studies reporting normal [90], and others increased PMN oxidative burst [89]. In our cohort of ambulatory cirrhotics without evidence of acute decompensation, both resting and *E.coli* stimulated oxidative burst of PMNs were increased compared with healthy controls. Similarly, circulating monocytes showed increased *E.coli* stimulated oxidative burst in line with a previous study on hospitalized cirrhotics [141]. Phagocytic function was impaired both in PMNs and monocytes, but this reached statistical significance only in the former. Although PMN phagocytosis has been reported to be normal or even increased in some studies [90, 142, 143], our findings are in keeping with two recent reports in which PMNs showed impaired phagocytosis [86, 89]. Differences among published studies regarding PMN phagocytosis in cirrhosis may be attributed to the complexity of neutrophil function that may be influenced by several factors (including plasma complement, immunoglobulin, endotoxin, sodium and ammonia concentration, the presence of hepatic encephalopathy, endotoxin levels) [144], and warrants further investigation.

A higher resting and PMA stimulated monocyte oxidative burst were observed in patients with compared to those without prior or current decompensation, which is a novel finding. Also, phagocytic capacity of PMNs was lower in patients with more severe liver disease which, although it did not reach statistical significance, is in keeping with previously reported data [84, 86, 87, 89]. The lack of statistical significance of the latter finding could, possibly, be explained by the fact that patients in our cohort had predominantly Child-Pugh A cirrhosis and low MELD scores, compared to the wider distribution of patients across the cirrhosis severity spectrum in previous reports [86, 89]. The finding that both increased resting oxidative burst and impaired phagocytic capacity of PMNs correlated to increasing pro-inflammatory cytokine levels is in accordance with previous observations [89]. However, the observed correlations between monocyte function and pro-inflammatory cytokine levels in paper IV were weak and it remains unclear to which extent monocyte functional impairment in cirrhosis may be linked to inflammation (which is a characteristic of cirrhosis). Recently, inflammation in cirrhosis has been shown to be associated with higher PMN expression of toll-like receptors, due to binding of ligands, such as endotoxin or

other pathogens, present in cirrhotic plasma, which in turn enhances the production of pro-inflammatory cytokines [85, 89]. Further studies are warranted to fully delineate the pathophysiology of monocyte impairment in cirrhosis.

Chronic alcohol intake may have an impact on PMN [145] and monocyte function [146] in non-cirrhotic individuals. The prevalence of endotoxemia has been shown to be higher in alcoholic cirrhotics when compared to non-alcoholics [42]. However, circulating PMN function has been reported to be similar in ALD and non-ALD cirrhosis in a recent report [89]. Similarly, we did not find any impact of cirrhosis etiology (ALD vs. non-ALD) on circulating PMN or monocyte function. This finding is in line with the results of paper III showing no independent relation between alcoholic etiology of cirrhosis and occurrence of bacterial infection. However, the study presented in paper IV was not designed to address any potential relation between cirrhosis etiology and PMN or monocyte dysfunction. In addition, the ALD and non-ALD groups were not matched. Finally, the heterogeneity of patients with non-ALD cirrhosis may have also impacted our findings.

Defects in PMN function have been shown to be associated with a significantly greater risk of infection and mortality in hospitalized cirrhotics with alcoholic hepatitis [85]. Also, increased resting oxidative burst has been reported to be related to worse mortality in cirrhosis in a previous study including both stable cirrhotics and patients with ACLF [86]. The data presented in paper IV extend these findings to only ambulatory cirrhotics without acute decompensation by showing that stimulated PMN oxidative burst predicts the occurrence of severe sepsis and ACLF. The products of oxidative burst, though effective in first line defense against infection, may lead to bystander tissue damage and inflammation. Excessive activation of neutrophils has been shown to play a key role in the pathogenesis of organ failure in severe sepsis in non-cirrhotic patients [147]. We hypothesize therefore that increased circulating ROS in cirrhosis may lead to considerable damage to the endothelium which, in turn, may be responsible for secondary organ impairment. Furthermore, impaired monocyte function has been shown to be associated with poor clinical outcome and may account for the predisposition to infectious complications in both acute liver failure [148] and ACLF [93, 149]. Our findings that increased stimulated oxidative burst and reduced phagocytosis of monocytes are predictors of severe sepsis, ACLF, and mortality indicate a potential role for monocyte function as a prognostic biomarker in ambulatory cirrhotic patients without acute decompensation. To our knowledge, paper IV presents the first study assessing the impact of monocyte dysfunction on patient outcome in this patient group.

Limitations

Papers I-III

Certain limitations should be considered when interpreting the findings presented in papers I-III. First, these studies are retrospective in nature.

Thus, we cannot exclude a potential misclassification of patients into compensated or decompensated stage since performance of diagnostic tests, such as transabdominal ultrasound and endoscopy, was not standardized, potentially leading to variation in the timing of the diagnosis of varices and ascites. Although the proportion of patients with ALD cirrhosis in our cohort is similar to that in previous studies from Sweden [150, 151], it is conceivable that some patients may have concealed their alcohol consumption leading to misclassification. Similarly, patients considered to be abstinent following cirrhosis diagnosis, may have had ongoing alcohol consumption, not captured by laboratory tests performed at the discretion of the caring physician or at other healthcare episodes during follow-up.

Also, infection diagnosis may have been influenced by clinician practice and thus in some cases it may have been delayed, potentially affecting our findings. SIRS is a hallmark of advanced cirrhosis and difficulties in interpretation of its components may have influenced the occurrence of sepsis in the current study. Nevertheless, the same definition of sepsis has been used as in previous large studies in this field [152-154].

Although the occurrence of AKI in papers I-III was similar to or higher than that reported by prospective studies in cirrhotic patients with bacterial infections [10, 47], the incidence of AKI may have been underestimated since serum creatinine was not measured at least every 48h in all patients. For similar reasons, the diagnosis of infection-related ACLF (although it was the same as that in CANONIC study [72]) may have been underestimated.

Furthermore, specimens were sent for culture according to the clinical judgment of the team caring for the patients enrolled. Although the proportion of culture-positive infections (approximately 66%) was not lower than in previous prospective studies (48-69%) [5, 11, 83], it cannot be excluded that the occurrence of antibiotic resistance may have been under- or over-estimated in papers I and III.

Also, the results of papers I-III apply only to serious bacterial infections occurring in cirrhotic patients, i.e. those requiring hospital care or leading to hospitalization. Infections not requiring hospitalization were not included in these studies (as in essentially all previous reports in the field [3, 5, 10, 11, 83, 107, 110]), which may have biased the results of paper I underestimating the potential role of PPIs in CA infections and/or infections not requiring hospital care. Furthermore, in the same paper data on the exact duration of PPI therapy could not be extracted in a reliable and consistent manner from medical records.

Finally, papers I-III covered a period of 10 years. Although this made conclusions on long-term prognosis possible, it is conceivable that treatment practices may have changed during this period of time, which may have influenced patient outcome and, consequently, our findings. In these papers, Charlson comorbidity index was measured as a measure of the burden of comorbid illness [108]. In 2014, after studies presented in papers I-III were conducted, a new comorbidity index was developed by Jepsen et al specifically for patients with liver cirrhosis [140]. Although the Charlson comorbidity index has been widely used in general [155-157] and in cirrhosis in particular [139, 158-161], we cannot exclude that our results could have been different if this cirrhosis-specific comorbidity index had been used, instead.

Another limitation of the studies presented in papers I-III is that survival analyses (Kaplan Meier) were performed without taking potential competing risks into account. As multistate models are highly relevant for studies of patients with cirrhosis [162], we are aware that this fact could potentially have biased our findings. Survival analyses presented in these papers were repeated as competing risks analyses, considering death as competing risk. The main results, however, remained unchanged (data not shown).

Larger prospective population-based studies including patients from different geographical areas are needed to confirm the findings presented in papers I-III.

Paper IV

The majority of patients included were classified as Child-Pugh A. Although this makes it possible to draw conclusions regarding ambulatory patients without acute decompensation and less severe cirrhosis, it limits the results of paper IV to this group. Also, analyses on phagocytosis were performed in a subgroup of patients (46/60, 76%), which may also have influenced our findings. Pro-inflammatory cytokines were not measured in controls and thus, it may be difficult to interpret the observed correlation between their levels and PMN function. The groups of controls and cirrhotics were not matched for age, sex and bone mass index and thus, we cannot exclude age-, sex- or bone mass index-related differences in impairment of PMN or monocyte function between these groups. However, the findings on impaired phagocytosis and increased oxidative burst of PMNs as well as on the correlation between PMN function and pro-inflammatory cytokine levels in patients with cirrhosis are in keeping with recent reports [86, 89, 141]. Hence larger prospective studies are warranted in order to elucidate the pathophysiology of PMN and monocyte dysfunction in cirrhosis.

Conclusions

On the basis of the results of the four papers in the thesis, the following conclusions can be drawn:

- I. The occurrence of HCA and HA bacterial infections increases during the course of liver cirrhosis. Use of PPIs is a predictor of these infections in a dose-related manner, along with advanced liver disease and previous antibiotic therapy. HCA and HA infections often have a severe clinical course as they are associated to infection-related ACLF. Furthermore, bacteria responsible for HA infections are often resistant to first-line antibiotics even in areas of low prevalence of background bacterial resistance to antibiotics like Sweden, making empiric antibiotic treatment decisions challenging.
- II. Bacterial infections, although frequent during the whole course of liver cirrhosis, do not seem to have any major prognostic impact in patients with compensated disease. Infection-related ACLF has, on the other hand, a significant impact on the course of decompensated cirrhosis. The severity of liver disease along with specific infection characteristics, in particular mixed infection and HCA or HA acquisition types, are major predictors of infection-related ACLF. Moreover, the number of organ failures as well as comorbid illness rather than the severity of liver dysfunction determine short-term outcome in patients with infection-related ACLF.
- III. Bacterial infections are more common in ALD cirrhotics, but this appears to be related to the increased cirrhosis severity in these patients. However, ALD cirrhosis is related to the development of pneumonia as well as infections caused by gram-positive bacteria with more challenging resistance patterns. Although the etiology of cirrhosis *per se* does not appear to have any major impact on infection outcome, infections in ALD cirrhosis may lead to prolonged length of hospital stay, which in turn could lead to increased resource utilization. This is of particular concern in the light of the observed increasing burden of bacterial infections in cirrhosis over time, particularly in ALD patients.
- IV. The function of both PMNs and monocytes is altered in ambulatory cirrhotic patients without acute decompensation. Defects not only in PMN but also in monocyte function may be predictors of the development of severe bacterial infections and of poor prognosis in these patients.

Perspectives

Clinical lessons

It is clear that bacterial infection constitutes a major and frequent complication during the whole course of liver cirrhosis. Despite significant advances in our understanding of the pathogenesis of infection in cirrhosis, the outcome remains poor, especially once ACLF occurs. As bacterial infections can rapidly change a patient's suitability for transplant [138], prevention of infections and subsequent organ failure is critical. Nosocomial infections are associated with the development of ACLF and were recently reported to be associated with three-month readmissions in cirrhotic patients [163]. Up to one-third of these infections should be preventable [68]. The reduction of unnecessary instrumentation and the limited use of antibiotics to data-supported subgroups of cirrhotic patients are key areas.

Although infections will probably continue to occur in patients with cirrhosis, physicians should ensure that these future infections do not become multidrug-resistant infections. The prevention of a substantial proportion of HA infections which are often caused by drug-resistant organisms could help to reach this goal. Causative organisms in nosocomial infections are frequently drug-resistant even in countries with low rates of bacterial resistance, such as Sweden. As a result, empiric antibiotic treatment appropriate for CA infections is often inadequate for HCA and HA infections. Current guidelines of empiric antibiotic therapy in cirrhosis in Sweden are based on older data [164], and should take this feature into account. This is particularly important in light of recent data showing that a broad spectrum antibiotic therapy as empirical treatment in HCA infections reduces in-hospital mortality in cirrhotic patients [165]. Susceptibility of bacteria causing infections in cirrhosis should be periodically tested in each hospital and the empirical antibiotic schedules should be properly adapted. The high rates of pneumonia and gram-positive infections in ALD cirrhosis should also be kept in mind when treating infected alcoholic cirrhotic patients.

An additional way to prevent the development of increasingly resistant HCA/HA infections is to avoid the misuse and overuse of medications such as PPIs in cirrhotic patients. This is of particular importance in the light of recent data showing an independent relation between PPI use and mortality in cirrhosis [166].

As PPIs are frequently prescribed without clear indications in cirrhosis [52, 53], physicians should critically evaluate their use in these patients.

It is known that cirrhosis associated immune dysfunction plays a critical pathogenetic role in several clinical manifestations of cirrhosis, including bacterial infections, hemodynamic derangement, and organ inflammatory damage [27, 30, 39]. Identification and grading of immune dysfunction in cirrhosis has been pursued by measuring the activity of phagocytic, cytotoxic and regulatory immune cells and serum levels of circulating cytokines. As a result, PMN and monocyte function, in particular, have emerged as prognostic tools in liver cirrhosis [85, 86, 89]. This new knowledge could potentially help physicians to early identify early subgroups of patients at high risk for developing severe bacterial infections and organ failures.

Future research

The reported lack of sensitivity and specificity of the conventional criteria for the definition of SIRS makes it difficult to diagnose sepsis in cirrhotic patients [61]. Meanwhile, mortality progressively increases for each hour of delay before antibiotic treatment after onset of hypotension [167]. Clinical trials are needed to identify useful early diagnostic biomarkers of the presence of the infection and its severity in patients with cirrhosis. Elevated serum levels of procalcitonin and C-reactive protein have been shown to correlate with the presence and severity of sepsis in patients with cirrhosis [168]. However, the usefulness of these acute phase proteins in the early diagnosis and definition of infections in these patients has yet to be investigated.

Development of non-antibiotic strategies as a potential alternative to antibiotic prophylaxis in cirrhotic patients could improve patient outcome by reducing the occurrence of nosocomial infections and the rates of bacterial resistance. Rifaximin, a minimally absorbed antibiotic, has emerged as a potential alternative to norfloxacin in the prophylaxis of bacterial infections in cirrhosis, with lower risk for bacterial resistance development [169]. A randomized, controlled study comparing the efficacy and safety of rifaximin vs. norfloxacin in the prevention of SBP in cirrhosis has, however, not been conducted yet. Similarly, it has been suggested that the use of probiotics, prokinetic agents and supplementation with oral bile acids could help reduce the incidence of infections in cirrhosis by affecting BT [170-173]. Further studies comparing these treatments with norfloxacin in prevention of SBP are warranted.

The debate on whether the use of PPIs is related to the development of infections in cirrhotic patients still goes on with the latest data on this issue being conflicting [58, 124, 129, 174-176]. Further large prospective studies are needed to

investigate the specific role of frequently prescribed medications in cirrhotic patients such as PPIs, in development of infections. It has recently been reported that PPIs significantly decrease cellular oxidative burst in cirrhosis [177]. Further prospective studies investigating the potential impact of PPIs on PMN/monocyte function and attempting to provide a pathogenetic explanation to the reported high rates of bacterial infections in this setting are needed.

Although the effects of alcohol on human immunology, including the local immunity of the lung, have been well-studied, there is a paucity of data on the impact of alcoholic etiology of cirrhosis on the occurrence, outcome and microbiology of infections in cirrhotic patients. Further prospective studies are needed to address these questions and to investigate if change of empirical antibiotic treatment is justified in infected alcoholic cirrhotics. Whether the higher prevalence of endotoxemia in ALD cirrhosis [42] results to more impaired PMN/monocyte function and increased susceptibility to infection in alcoholic cirrhotics still remains unclear and needs to be elucidated by further, prospective studies.

Currently, there is no doubt that the presence of extrahepatic organ failures in infected cirrhotic patients is related to adverse outcome [72, 80]. However, reliable diagnostic biomarkers of infection-related ACLF as well as scoring systems to determine which patients would benefit from extracorporeal liver support devices and/or early liver transplantation are lacking. Further prospective studies are needed to address these issues. A universally accepted definition of ACLF in infected cirrhotic patients will help with research and intervention trials and could be possible with extensive, prospectively collected and validated data applicable in geographically diverse populations. Although the clinical benefit of human albumin in the host response against bacterial infections has been largely discussed [178-181], the potential role of human albumin preventing organ failure in cirrhotic patients with infections other than SBP needs to be explored in future studies. The development of tools to identify subgroups of cirrhotic patients at greatest risk for organ failures at an early stage and introduction of effective interventions to prevent this development, would probably contribute the most to the improvement of survival of these patients.

The results of paper IV indicate that elements of PMN and monocyte dysfunction could be used as such tools. Further large prospective studies are warranted to determine whether features of cirrhosis associated immune dysfunction could predict the occurrence of extrahepatic organ failures in these patients and tailor appropriate new therapies. Although there is accumulating evidence supporting PMN and monocyte dysfunction in chronic liver disease, factors that underpin this dysfunction are unclear and probably multiple [144]. Further studies determining these factors could be crucial for the prevention of bacterial infections and the reduction of the infection-related morbidity and mortality in cirrhosis.

Populärvetenskaplig sammanfattning

Bakteriella infektioner är en väldigt vanlig komplikation hos patienter med skrumplever. Upp till 30%-40% av skrumpleverpatienter som vårdas inom slutenvården på sjukhuset har eller utvecklar bakteriell infektion under vårdtiden. Detta resulterar i allvarliga konsekvenser som organsvikt, förlängd vårdtid, ytterligare infektioner och död. Enligt en nyligen genomgång av litteraturen orsakar bakteriella infektioner en fyrfaldig ökning av dödligheten hos dessa patienter. Akut-på-kronisk leversvikt är en prognostiskt ogynnsam komplikation till bakteriella infektioner som karakteriseras av akut försämring av leversjukdomen, svikt av flera organ och ökad dödlighet på kort tid. Det övergripande syftet med de fyra delarbete som ingår i denna avhandling var att undersöka förekomsten och påverkan av bakteriella infektioner hos skrumpleverpatienter.

I delarbete I, II och III inkluderades alla patienter med förstagångs skrumpleverdiagnos under perioden 2001-2010 bosatta i ett geografiskt avgränsat område (Malmös och Lunds upptagningsområden). Patienterna följdes upp till de blev levertransplanterade, tills de avled, eller till slutet av 2011. Alla bakteriella infektioner som föranledde inläggning på sjukhuset eller utvecklades under sjukhusvistelse under studietiden, registrerades genom journalgranskning.

Målsättningen med delarbete I var att beskriva förekomsten av vårdrelaterade bakteriella infektioner hos patienter med skrumplever, faktorer som kan prediktera infektionerna, vilka bakterier som orsakar dem, samt påverkan av dessa infektioner på överlevnad. I detta delarbete har man kunnat bevisa att 1. majoriteten av allvarliga infektioner hos patienter med skrumplever är vårdrelaterade, 2. att vårdrelaterade infektioner är relaterade till användning av syrahämmande läkemedel och antibiotika, 3. att, även i ett område med relativt låg förekomst av antibiotikaresistens som Sverige, är dessa infektioner oftast orsakade av bakterier som är resistenta mot vanliga antibiotika, och 4. att vårdrelaterade infektioner kan leda till akut-på-kronisk leversvikt, vilket, i sin tur, resulterar i ökad dödlighet.

Målsättningen med delarbete II var att undersöka rollen som bakteriella infektioner har i naturalförloppet av skrumplever, förekomsten av akut-på-kronisk leversvikt hos skrumpleverpatienter med bakteriella infektioner, samt att identifiera prediktiva faktorer för ökad dödlighet bland dessa patienter som utvecklade infektionsrelaterad akut-på-kronisk leversvikt. Man har kunnat bevisa

att akut-på-kronisk leversvikt inträffar i c:a en fjärdedel av de bakteriella infektioner som drabbar skrumpleverpatienter, och att det är en viktig prediktiv faktor för dödlighet bland patienter med avancerad skrumplever. Däremot, verkar förekomst av bakteriella infektioner inte ha någon påverkan på överlevnad av patienter med tidig skrumplever innan leverkomplikationer inträffar. Bland patienter som utvecklar akut-på-kronisk leversvikt p.g.a. en bakteriell infektion, löper patienter med komorbiditeter (dvs andra sjukdomar förutom skrumplever) och de hos vilka flera organ sviktar, högst risk att avlida.

Målsättningen med delarbete III var att studera förekomsten och betydelsen av bakteriella infektioner hos patienter med alkoholorsakad respektive icke-alkoholorsakad skrumplever. Trots att alkoholkonsumtion teoretiskt kunde vara förknippad med en högre risk för bakteriella infektioner (p.g.a. de negativa effekter som alkoholkonsumtion har på immunförsvaret), kunde man inte identifiera orsaken till skrumplevern som en oberoende faktor för förekomsten av bakteriella infektioner. Däremot, kunde man visa att alkoholorsakad skrumplever är relaterad till förekomsten av lunginflammation och infektioner orsakade av bakterier som oftast inte svarar på vanliga antibiotika, antibiotika som ofta används i denna patientpopulation. I detta delarbete kunde man också visa att förekomsten av bakteriella infektioner har ökat under de senaste 10 åren hos skrumpleverpatienter, mest p.g.a. en ökning av vårdrelaterade infektioner.

I delarbete IV inkluderades 60 patienter från öppenvården med skrumplever utan andra faktorer som skulle kunna påverka immunsystemet. Inklusion av patienterna skedde på levermottagning under 2013 och patienterna följdes upp till de blev levertransplanterade, tills de avled, eller till slutet av 2014. Målsättningen med delarbete IV var att beskriva funktionen av immunceller samt eventuella samband med utveckling av bakteriella infektioner hos öppenvårdspatienter med skrumplever. Det har tidigare beskrivits att skrumpleverpatienter som vårdas inom slutenvården med akut försämring av leversjukdomen har immunceller som är dysfunktionella. I detta delarbete har man kunnat bevisa att detta även gäller patienter i öppenvården med skrumplever, och att det är flera typer av immunceller som uppvisar funktionsstörningar. Man har också kunnat visa att denna immuncellsdysfunktion kan prediktera förekomst av sepsis, akut-på-kronisk leversvikt och död hos öppenvårdspatienter med skrumplever.

Sammanfattningsvis, belyser denna avhandling att bakteriella infektioner och konsekvenserna av dem spelar en viktig roll i naturalförloppet av avancerad skrumplever. Dessvärre, ökar förekomsten av bakteriella infektioner, särskilt av de vårdrelaterade infektionerna, hos dessa patienter. Det är viktigt att försöka förebygga en del av vårdrelaterade infektioner som drabbar patienter med skrumplever, eftersom det skulle kunna leda till lägre förekomst av antibiotikaresistens och akut-på-kronisk leversvikt. Detta skulle man kunna uppnå genom att undvika att utsätta dessa patienter för onödiga undersökningar och antibiotika kurer. Att använda syrahämmande läkemedel endast när det finns en

verklig indikation skulle också kunna bidra till att minska förekomsten av vårdrelaterade infektioner hos skrumpleverpatienter. Att tidigt kunna identifiera dessa skrumpleverpatienter som löper hög risk för organsvikt i samband med en bakteriell infektion är av stor betydelse för att förbättra överlevnaden hos dessa patienter. Genom att testa funktionen av immunceller kan man eventuellt få viktig information som hjälper uppnå detta mål, men detta får studeras och bekräftas av större studier i framtiden.

Acknowledgments

I would like to express my sincere gratitude to everyone that contributed to this thesis. In particular, I would like to thank:

All patients included in the studies, without whom there had been no studies.

My main supervisor, Evangelos Kalaitzakis, for excellent mentorship through every stage, for his endless support, humor, curiosity and patience in teaching me the ways of scientific research. Thank you for being the supervisor every PhD student wishes for, for believing in me from the very beginning and for becoming a good friend.

Hanne Prytz and Daniel Klintman, my two co-supervisors, for generously sharing their knowledge with me, for critical thinking and valuable comments.

My co-authors Sara Bertilsson, Åsa Johansson, Emma Nilsson and Anna Strand for fruitful collaboration and interesting scientific discussions.

Hans Verbaan and Berit Sternby for their kind and valuable contributions at my mid-seminar.

Johan Rydberg at Department of Microbiology for his kind help with the interpretation of culture results.

All my colleagues and the staff at the Department of Gastroenterology in Skåne University Hospital for a warm and friendly work environment.

All my friends, my parents, and my sister who have supported me throughout.

Jose for being my biggest fan and supporter and for showing me what life is all about.

The studies in the thesis were supported by a grant from Region Skåne.

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Paper I

CIRRHOSIS AND LIVER FAILURE

Healthcare-associated and nosocomial bacterial infections in cirrhosis: predictors and impact on outcome

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Keywords

acute-on-chronic liver failure – bacterial infections – bacterial resistance – cirrhosis – healthcare-associated infection – hospital-acquired infection – outcome – proton pump inhibitors

Abbreviations

ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; CA, community-acquired; CI, confidence interval; DDD, defined daily dose; ESBL, extended-spectrum β -lactamase; HA, hospital-acquired; HCA, healthcare-associated; KM, Kaplan–Meier; MELD, model for end-stage liver disease; MRSA, methicillin-resistant *Staphylococcus aureus*; OR, odds ratio; PPI, proton pump inhibitors; QRGNR, quinolone-resistant Gram-negative rods; SBP, spontaneous bacterial peritonitis; VRE, vancomycin-resistant *Enterococcus*.

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Received 18 April 2014

Accepted 19 June 2014

DOI:10.1111/liv.12625

Liver Int. 2015; 35: 391–400

Healthcare-associated (HCA) and hospital-acquired (HA) infections are of concern in cirrhosis (1–4). Although their incidence is thought to be increasing (1, 5–7), there are no data on their occurrence in longitudinal population-based cirrhotic cohorts. Furthermore, invasive procedures predispose patients to HCA and HA infections (3), but there are few data on other potential predictors of these infection types in cirrhosis.

Proton pump inhibitors (PPIs) are commonly used in these patients, often with unclear or inadequate indications (8, 9). A relation between PPIs and the occurrence of bacterial infections has been reported in

Abstract

Background & Aims: Population-based data on the occurrence of healthcare-associated (HCA) and hospital-acquired (HA) bacterial infections in cirrhosis, their predictors, and their impact on outcome are limited. **Methods:** All patients with incident cirrhosis in 2001–2010 residing in an area of 600 000 inhabitants were retrospectively identified. All serious bacterial infections (resulting in or occurring during an inpatient hospital episode) during this period were registered. Acquisition type, site of infection, occurrence of infection-related acute-on-chronic liver failure (ACLF), acute kidney injury (AKI) and bacterial resistance were analysed. Patients were followed longitudinally until death, transplant or end of 2011. **Results:** A total of 398 serious infections occurred in 241/633 (38%) patients. Forty-seven per cent were HCA and 21% HA. Proton pump inhibitor (PPI) use was more common in HA (80%) vs. HCA (64%) vs. community-acquired (44%) infections ($P < 0.001$). In regression analysis, decompensated status, use of antibiotics and PPIs at infection diagnosis were independent predictors of HCA/HA infections ($P < 0.05$). After adjustment for confounders, HCA/HA infections were significantly related to infection-related ACLF ($P < 0.05$), but not severe sepsis, AKI or infection-related mortality ($P > 0.05$). Antibiotic-resistant infections were more frequent among HA (17%) than HCA (6%) or community-acquired (8%) infections ($P < 0.05$). Antibiotic-resistant HCA/HA infections were independently related to severe sepsis ($P < 0.05$). **Conclusions:** In a population-based cirrhotic cohort, two-thirds of serious bacterial infections were HCA or HA. Decompensated liver disease, antibiotics and PPIs were predictors of serious HCA/HA infections, which were associated with the development of ACLF. Antibiotic resistance was frequent, especially in HA infections, and contributed to risk of severe sepsis.

cirrhosis (9, 10), and HCA and HA bacterial infections in non-cirrhotic individuals are related to antacid administration (11, 12). However, a potential relationship between infection acquisition type and PPI use is unexplored in cirrhosis.

Bacterial infections in cirrhosis may result in acute kidney injury (AKI) and/or acute-on-chronic liver failure (ACLF) leading to poor outcome (4, 13, 14). Although infection acquisition type [community-acquired (CA) vs. HCA/HA] is considered to be related to infection-related morbidity and mortality in cirrhosis (1–4, 7, 15), published data are not unanimous (6, 16,

17) and it remains unclear whether it is associated with the occurrence of AKI or ACLF.

Studies from Southern Europe and the US have shown high occurrence of antibiotic-resistant infections in cirrhotic patients, particularly in those with HCA and HA infections (3, 7, 18). However, there are only few data on the relation of antibiotic-resistant infections with infection acquisition type in cirrhotic cohorts from areas of low prevalence of bacterial resistance.

In a population-based cirrhotic cohort, we aimed to investigate the occurrence of HCA/HA infections and their predictors, and, in particular, whether they are related to PPI use; and the potential association of infection acquisition type with patient outcome and bacterial resistance patterns in an area of low background prevalence of bacterial resistance to antibiotics.

Patients and methods

Patients

All adult patients (≥ 18 years) diagnosed with cirrhosis in the period 2001–2010 at our institution were identified by means of a search of the computerized discharge diagnosis register of the hospital. Cirrhosis diagnosis was based on liver biopsy and/or compatible clinical, laboratory and imaging findings. Only patients with first-time cirrhosis diagnosis during this period were included. Patients with a previous liver transplant and those residing outside the primary catchment area of the hospital were excluded. Patients with chronic liver disease are in effect exclusively diagnosed and followed up at their local public healthcare institutions in Sweden. Thus, all patients with incident cirrhosis in the primary catchment area of our institution (population of 600 000) were included. Patients were followed longitudinally until death, liver transplantation or the end of 2011. The study protocol was approved by the local ethics committee.

Infection diagnosis

The electronic medical records of patients identified in our institution as well as any records these patients had in any hospital in the healthcare region were scrutinized. All serious bacterial infections (i.e. those resulting in or occurring during an inpatient hospital episode) were registered. Spontaneous bacterial peritonitis (SBP) and other infections were defined according to conventional criteria (see supplementary file) (7, 19, 20). Mixed bacterial infections (bacterial infections at different sites diagnosed simultaneously) were considered as a single infection episode. Further infections diagnosed following a first infection during the same hospitalization were considered separate, as they may have a different clinical course (17). HCA, HA and CA infections were defined as previously described (see supplementary file) (11).

Data collection

Medical records are fully computerized in Sweden and data for at least vital signs, medications as well as clinical status were registered on a daily basis for inpatients. It is compulsory for clinicians to register details of all patient contacts in the records. At the time of cirrhosis diagnosis and at diagnosis of each serious infection episode, data were extracted with regard to patient demographics and aetiology, severity and complications of liver disease. At diagnosis of each serious infection episode, medications and comorbid illness were also recorded. The Charlson comorbidity index was calculated as a measure of the burden of comorbid illness (21), excluding liver disease.

PPI use

Data on PPI use and dose at cirrhosis diagnosis and upon each serious infection episode were registered. PPI use was defined as regular use of PPIs prior to cirrhosis and infection diagnosis, respectively, independent of the treatment duration. Commencement of PPIs during the same hospital episode as the infection but prior to infection diagnosis was registered as new PPI therapy. Patients receiving PPIs were classified into those receiving less than, equal to, or more than one defined daily dose (DDD). One DDD was 20 mg for omeprazole, esomeprazole, rabeprazole and pantoprazole and 15 mg for lansoprazole (22).

Patient outcome

The outcomes recorded for each serious infection episode were sepsis, severe sepsis, AKI, ACLF [as defined in the CANONIC study (14)] and in-hospital mortality (see supplementary file for definitions) (23, 24). Infection-related mortality was defined as death that could be attributed, at least partly, to a serious infection. Data on date and cause of death were obtained from medical records and were confirmed through linkage to the Cause of Death Register by means of the unique national registration number assigned to all Swedish residents.

Microbiology

In the case of culture-positive infections, all bacteria and their antibiotic susceptibility patterns were registered. The following bacteria were considered to cause antibiotic-resistant infections: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), ampicillin-resistant *Enterococcus*, extended-spectrum β -lactamase-producing *Enterobacteriaceae* (ESBL), quinolone-resistant Gram-negative rods (QRGNR), or isolates resistant to three or more classes of antibiotics (7, 18).

Statistical analysis

Data were expressed as mean and standard deviation or *n* and percentage as appropriate. When comparing groups, the chi-square test was used for categorical variables and ANOVA for continuous variables. In an attempt to identify independent predictors of HCA/HA infections and to investigate whether infection acquisition type was independently related to infection episode outcome (severe sepsis, infection-related ACLF and infection-related AKI stage 3), all parameters related to the development of HCA/HA infections and infection episode outcome, respectively, with a *P*-value ≤ 0.1 in univariate analysis, were entered into multivariate logistic regression analyses. Predictors of infection-related mortality were assessed with survival analysis (Kaplan–Meier) and groups were compared with log-rank test. Cox regression analysis was used to identify independent predictors of infection-related mortality. All tests were 2-tailed and were conducted at a 5% significance level.

Results

A total of 633 patients with a first-time diagnosis of cirrhosis during the study period were identified and followed under 2276 patient-years (Table 1). Twelve patients were lost to follow-up. During a median follow-up of 36 months (range: 0.1–134 months), 241/633 patients (38%) experienced a total of 398 serious bacterial infection episodes (median 1, range 1–8) (853 patient-years). In all, 174/633 (27%) developed at least one HCA (*n* = 186 infections in 133 patients; median 1, range: 1–5) and/or HA infection (*n* = 83 infections in 61 patients; median 1, range: 1–7).

HCA and HA infection episodes

Most infection episodes were HCA (47%), followed by CA (32%) and HA (21%) (Table 2). The occurrence of HCA/HA serious infections increased with time from cirrhosis diagnosis with 58%, 71%, and 92% of first,

second and third serious infection episodes experienced by the patients in our cohort being HCA or HA infections (*P* < 0.001). Liver impairment was more severe in HCA/HA infections compared with CA (Table 2). HA infections were more frequently related to recent invasive procedures or gastrointestinal bleeding and were less commonly as a result of SBP or mixed infections in comparison to the other groups. Neither patient age or gender, nor cirrhosis aetiology or infection occurrence in other sites differed significantly among the three infection acquisition groups (Table 2).

Medications

Therapy with steroids and antibiotics was more frequent in HCA/HA than CA infections (Table 2). Steroids were given mainly for autoimmune hepatitis (*n* = 7/26) or coexisting medical conditions (*n* = 17/26) and less commonly for alcoholic hepatitis (*n* = 2/26). The most frequent indication for antibiotic therapy at infection diagnosis was another ongoing infection (commonly another serious infection treated prior to the diagnosis of a subsequent HA infection, *n* = 16/49; but also skin/soft-tissue infections diagnosed and treated in an outpatient setting prior to the serious infection considered in the current study, *n* = 12/49) followed by prophylactic therapy (*n* = 21/49) mainly for hepatic encephalopathy or recurrent cholangitis.

Patients with HCA/HA infections used PPIs regularly more often and at a higher daily dose compared with those with CA infections (Table 2). These results did not change after exclusion of patients on steroids (44% vs. 64% vs. 78%, respectively, *P* < 0.001 for PPI use and 25% vs. 36% vs. 41%, respectively, *P* = 0.039 for PPI > 1 DDD). PPI use was still more common in HCA/HA vs. CA infections when compensated (55% vs. 34%, *P* = 0.036) and decompensated patients (73% vs. 51%, *P* = 0.001) were analysed separately. PPI use increased from the first to subsequent serious HCA/HA infections experienced by patients in our cohort (64% vs. 79%, *P* = 0.011), which was also true for their dose (33% vs. 45%, *P* = 0.05 of patients treated with >1 DDD).

Table 1. Patient characteristics at cirrhosis diagnosis (*n* = 633)

Age (y)	59 (12)
Sex (male)	412 (65)
MELD score	10.4 (7)
Decompensated status*	301 (48)
Aetiology	
ALD-cirrhosis (or mixed)	363 (57)
Viral	98 (16)
Cryptogenic/NASH	84 (13)
Cholestatic	39 (6)
Other	49 (8)
Proton pump inhibitors	232 (37)

*Decompensated status was defined as the occurrence of ascites and/or variceal bleeding (25).

Data presented as mean (SD) or *n* (%) as appropriate.

ALD, alcoholic liver disease; NASH, non-alcoholic steatohepatitis.

Predictors of HCA/HA infections

In regression analysis, decompensated status [defined as the occurrence of ascites and/or variceal bleeding according to the Baveno IV consensus conference (25)] (odds ratio (OR) 2.79, 95% confidence interval (CI) 1.69–4.61), use of antibiotics (OR 2.77, 95% CI 1.16–6.64) and PPIs (OR 2.30, 95% CI 1.44–3.68) at infection diagnosis but not infection site, recent invasive procedures, gastrointestinal bleeding or steroid use were independent predictors of HCA/HA infections. PPI use was still predictive of serious HCA/HA infections when analysis was repeated after exclusion of patients with concomitant therapy with steroids (OR 2.17, 95% CI 1.34–3.15) or new PPI users (OR 2.49, 95% CI 1.54–

Table 2. Characteristics of community-acquired, healthcare-associated and hospital-acquired serious bacterial infection episodes ($n = 398$)

	Community- acquired ($n = 129$)	Healthcare-associated ($n = 186$)	Hospital-acquired* ($n = 83$)	<i>P</i> -value
Age (y)	63 (9)	62 (11)	60 (11)	0.616
Sex (male)	81 (63)	130 (70)	55 (66)	0.417
Decompensated status†	74 (57)	146 (78)	67 (81)	<0.001
ALD-cirrhosis	84 (65)	126 (68)	62 (75)	0.333
MELD score at infection diagnosis	15 (8)	17 (7)	18 (9)	0.005
Charlson comorbidity index>1‡	31 (24)	60 (32)	22 (26)	0.082
Gastrointestinal bleeding§	17 (13)	9 (5)	15 (18)	0.002
Invasive procedures ≤24 h before infection diagnosis¶	2 (2)	9 (5)	17 (20)	<0.001
Sites of infection				
Urinary tract infection	25 (19)	30 (16)	21 (25)	0.209
Spontaneous bacterial peritonitis	22 (17)	34 (18)	5 (6)	0.029
Pneumonia	19 (15)	29 (16)	7 (8)	0.272
Skin/soft-tissue infection	20 (15)	22 (12)	9 (11)	0.526
Spontaneous bacteraemia	16 (12)	17 (9)	15 (18)	0.114
Mixed	5 (4)	14 (7)	0 (0)	0.024
Other	22 (17)	40 (21)	26 (31)	0.049
Medications				
Steroids	4 (3)	12 (6)	10 (12)	0.036
Immunosuppressive drugs other than steroids	1 (1)	4 (2)	0 (0)	0.284
Antibiotics	7 (5)	20 (11)	22 (26)	<0.001
PPI user at infection diagnosis**	57 (44)	120 (64)	66 (80)	<0.001
New PPI therapy††	6 (5)	2 (1)	15 (18)	<0.001
>1 DDD of PPIs	32 (25)	66 (35)	35 (42)	0.023

*Twenty-four out of 83 (29%) of HA infections occurred as subsequent (new) infections following a first infection episode during the same hospitalization.

†Decompensated status was defined as the occurrence of ascites and/or variceal bleeding (25).

‡A score of 1 was the median value of Charlson comorbidity index (excluding liver disease).

§Gastrointestinal bleeding occurring during the same hospital episode as the infection.

¶see supplementary file.

**Regular PPI use prior to infection diagnosis.

††Commencement of PPI therapy during the same hospital episode as the infection but prior to infection diagnosis.

Data presented as mean (SD) or n (%) as appropriate.

ALD, alcoholic liver disease; DDD, defined daily dose; PPI, proton pump inhibitors.

4.01). PPI users, in particular those with >1 DDD of PPIs, suffered second serious HCA/HA infections more commonly following a first serious HCA/HA infection episode (Fig. 1A, B). These results did not change when sensitivity analysis was performed excluding new PPI users (Fig. 1, footnote). Small numbers in the new PPI user group (i.e. those who started PPIs during the same hospital episode as the infection but prior to infection diagnosis; Table 2, $n = 17$) did not allow a direct comparison of this group with the non-PPI user group or that of patients who used PPIs prior to hospitalization.

Impact of infection acquisition type on outcome

Serious HCA and HA infection episodes were more frequently complicated by severe sepsis, ACLF, stage 3 AKI and in-hospital mortality (Table 3). After adjustment for confounders by means of logistic regression analysis, HCA/HA infections were found to be independent pre-

dictors of infection-related ACLF (OR 2.78, 95% CI 1.31–5.86), but not severe sepsis (OR 1.39, 95% CI 0.75–2.56) or stage 3 infection-related AKI (OR 1.68, 95% CI 0.63–4.49). Among patients experiencing serious bacterial infections in our cohort, infection-related ACLF developed more commonly among those with HCA and HA infections (Fig. 2A, B). Redefining infection-related ACLF according to a recent prospective study in which only cirrhotic patients with bacterial infections were included (26) yielded very low numbers of infection-related ACLF in our cohort (data not shown) and thus no further analysis was attempted.

Among patients with at least one bacterial infection episode during follow-up, patients who developed HCA/HA infections had significantly higher infection-related mortality [Kaplan–Meier (KM), $P = 0.001$; data not shown]. After adjustment for confounders with Cox regression analysis, HCA/HA infections were not independently related to infection-related mortality ($P > 0.05$).

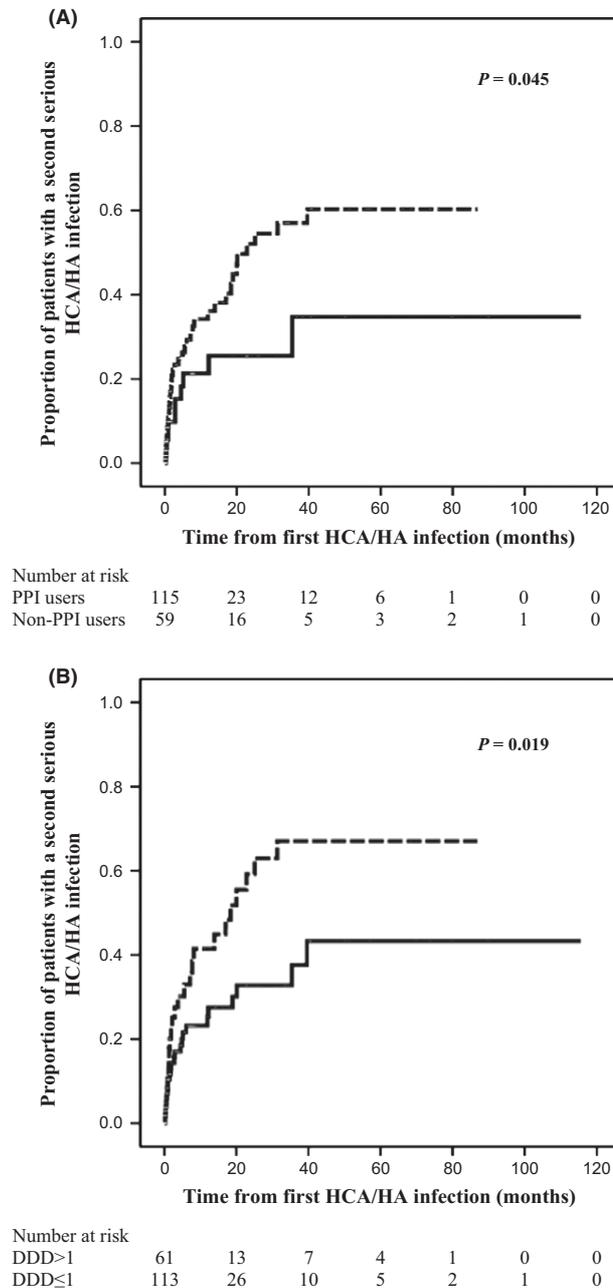


Fig. 1. Relationship between PPI use and serious HCA/HA infection development (A) Proportion of PPI users (dashed line) vs. non-PPI users (continuous line) developing a second serious HCA/HA infection during follow-up following a first serious HCA/HA infection (B) Proportion of PPI users with >1 DDD (dashed line) vs. non-PPI users/PPI users with ≤1 DDD (continuous line) developing a second serious HCA/HA infection during follow-up following a first serious HCA/HA infection. Patients were censored at last follow-up until end of 2011, liver transplantation or death. When new PPI users (those starting PPI therapy during the same hospital episode as the infection prior to infection diagnosis, $n = 14$) were excluded from analysis: $P = 0.015$ (Fig. 1A) and $P = 0.011$ (Fig. 1B). HCA, healthcare-associated; HA, hospital-acquired; DDD, defined daily dose.

Culture-positive bacterial infection episodes

In all, 261/398 of bacterial infections (66% of CA, 66% of HCA and 65% of HA infections, $P = 0.992$) had a

positive bacterial culture. The proportion of infections caused by Gram-positive or Gram-negative bacteria did not differ significantly between the three infection acquisition groups (Table 4). However, resistance to

Table 3. Infection outcome in community-acquired, healthcare-associated and hospital-acquired serious bacterial infections ($n = 398$)

	Community-acquired ($n = 129$)	Healthcare-associated ($n = 186$)	Hospital-acquired ($n = 83$)	<i>P</i> -value
Sepsis	94 (72)	140 (75)	62 (75)	0.889
Severe sepsis	39 (30)	75 (40)	37 (45)	0.072
Infection-related ACLF	19 (15)	51 (27)	25 (30)	0.011
Infection-related AKI stage 1	36 (28)	46 (25)	12 (15)	0.106
Infection-related AKI stage 2	13 (10)	32 (18)	9 (11)	0.141
Infection-related AKI stage 3	10 (8)	22 (12)	20 (26)	0.001
In-hospital mortality	13 (10)	44 (24)	19 (23)	0.007
Length of stay (days)	15 (13)	15 (16)	33 (27)	<0.001

Data presented as mean (SD) or n (%) as appropriate.

ACLF, acute-on-chronic liver failure; AKI, acute kidney injury.

commonly used antibiotics was more common in serious HA vs. HCA vs. CA infections. This was true for third-generation cephalosporins, piperacillin–tazobactam and quinolones, although it did not reach statistical significance in the latter (Table 4). Bacteria causing antibiotic-resistant infections were more frequently isolated in serious HA infections (14/83, 17%), as compared with HCA (12/186, 6%) and CA (10/129, 8%) episodes ($P < 0.05$). In logistic regression analysis, HCA/HA antibiotic-resistant infection was found to be related to the occurrence of severe sepsis (OR 3.58, 95% CI 1.09–11.80), but not AKI, ACLF or in-hospital mortality ($P > 0.05$ for all).

Bacterial resistance patterns following a first serious HCA or HA infection

Subsequent serious HCA/HA infections following a first serious HCA/HA bacterial infection episode were more frequently caused by third-generation cephalosporin-resistant strains (49% vs. 29%, $P = 0.010$), piperacillin–tazobactam-resistant strains (17% vs. 7%, $P = 0.075$), ampicillin-resistant enterococcus (12% vs. 4%, $P = 0.074$) or enterobacteriaceae resistant to at least one β -lactam (11% vs. 3%, $P = 0.04$). However, they did not differ significantly in the occurrence of Gram-positive bacteria (54% vs. 48%, $P = 0.532$) nor were they significantly more often antibiotic-resistant infections (18% vs. 13%, $P = 0.379$).

Discussion

In this 10-year population-based cirrhotic cohort, two-thirds of serious bacterial infection episodes were HCA or HA infections, with 27% of patients experiencing at least one such infection during 2276 patient-years. The likelihood of serious infections being HCA or HA increases with time from cirrhosis diagnosis and use of PPIs. HCA and HA infections have an impact on patient outcome as they are often, particularly in the case of HA infections, caused by bacteria resistant to commonly used antibiotics, but also, as they are associated with infection-related ACLF. Our findings are in keeping with previously published data showing that the majority of serious infections in cirrhosis are HCA or HA (2, 3, 6, 7, 16, 17), frequently associated with previous antibiotic use and antibiotic-resistant bacteria (1–3, 7, 15, 16). To our knowledge, this is the first population-based study on a cohort of incident cases of cirrhosis in whom all serious bacterial infections diagnosed during follow-up are studied longitudinally.

Our observation that PPI use is related to the occurrence of HCA or HA infections in cirrhosis is novel. Several studies have reported an increased risk of bacterial infections, in particular gastroenteritis and pneumonia, in non-cirrhotic PPI users (11, 12, 27). Also, the risk of SBP and *Clostridium difficile* disease is reported to be increased in cirrhotic patients using PPIs (9, 10, 28). PPI treatment facilitates intestinal bacterial proliferation by acid suppression and may impair gastrointestinal motility, predisposing to bacterial translocation, and neutrophil function by decreasing the production of reactive oxygen species (29–31). In the current cohort, the association between PPI use and the occurrence of serious HCA and HA infections could not be explained by concomitant immunosuppressive drug therapy, comorbid conditions, liver disease severity or age. Also, this association persisted when new PPI users were excluded from analysis. Our finding that more intense PPI treatment was associated with an increased risk for serious HCA and HA infection in cirrhosis is in line with those of a study in non-cirrhotic patients with HA *Clostridium difficile* disease showing a dose–response relationship between PPI use and infection (32). PPIs are frequently overprescribed in cirrhosis, with an adequate indication for PPI use being present in fewer than half of cirrhotic PPI users (8, 9). Thus, clinicians should routinely review the indications of PPI treatment in patients with cirrhosis under their care, as PPI discontinuation, if appropriate, might potentially improve the infection risk profile of these patients.

Our patients with serious HCA and HA infections had an increased risk for in-hospital mortality, in line with previous studies (1, 3, 4, 7). Although infection acquisition type *per se* did not have an impact on infection-related mortality in multivariate analysis, it was found to be independently related to infection-related ACLF, which, in turn, is known to be a predictor of

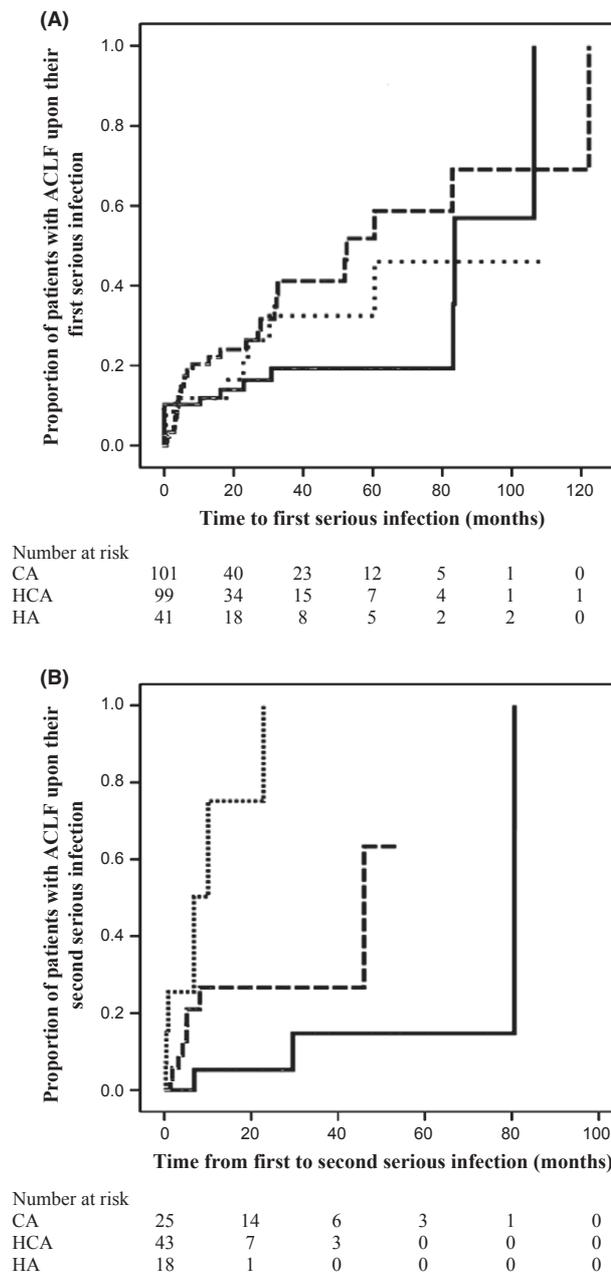


Fig. 2. Relationship between infection-related ACLF and infection acquisition type (A) Proportion of patients with at least one serious infection developing infection-related ACLF upon their first serious bacterial infection (B) Proportion of patients with a second serious infection developing infection-related ACLF upon their second serious bacterial infection. Community-acquired (continuous line), healthcare-associated (dashed line) or hospital-acquired (dotted line). (A) Patients were censored at development of a first serious infection (log-rank test: HCA vs. CA $P = 0.077$; HA vs. CA $P = 0.792$; HCA vs. HA $P = 0.416$). (B) Patients were censored at development of a second serious infection (log-rank test: HCA vs. CA $P = 0.029$; HA vs. CA $P < 0.001$; HCA vs. HA $P = 0.001$). ACLF; acute-on-chronic liver failure.

mortality (13, 14). These data are in line with the results of a recent study, demonstrating an independent relation between infection-related ACLF and HA infections (26). A possible explanation for this observation could be that patients developing HCA or HA infections have more

severe liver disease, with an excessive imbalanced cytokine response leading to sepsis-related organ failures (33). Thus, not surprisingly, our patients with HCA or HA infections also had a higher frequency of severe sepsis and AKI compared with those with CA infections.

Table 4. Microbiological findings in culture-positive serious bacterial infections (*n* = 261)

	Community-acquired (<i>n</i> = 85)	Healthcare-associated (<i>n</i> = 122)	Hospital-acquired (<i>n</i> = 54)	<i>P</i> -value
Gram-negative bacilli	39 (46)	50 (41)	25 (46)	0.745
Enterobacteriaceae resistant to at least one β-lactam	1 (1)	4 (3)	6 (11)	0.014
QRGMR	3 (3)	6 (5)	2 (4)	0.868
Gram-positive cocci	42 (49)	66 (54)	23 (43)	0.393
Ampicillin-resistant Enterococcus	6 (7)	5 (4)	8 (15)	0.041
Gram-negative and Gram-positive strains	4 (5)	6 (5)	6 (11)	0.234
Third-generation cephalosporin-resistant strains	21 (25)	39 (32)	25 (46)	0.034
Piperacillin–tazobactam-resistant strains	8 (9)	7 (6)	12 (23)	0.004
Quinolone-resistant strains	18 (25)	34 (34)	19 (41)	0.183
Bacteria causing antibiotic-resistant infections*	10 (12)	12 (10)	14 (26)	0.014

*see methods for definition.

Data presented as *n* (%).

QRGMR, quinolone-resistant Gram-negative rods.

Another potential explanation of the impact of HCA and HA infections on patient outcome is that they are more often caused by bacteria resistant to commonly used antibiotics (3, 7). Severe sepsis rates were significantly higher in HCA/HA antibiotic-resistant infections in our study indicating a more severe clinical course. Studies from Italy and Spain have recently reported a high prevalence of antibiotic-resistant bacteria in cirrhosis, especially in infections acquired in the healthcare environment: 23–35% in HA and 14–41% in HCA infections (3, 7). Sweden is a country with sustained low antibiotic use and bacterial resistance (34). Despite this, in the current study, 17% of HA infections were caused by antibiotic-resistant bacteria, a proportion lower than previously reported in studies from Southern Europe (3, 7), but undoubtedly high. Nearly half of culture-positive HA infections were caused by strains resistant to third-generation cephalosporins, which is half of that described in a recent study from Spain (7), but similar to data from Denmark (35), a country with low rates of bacterial resistance like Sweden (36). Resistance rates to quinolones in HA infections in the current study were as high as to third-generation cephalosporins, and similar to those reported for HCA/HA SBP in Korea, Germany or Spain (2, 6, 15). Resistance to third-generation cephalosporins and to piperacillin-tazobactam in our cohort was more frequent in subsequent serious HCA/HA infections following a first HCA/HA infection episode, suggesting that bacterial resistance may develop as patients are increasingly exposed to invasive procedures and to various antibiotic treatments. The results of our study indicate that neither quinolones nor cephalosporins should be considered clinically effective in infections of nosocomial origin, particularly in those occurring in patients with previous HCA or HA infections, and suggest that piperacillin-tazobactam could be an alternative as empiric therapy.

Certain limitations should be considered when interpreting the findings of the current study. Firstly, it is retrospective in nature. Thus, specimens were sent for culture according to the clinical judgment of the team caring for the patients enrolled. Although the proportion of culture-positive infections (approximately 66%) was not lower than in previous prospective studies (48–69%) (3, 7, 17), we cannot exclude that the occurrence of antibiotic resistance may have been under- or overestimated in our cohort. Also, our results apply only to serious bacterial infections occurring in cirrhotic patients, i.e. those requiring hospital care or leading to hospitalization. Infections not requiring hospitalization were not included in the current study (as in essentially all previous reports in the field (3–5, 7, 17, 18, 26)), which may have biased our results underestimating the potential role of PPIs in CA infections and/or infections not requiring hospital care. Furthermore, data on the exact duration of PPI therapy could not be extracted in a reliable and consistent manner from medical records. Although the occurrence of AKI in the current study was similar to or higher than that reported by prospective studies in cirrhotic patients with bacterial infections (4, 37), the incidence of AKI may have been underestimated as serum creatinine was not measured at least every 48 h in all patients. For similar reasons, the diagnosis of infection-related ACLF [although it was the same as that in a previous large study of hospitalized patients with cirrhosis and infection (14)] may have been underestimated. Finally, infection diagnosis may have been influenced by clinician practice and thus in some cases it may have been delayed, potentially affecting our findings. Larger prospective population-based studies including patients from different geographical areas are needed to confirm our findings and investigate further the incidence and risk factors of HCA and HA infections as well as their bacterial resistance patterns and impact on patient outcome.

In summary, the current study provides longitudinal population-based data confirming that the occurrence of HCA and HA infections increases during the course of cirrhosis. The use of PPIs is a predictor of these infections in a dose-related manner, along with advanced liver disease and previous antibiotic therapy. HCA and HA infections often have a severe clinical course as they are associated with infection-related ACLF. Furthermore, bacteria responsible for HA infections are often resistant to first-line antibiotics even in areas of low prevalence of background bacterial resistance to antibiotics like Sweden, making empiric antibiotic treatment decisions challenging.

Acknowledgements

Financial support: This study was supported by a grant from Region Skåne.

Conflict of interest: The authors do not have any disclosures to report.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary file.

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Paper II

ORIGINAL ARTICLE

Predictors of mortality among patients with compensated and decompensated liver cirrhosis: the role of bacterial infections and infection-related acute-on-chronic liver failure

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Abstract

Objective. Population-based data on the impact of bacterial infections on the course of compensated and decompensated cirrhosis as well as the occurrence, predictors of infection-related acute-on-chronic liver failure (ACLF) and its fatal outcome are limited. **Material and methods.** All patients with incident cirrhosis in the period 2001–2010, residing in an area of 600,000 inhabitants, were retrospectively identified. All serious bacterial infections (resulting in or occurring during an inpatient hospital episode) during this period were analyzed. Infection site and acquisition type, comorbid illness (Charlson comorbidity index) and infection severity features were analyzed. Patients were followed up until death, transplant, or the end of 2011. **Results.** Overall, 398 serious bacterial infections occurred in 241/633 (38%) patients (106/332 diagnosed with compensated and 135/301 with decompensated disease; follow-up time was 2276 patient-years). ACLF occurred in 95/398 (24%) serious infections with an in-hospital mortality of 50%. In logistic regression analysis, the model for end-stage liver disease score, active alcohol misuse and healthcare-associated infections were predictors of infection-related ACLF ($p < 0.05$ for all). In-hospital mortality in infections with ACLF was related to albumin levels, Charlson comorbidity index >1 and occurrence of one or more organ failures ($p > 0.05$ for all). In Cox regression analysis, infection-related ACLF was an independent negative predictor of transplant-free survival in decompensated patients ($p = 0.049$). **Conclusions.** In a population-based cirrhotic cohort, infection-related ACLF was a negative predictor of survival in decompensated disease. Infection-related ACLF was frequent and related to cirrhosis severity and infection acquisition type, as well as to high inpatient mortality, in particular in patients with significant comorbidity.

Key Words: acute-on-chronic liver failure, bacterial infection, comorbidity, compensated cirrhosis, decompensated cirrhosis, healthcare-associated infection, hospital-acquired infection, organ failure, prognosis

Introduction

Bacterial infections are increasingly frequent in patients with cirrhosis and account for significant morbidity and mortality [1–3]. Compensated and decompensated cirrhosis have markedly different survival as well as different predictors of mortality and, therefore, are considered to be prognostically separate entities [4,5]. A recent systematic review proposes that sepsis has prognostic importance mostly in

decompensated patients, thus representing a separate cirrhosis stage, following decompensation [6]. However, longitudinal population-based data on the impact of bacterial infection development and its severity on survival in both compensated and decompensated cirrhosis are scarce.

Recently published data have shown that acute-on-chronic liver failure (ACLF) is a distinct syndrome from acute decompensation in liver cirrhosis, leading to significant short-term mortality [7–9]. Bacterial

infections are well-recognized precipitating factors of this syndrome [7–11]. In a recent multicenter study from North America, infection-related ACLF was shown to be independently related to nosocomial infections and severity of liver disease as well as infections other than spontaneous bacterial peritonitis [12]. Population-based data, however, on the occurrence, predictors and outcome of infection-related ACLF are lacking.

The aim of the current study was to investigate the independent predictors of survival in compensated and decompensated cirrhosis in a longitudinal population-based incident cohort of cirrhotic patients, with a specific focus on infection-related factors. We also aimed to specifically investigate the occurrence as well as predictors of infection-related ACLF and its fatal outcome in cirrhosis.

Materials and methods

Patients and follow-up

All adult patients (≥ 18 years) diagnosed with cirrhosis in the period 2001–2010 at our institution, which is the only hospital in our primary catchment area (population 600,000), were identified by means of a search of the computerized discharge diagnosis register of the hospital. Patients with chronic liver disease are in effect exclusively diagnosed and followed up at their local public healthcare institutions in Sweden. Private or public community-based hepatology practice is extremely rare in the country, and this is also the case for outpatient/day hospital activities regarding these patients. By means of the initial computerized search in the discharge diagnosis register, all patients with at least one outpatient or inpatient episode with the diagnosis of cirrhosis were identified, but the final cohort included in the study comprised only of patients with first-time cirrhosis diagnosis during the study period. Although our institution accepts referrals of patients with cirrhosis, no inpatients are transferred to other hospitals. Patients with a previous liver transplant and those residing outside the primary catchment area of the hospital were excluded. Thus, all patients with incident cirrhosis in the primary catchment area of our institution were included. Medical records were systematically reviewed, and the diagnosis of cirrhosis was re-evaluated and confirmed. In Sweden, all healthcare contacts of patients are registered in the electronic patient record system, and notes of clinicians and, in the event of emergency or inpatient care, of nurses regarding the care patients receive are also stored electronically. Thus, medical records, including clinician and nurse notes for both outpatient and inpatient episodes, laboratory tests, imaging and

endoscopy exams, and histopathology results, are computerized. It is obligatory for clinicians and nurses to register any patient contact, outpatient or inpatient (including diagnosis codes but also mandatory structured text), in medical records. For example, in the case of inpatients, data for at least vital signs as well as clinical status were registered on, essentially, a daily basis. The discharge diagnosis register was used merely for identification of patients who had received the diagnosis of liver cirrhosis, but, subsequently, medical records scrutinization included all the components of medical records for both inpatient and outpatient patient episodes. Cirrhosis diagnosis was based on liver biopsy and/or compatible clinical, laboratory and imaging findings. Patients were longitudinally followed up until death, liver transplantation or till the end of 2011. The study was approved by the local ethics committee. The same patient cohort has been recently used in a study on healthcare-associated (HCA) and hospital-acquired (HA) infections in cirrhosis [13].

Diagnosis of infection

The electronic medical records of patients identified in our institution as well as any records these patients had in any public hospital in the whole of the healthcare region (population 1.3 million) were scrutinized (the same electronic system of medical records is used by all hospitals in the healthcare region). There are no private hospitals in the region. All serious bacterial infections (i.e. those resulting in or occurring during an inpatient hospital episode) were identified and registered after a detailed review of all the components of medical records (and not by means of a search of the computerized discharge diagnosis register in order to avoid misclassification) and registered. Spontaneous bacterial peritonitis and infections in other sites were defined according to conventional criteria [14]. Mixed bacterial infections (bacterial infections at different sites diagnosed simultaneously) were considered as a single infection episode. Further infections diagnosed following a first infection during the same hospitalization were considered separate and were defined as second infections [15]. Infections were classified according to their acquisition type as HCA, HA or community-acquired, as previously described [16].

Data collection

At the time of cirrhosis diagnosis and at diagnosis of each serious infection episode, data were extracted with regard to patient demographics and etiology, active alcohol misuse, severity and complications of liver disease. Patients were classified into a compensated (absence of variceal bleeding and ascites) and a

decompensated group (variceal bleeding and/or ascites) at cirrhosis diagnosis, as agreed upon in the Baveno IV consensus conference [17]. During follow-up, data on the development of hepatocellular carcinoma (HCC) and decompensation, defined as transition from a compensated to a decompensated stage (ascites and/or variceal bleeding occurrence [17]), as documented in the medical records and re-evaluated during medical record scrutinization, were also registered. Ascites was considered to be present when documented by means of an imaging test, which was also available in the medical record system and scrutinized. At diagnosis of each serious infection episode, data on ongoing alcohol misuse, vital signs and relevant laboratory values were collected. Comorbid illness was also recorded, and the Charlson comorbidity index was computed [18], excluding liver disease.

Clinical outcomes

The primary endpoint was death or liver transplantation. Outcomes recorded for each serious infection episode were sepsis, severe sepsis, acute kidney injury (AKI), ACLF and in-hospital mortality. Sepsis was defined as two or more systemic inflammatory response syndrome criteria associated with a confirmed bacterial infection and severe sepsis as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion [19]. AKI was diagnosed as a 50% increase in serum creatinine from a stable baseline [20]. ACLF was diagnosed and classified into grades 1–3 according to the criteria established by the CANONIC study [7]. Infection-related mortality was defined as death that could be attributed to a serious infection. Data on date and cause of death were obtained from medical records and were confirmed through linkage to the Cause of Death Register by means of the unique national registration number assigned to all Swedish residents.

Statistical analysis

Data were expressed as mean and standard deviation or number and percentage as appropriate. When comparing groups, the Fisher exact test was used for categorical variables and the student T test for continuous variables. Predictors of of transplant-free survival in compensated and decompensated patients at cirrhosis diagnosis were assessed with survival analysis (Kaplan-Meier), and groups were compared with the log-rank test. Cox regression analysis was subsequently used to identify independent predictors of survival in the two groups. In an attempt to identify independent predictors of infection-related ACLF and its short-term mortality, all parameters related to them with a p -Value ≤ 0.1 in univariate analysis

were entered into multivariate logistic regression analyses (performed among infection episodes; each patient may have experienced one or more episodes). All tests were two-tailed and were conducted at a 5% significance level.

Results

A total of 633 patients diagnosed with cirrhosis (incident cases; 332 compensated and 301 decompensated at cirrhosis diagnosis) during the study period were retrospectively enrolled and followed for 2276 patient-years (Table I). Twelve patients were lost to follow-up. During a median follow-up of 36 months (range: 0.1–134 months), 241 patients (38%) experienced a total of 398 serious bacterial infection episodes (median 1, range 1–8). The flow of patients from cirrhosis diagnosis through serious bacterial infections to mortality, liver transplant or end of follow-up is shown in Figure 1. Infection-related morbidity and mortality were significantly more frequent in the decompensated compared to the compensated group (Table I).

Predictors of survival in patients with compensated disease at cirrhosis diagnosis

A total of 106 (32%) patients with compensated disease at cirrhosis diagnosis experienced at least one serious bacterial infection during the whole follow-up (Figure 1). Independent predictors of mortality or transplantation (combined outcome) in compensated patients at cirrhosis diagnosis (with or without decompensation during follow-up) selected by Cox regression analysis were: Model for end-stage liver disease (MELD) score (hazard ratio [HR] 1.05, 95% confidence interval [CI] 1.02–1.08) upon cirrhosis diagnosis and decompensation during follow-up (HR 2.10, 95% CI 1.44–3.06) but not age, diabetes mellitus upon cirrhosis diagnosis, varices, gender, etiology of cirrhosis, occurrence of serious bacterial infection, HCA/HA infection, sepsis, severe sepsis, infection-related AKI, infection-related ACLF, HCC or encephalopathy (data not shown). Similar results were also obtained when compensated patients with HCC were excluded from analysis (data not shown).

Predictors of survival in patients with decompensated disease at cirrhosis diagnosis

A total of 135/301 (45%) patients in our cohort presenting with decompensated cirrhosis had at least one serious bacterial infection episode (Figure 1). Out of 301, 42 (14%) were diagnosed with a serious bacterial infection during their hospitalization upon

Table I. Baseline characteristics and infection-related features in patients with compensated and decompensated disease at cirrhosis diagnosis.

Parameter	Compensated at cirrhosis diagnosis (<i>n</i> = 332)	Decompensated at cirrhosis diagnosis (<i>n</i> = 301)	<i>p</i> -Value
Age (year)	58 (12)	61 (11)	< 0.001
Male gender	213 (64)	199 (66)	0.617
MELD at cirrhosis diagnosis	7.8 (5.7)	13.1 (7.2)	< 0.001
<i>Etiology</i>			
Alcoholic cirrhosis (or mixed)	136 (41)	227 (75)	< 0.001
Viral	81 (24)	17 (6)	< 0.001
Cryptogenic/NASH	47 (14)	37 (13)	0.558
Cholestatic	29 (9)	10 (3)	0.005
Other	40 (12)	10 (3)	< 0.001
HCA/HA infection (incidence rate)	39 (3.5)	100 (10.6)	< 0.001
Sepsis (incidence rate)	90 (6.7)	108 (11.4)	< 0.001
Severe sepsis (incidence rate)	51 (3.8)	68 (7.2)	< 0.001
Infection-related AKI (incidence rate)	59 (4.4)	85 (9.0)	< 0.001
Infection-related mortality (incidence rate)	28 (2.1)	42 (4.4)	0.002
Overall mortality/OLT (incidence rate)	155 (11.6)	200 (21.2)	< 0.001

Abbreviations: AKI = Acute kidney injury; HCA/HA = Healthcare-associated/hospital-acquired; HCC = Hepatocellular carcinoma; MELD = Model for end-stage liver disease; NASH = Non-alcoholic steatohepatitis; OLT = Orthotopic liver transplantation.

Data are expressed as mean (standard deviation) or number of patients (%) or number of patients with incidence rate per 100 patient-years as appropriate.

Patients with variceal bleeding or/and ascites at cirrhosis diagnosis were classified as decompensated and those without these features as compensated [17].

first presentation with cirrhosis (12/42 with infection-related ACLF). Decompensated patients without serious bacterial infections during follow-up had similar transplant-free survival to those with serious bacterial infections without associated ACLF (Figure 2).

However, compared to both groups, decompensated patients with infection-related ACLF during follow-up had significantly worse transplant-free survival (Figure 2). In Cox regression analysis, male gender (HR 1.40, 95% CI 1.01–1.94), age (HR 1.04/year,

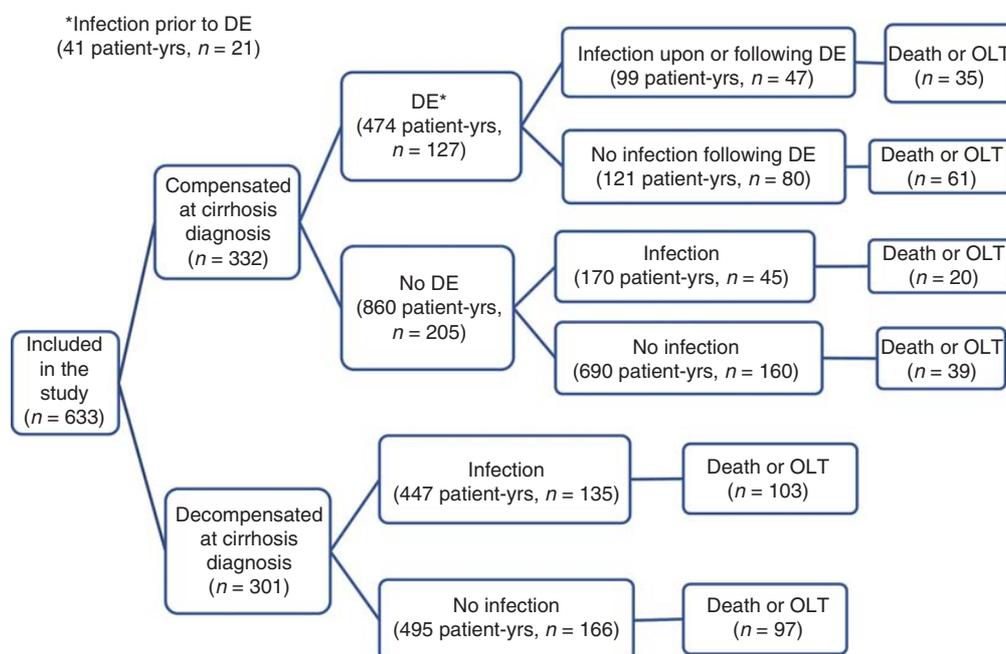
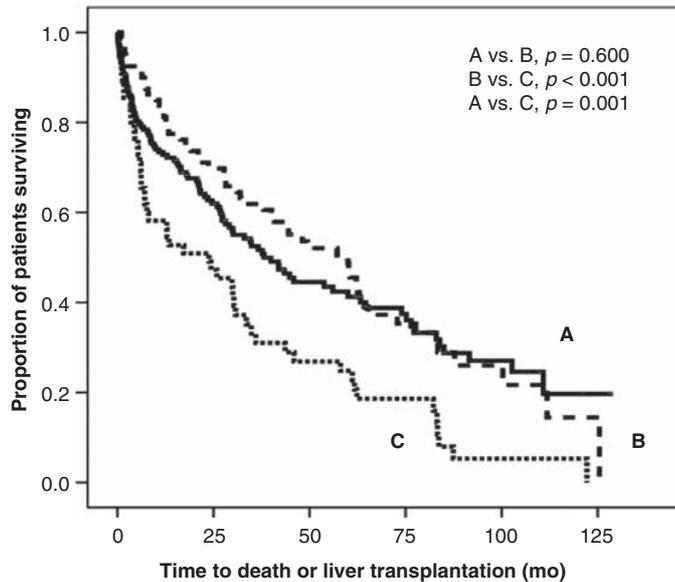


Figure 1. Flow of patients from cirrhosis diagnosis through serious bacterial infection episodes to mortality or liver transplantation. Seven of 21 initially compensated patients with infection prior to decompensation experienced even infection upon or following decompensation. Abbreviations: DE = Decompensation; infection, serious bacterial infection (i.e., bacterial infection episode resulting in or occurring upon an inpatient hospital episode); OLT = Orthotopic liver transplantation.



Patients at risk						
A	166	84	45	27	12	3
B	80	54	34	18	6	1
C	55	26	13	7	1	0

Figure 2. The impact of infection-related acute-on-chronic liver failure on survival in decompensated cirrhosis. Transplant-free survival in decompensated patients without serious bacterial infection (A; $n = 166$), with serious bacterial infection not complicated by acute-on-chronic liver failure (B; $n = 80$) or with infection-related acute-on-chronic liver failure (C; $n = 55$) during follow-up. Death or liver transplantation were considered events. Patients were censored at the end of follow-up.

95% CI 1.03–1.06), MELD score (HR 1.05, 95% CI 1.03–1.08) and diabetes mellitus (HR 1.48, 95% CI 1.04–2.09) upon cirrhosis diagnosis, hepatic encephalopathy during follow-up (HR 1.47, 95% CI 1.08–2.00) and infection-related ACLF (HR 1.41, 95% CI 1.01–1.20) were found to be independent predictors of

mortality or transplantation in decompensated patients but not etiology of cirrhosis, serious bacterial infection, HCA/HA infection, sepsis, severe sepsis, infection-related AKI or HCC (data not shown) (Figure 3). After exclusion of patients with HCC, transplant-free survival was still worse among decompensated

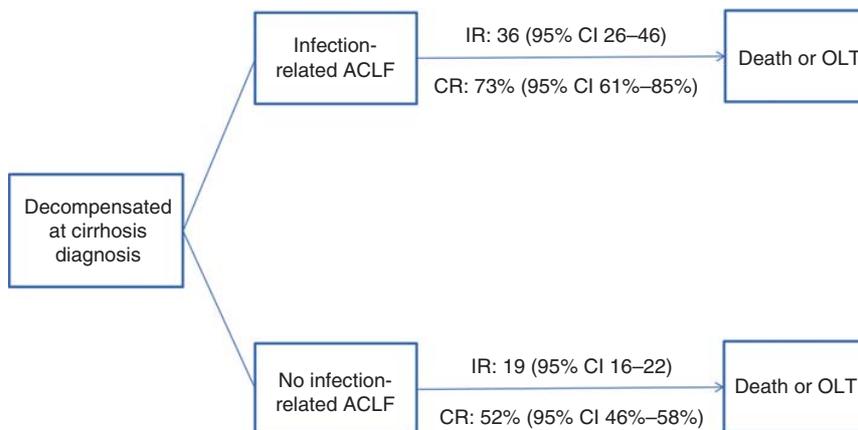


Figure 3. Overall mortality/transplant incidence rates and 3-year overall mortality/transplant cumulative risk in patients presenting with decompensated cirrhosis according to the occurrence of infection-related ACLF. Abbreviations: ACLF = Acute-on-chronic liver failure; IR = Incidence rate per 100 patient-years; CI = Confidence interval; CR: 3-year cumulative risk; OLT = Orthotopic liver transplantation.

Table II. Patient characteristics in serious bacterial infection episodes with or without ACLF and in those complicated by infection-related ACLF with or without in-hospital mortality.

Parameter	No ACLF (n = 303)	ACLF (n = 95)	p-Value	ACLF without in-hospital mortality (n = 48)	ACLF with in-hospital mortality (n = 47)	p-Value
Age (year)	63 (11)	63 (10)	0.573	61 (10)	66 (9)	0.011
Male sex	190 (63)	76 (80)	0.002	40 (83)	36 (77)	0.452
Alcoholic cirrhosis	197 (65)	75 (79)	0.011	40 (83)	35 (74)	0.324
Active alcohol misuse at infection diagnosis	119 (39)	54 (58)	0.003	29 (60)	25 (54)	0.677
Charlson index >1 ^a	90 (30)	23 (25)	0.361	6 (12)	17 (36)	0.009
MELD at infection diagnosis	14.1 (6.9)	24.5 (6.6)	< 0.001	24.9 (6.5)	23.9 (6.8)	0.506
HCA/HA infection	193 (64)	76 (79)	0.004	35 (73)	41 (87)	0.123
Second infection	15 (5)	9 (9)	0.136	7 (15)	2 (4)	0.159
<i>Laboratory data at infection diagnosis</i>						
Leukocyte count ($\times 10^9/L$)	10.8 (8.5)	13.5 (7.6)	0.007	13.6 (7)	13.4 (8.2)	0.990
CRP (mg/L)	76 (75)	90 (89)	0.127	73 (72)	108 (101)	0.059
Sodium (mmol/L)	134 (5)	132 (6)	0.002	131 (6)	132 (6)	0.656
Albumin (g/L)	25 (6)	23 (6)	0.004	24 (7)	21 (4)	0.029
<i>Site of infection</i>						
Urinary tract infection	63 (21)	13 (13)	0.137	9 (19)	4 (8)	0.232
Spontaneous bacterial peritonitis	47 (15)	14 (15)	1.000	9 (19)	5 (10)	0.386
Pneumonia	43 (14)	12 (13)	0.865	2 (4)	10 (21)	0.014
Skin/soft-tissue infection	44 (15)	7 (7)	0.079	3 (6)	4 (8)	0.714
Spontaneous bacteremia	33 (11)	15 (16)	0.209	6 (13)	9 (19)	0.412
Mixed infection ^b	9 (3)	10 (10)	0.005	5 (10)	5 (11)	1.000
Other	63 (21)	25 (26)	0.260	14 (29)	11 (23)	0.642
Mean arterial pressure (mm/Hg)	76 (34)	75 (26)	0.935	79 (27)	72 (25)	0.239
ICU care	5 (2)	19 (20)	< 0.001	6 (12)	13 (28)	0.077
In-hospital mortality	29 (10)	47 (49)	< 0.001			
<i>Organ failures</i>						
Liver				13 (27)	8 (17)	0.324
Kidney				34 (71)	42 (89)	0.039
Cerebral				10 (21)	16 (34)	0.173
Coagulation				9 (19)	7 (15)	0.785
Circulation				3 (6)	10 (21)	0.040
Lungs				4 (8)	11 (23)	0.053
ACLF grade > 1				12 (25)	27 (57)	0.002

Abbreviations: ACLF = Acute-on-chronic liver failure; CRP = C-reactive protein; HCA/HA = Healthcare-associated/hospital-acquired; ICU = Intensive care unit; MELD = Model for end-stage liver disease.

Data are expressed as mean (standard deviation) or number of patients (%) as appropriate.

^aA score of 1 was the median value of Charlson comorbidity index (excluding liver disease).

^bThe following sites of infection were involved in mixed infections: urinary tract infection, spontaneous bacterial peritonitis, skin/soft-tissue infection, pneumonia, spontaneous bacteremia and gastroenteritis.

patients with infection-related ACLF versus those without (log-rank test $p < 0.001$; data not shown). The majority of deaths or transplants (65%) occurred during the first 3 months following the infection episode complicated by ACLF.

Predictors of infection-related ACLF occurrence

In all, 95/398 (24%) of all serious bacterial infection episodes occurring in the study period were complicated by ACLF. Patient characteristics in serious bacterial infections with and without ACLF are summarized in Table II. In logistic regression analysis, only the MELD score at infection diagnosis (odds ratio [OR] 1.27, 95% CI 1.20–1.35), mixed infection (OR 6.41, 95% CI 1.97–20.85), active alcohol misuse

at infection diagnosis (OR 2.73, 95% CI 1.42–5.26) and serious HCA or HA infection (OR 2.12, 95% CI 1.02–4.40) were shown to be independent predictors of infection-related ACLF.

Predictors of in-hospital mortality in serious bacterial infection episodes with ACLF

In-hospital mortality was significantly higher in serious bacterial infection episodes with versus without ACLF and increased with increasing number of organ failures (Table II). In logistic regression analysis, at least one comorbidity in the Charlson comorbidity index (OR 3.29, 95% CI 1.049–10.37), albumin levels at infection diagnosis (OR 0.90, 95% CI 0.82–0.98) and ACLF grade >1 (OR 3.81, 95% CI 1.45–10.05), but

not C-reactive protein, age at infection diagnosis, respiratory, renal or circulatory failure ($p > 0.05$ for all; data not shown), were shown to be independent predictors of fatal infection-related ACLF.

Discussion

In our 10-year longitudinal population-based cohort, the severity of serious bacterial infections had an impact on survival in decompensated cirrhosis. Infection-related ACLF occurred in 24% of serious bacterial infections and was associated with significant mortality. Independent predictors of infection-related ACLF in our study were severity of liver disease, expressed as the MELD score, active alcohol misuse and mixed as well as HCA/HA infections. Predictors of in-hospital mortality in serious infections complicated by ACLF were low albumin levels, significant comorbidity, as captured by the higher Charlson comorbidity index, and more than one organ failure. To our knowledge, this is the first population-based longitudinal study assessing the impact of bacterial infections, in particular infection-related ACLF, on the course of liver cirrhosis.

The current study failed to show any significant impact of bacterial infection development, regardless of its severity, on survival of patients diagnosed with compensated cirrhosis. It seems that it is rather the severity of liver disease at cirrhosis diagnosis and the occurrence of decompensation that have major prognostic importance in this group of patients. A possible explanation for this finding could be provided by recently published data showing a more severe functional failure of neutrophils in patients with more advanced cirrhosis [21,22]. Our findings suggest that although bacterial infections frequently develop in patients diagnosed with compensated cirrhosis, they seem to independently affect survival predominantly in those diagnosed with decompensated disease in keeping with numerous previous studies [6,16,23–27].

In patients with decompensated cirrhosis, infection-related ACLF, rather than sepsis *per se* as previously suggested [6], was found to be a major predictor of survival. Inpatient mortality in infection-related ACLF was about 50%, in line with previous studies in patients with ACLF due to various causes (34–65%) [7–12]. Also, these findings are in keeping with published data from unselected cirrhotic cohorts on the prognostic importance of extrahepatic organ failures upon acute decompensation both long term [10,28] and short term [7,9,12]. The prognostic impact of infection-related ACLF in our cohort was dramatic during the first 3 months following the infection episode, in which the majority of deaths or transplants occurred. This is in accordance with

the observations in the cohort in which the definition of ACLF used in the current study was developed [7]. However, further comparative studies are warranted in order to explore the usefulness of different definitions of ACLF [7,12,29] in the prediction of outcome in cirrhotic patients with bacterial infections.

To our knowledge, this is the first population-based study assessing the occurrence of infection-related ACLF in a cohort of patients with incident cirrhosis. Our findings showing that liver disease severity and HCA/HA infections predict ACLF development are in line with those from a recent study from North America [12]. Our observation that mixed infections (i.e. infections at different sites diagnosed simultaneously) are related to infection-related ACLF development is novel. A possible explanation for this could be that patients developing mixed infections had more severe immunological disturbances leading to both increased infection susceptibility and more pronounced inflammatory response with more severe tissue injury [30]. In the current study, significant comorbid illness was related to increased mortality in serious infections with ACLF. Although this is in accordance with previous reports demonstrating increased overall mortality in cirrhotics with multiple chronic diseases [31], our findings suggest that cirrhotic patients with comorbidities and bacterial infections should be treated rigorously as risk of poor outcome is high in this group.

Our study has certain limitations. First, it is retrospective in nature, which poses difficulties in the interpretation of findings. Systemic inflammatory response syndrome is a hallmark of advanced cirrhosis, and difficulties in interpretation of its components may have influenced the occurrence of sepsis in the current study. Nevertheless, the same definition of sepsis has been used as in previous large studies in this field [32–34]. Also, performance of diagnostic tests, such as transabdominal ultrasound and endoscopy, was not standardized, potentially leading to variation in the timing of the diagnosis of varices and ascites. For the same reason, the occurrence of infection-related ACLF may have been underestimated (although it was the same as that among infected patients in a large prospective study in cirrhotic patients with or without infection [7]). Second, the current study covered a period of 10 years. Although this made conclusions on long-term prognosis possible, it is conceivable that treatment practices may have changed during this period of time, which may have influenced patient outcome and, consequently, our findings. Moreover, not all data on comorbidity may have been available at cirrhosis diagnosis and during follow-up, which could have confounded our findings. Large prospective multicenter studies are

needed to further explore factors affecting prognosis in cirrhotics with bacterial infections.

In conclusion, the findings of the current study suggest that infection-related ACLF has an impact on the course of decompensated cirrhosis (Figure 3). The severity of liver disease along with specific infection characteristics, in particular mixed infection and healthcare or nosocomial infection origin, are major predictors of infection-related ACLF. Moreover, the number of organ failures as well as comorbid illness rather than the severity of liver dysfunction determine short-term outcome in decompensated patients with infection-related ACLF. Early diagnosis and proper treatment of bacterial infections is a crucial step in the management of patients with decompensated cirrhosis, in particular those with comorbid illness.

Declaration of interest: This study was supported by a grant from Region Skåne, the Swedish Society of Medicine and the Royal Physiographic Society in Lund. The authors alone are responsible for the content and writing of the paper. None of the authors has any conflict of interest to declare.

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Paper III

Bacterial infections in alcoholic and nonalcoholic liver cirrhosis

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Objectives Longitudinal, population-based data on the occurrence, localization, and severity of bacterial infections over time in patients with alcoholic compared with nonalcoholic cirrhosis are limited.

Materials and methods All patients with incident cirrhosis diagnosed in 2001–2010 (area of 600 000 inhabitants) were retrospectively identified. All bacterial infections resulting in or occurring during an inpatient hospital episode during this period were registered. The etiology of cirrhosis (alcoholic vs. nonalcoholic), infection localization, and outcome as well as bacterial resistance patterns were analyzed. Patients were followed until death, transplant, or the end of 2011.

Results In all, 633 cirrhotics (363 alcoholic, 270 nonalcoholic) experienced a total of 398 infections (2276 patient-years). Among patients diagnosed with cirrhosis each year from 2001 to 2010, increasing trends were noted in the occurrence of infection (from 13 to 27%, $P < 0.001$) and infection-related in-hospital mortality (from 2 to 7%, $P = 0.05$), the latter mainly in the alcoholic group. Although alcoholic etiology was related to the occurrence of more frequent infection (Kaplan–Meier, $P < 0.001$), this relationship was not significant after adjustment for confounders in Cox regression analysis ($P = 0.056$). Resistance to piperacillin-tazobactam and carbapenems was more common in infections occurring in alcoholic versus nonalcoholic cirrhosis (13 vs. 5%, $P = 0.057$ and 12 vs. 2%, $P = 0.009$). Alcoholic etiology predicted pneumonia and infections caused by Gram-positive bacteria in multivariate analysis ($P < 0.05$ for both).

Conclusion In a population-based cirrhotic cohort, bacterial infections increased over time, which, in the case of alcoholic cirrhosis, was associated with pneumonia and bacterial resistance to antibiotics. However, alcoholic etiology was not related independently to the occurrence of bacterial infections. *Eur J Gastroenterol Hepatol* 27:1080–1086

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Introduction

Bacterial infections increase mortality by four-fold in cirrhosis and they are a very common cause of admissions and increased healthcare costs in these patients [1]. Although the prevalence of infections among hospitalized cirrhotics appears to be increasing [2], there are few longitudinal, population-based data on the occurrence of bacterial infections in cirrhotic patients with different cirrhosis etiologies.

Bacterial infections develop partly as a consequence of immune dysfunction that occurs progressively during the course of cirrhosis [3]. Liver cirrhosis is frequently associated with alcoholism, which has also been shown to lead to defective innate and adaptive immunity [4]. An increased frequency of bacterial infections among cirrhotics with alcoholic liver disease (ALD) [5], in particular bacteremia [6,7] and meningitis [8], has been reported.

However, the potential role of alcoholic cirrhosis as a risk factor for the development of bacterial infections has not been confirmed in other studies [9–13]. Although alcohol misuse in noncirrhotic individuals has been reported to be associated with infections caused by bacteria resistant to common antibiotics [14–16], it is unclear whether this holds true for patients with ALD cirrhosis.

Infection-related acute-on-chronic liver failure (ACLF) appears to occur more frequently in patients with ALD compared with those with non-ALD cirrhosis [17,18]. Previous studies have reported similar proportions of alcoholic cirrhotics with and without infection-related acute kidney injury (AKI) [19] or systemic inflammatory response syndrome [20]. However, published data on the potential role of cirrhosis etiology in infection-related mortality are conflicting, with some studies reporting a worse outcome in infected ALD cirrhotics [7,8], others in non-ALD cirrhotics [21,22], and other studies showing no difference between the two groups [5,6].

We aimed to investigate the time trends in the incidence of bacterial infections and, in particular, the potential role of cirrhosis etiology (ALD vs. non-ALD) in the occurrence, localization, and outcome of bacterial infections in a population-based longitudinal cirrhotic cohort.

European Journal of Gastroenterology & Hepatology 2015, 27:1080–1086

Keywords: alcoholic liver disease, antibiotic resistance, bacterial infection, liver cirrhosis

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Received 19 February 2015 **Accepted** 16 April 2015

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (www.eurojgh.com).

Materials and methods

Patients

All adult patients (≥ 18 years) with first-time diagnosis of cirrhosis in the period 2001–2010 in our institution were retrospectively identified by a search in the computerized

discharge diagnosis register of the hospital. Patients with chronic liver disease are in effect exclusively diagnosed and followed up at their local public hospital healthcare institutions in Sweden. Only patients with incident cirrhosis residing in the primary catchment area of our institution (population of 600 000) were included. The diagnosis of cirrhosis was made on the basis of liver biopsy and/or a combination of at least two of the following: clinical, biochemical, and imaging data. Patients were followed longitudinally until death, transplant, or the end of 2011. The same patient cohort has been used previously in a study on healthcare-associated (HCA) and hospital-acquired (HA) infections in cirrhosis [23], as well as in a study on predictors of mortality among cirrhotic patients [24]. Patients were classified into two groups according to the etiology of cirrhosis: ALD and non-ALD cirrhosis. All patients who had a long history of heavy alcohol consumption (≥ 80 g ethanol per day for male patients and ≥ 60 g for female patients) for a duration of at least 10 years were considered to have ALD cirrhosis. Medical records of all patients were scrutinized to confirm the diagnosis of cirrhosis and its etiology. Patients were considered to have ongoing alcohol misuse if they (or their relatives) had reported alcohol ingestion following the diagnosis of cirrhosis and/or if alcohol consumption was suspected/registered in the medical records (e.g. admissions to the accident and emergency department for alcohol-related reasons, positive relevant blood test results, such as phosphatidyl-ethanol, etc.). The study protocol was approved by the local ethics committee.

Infection diagnosis and outcome

All severe bacterial infections (i.e. those resulting in or occurring during an inpatient hospital episode) during the study period were identified by a detailed review of the extensive electronic medical records of our health region (see text, Supplemental digital content 1, <http://links.lww.com/EJGH/A28>, which explains the details on the medical record system in Sweden). Infection site (see text, Supplemental digital content 1, <http://links.lww.com/EJGH/A28>) and acquisition type (HCA/HA vs. community-acquired) as well as second infections were defined according to conventional criteria [23,24]. Mixed bacterial infections (bacterial infections with different localization diagnosed simultaneously) were considered as a single infection episode. The outcomes recorded for each severe infection episode were sepsis, severe sepsis, AKI, and ACLF (defined as previously described [23, 24]; see text, Supplemental digital content 1, <http://links.lww.com/EJGH/A28>), as well as in-hospital mortality. Data on the date and cause of death were obtained from medical records and confirmed through linkage to the Cause of Death Register by the unique national registration number assigned to all Swedish residents. Infection-related mortality was defined as death that could be attributed, at least partly, to a severe infection.

Data collection at infection diagnosis

At the time of each severe infection episode, data were extracted with respect to patient demographics and etiology, cirrhosis severity and complications, ongoing alcohol misuse, medications, vital signs, and comorbid illness (as expressed by the Charlson comorbidity index [25], excluding liver disease).

All bacterial isolates in culture-positive infections and their antibiotic susceptibility patterns were registered. The following bacteria were considered to cause antibiotic-resistant infections: methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, ampicillin-resistant *Enterococcus*, extended-spectrum β -lactamase-producing Enterobacteriaceae, quinolone-resistant Gram-negative rods, or isolates resistant to three or more classes of antibiotics [26,27].

Statistical analysis

Data were expressed as mean and SD or n and % as appropriate. Density incidence rates for various outcome variables were computed by dividing the total number of infectious events occurring in patients (who were diagnosed with incident cirrhosis in each year from 2001 to 2010) during follow-up, by the number of person-years for which each patient group, i.e. the patients diagnosed with cirrhosis in each year, was followed. Thus, for each study year, only the infections occurring in patients – who were diagnosed with cirrhosis in this particular year – during their total follow-up were included, that is each patient was included once in the calculation of density incidence rates in the year of cirrhosis diagnosis. The rates were multiplied by 100 and compared with Pearson's correlation coefficient. When comparing groups, Fisher's exact test was used for categorical variables and student's t -test was used for continuous variables. To adjust for confounders in the relation between, on the one hand, cirrhosis etiology (ALD vs. non-ALD) and, on the other, infection severity features, pneumonia, length of stay, or microbiological patterns/bacterial resistance, all parameters related to each one of these variables with a P value 0.1 or less in univariate analysis were entered into multivariate logistic regression analyses. Length of hospital stay was assessed as a dichotomous variable using its mean value (days) in the study as a cut-off. Infection occurrence and infection-related mortality in patients with ALD versus non-ALD cirrhosis were assessed by survival analysis (Kaplan–Meier) and groups were compared using the log-rank test. The relationship between ALD cirrhosis and the occurrence of severe infections was evaluated further by Cox regression analysis. In this analysis, decompensation (at diagnosis of cirrhosis or during follow-up) [28] was entered as a time-dependent variable along with other potential confounders. All tests were two-tailed and were carried out at a 5% significance level.

Results

Alcoholic liver disease and nonalcoholic liver disease cirrhotic patients

A total of 633 patients with incident cirrhosis [$n=363$ (57%) with ALD; 1245 patient-years and $n=270$ (43%) with non-ALD; 1031 patient-years] were identified (Table 1). In all, 221/633 (35%) patients were diagnosed with cirrhosis by liver biopsy. Twelve patients were lost to follow-up. A total of 204/363 (56%) patients with ALD cirrhosis continued to misuse alcohol following the diagnosis of cirrhosis.

Table 1. Baseline characteristics in patients with alcoholic and nonalcoholic liver disease at diagnosis of cirrhosis

	ALD cirrhosis (n = 363)	Non-ALD cirrhosis (n = 270)	P-value
Age (years)	59 (9)	59 (14)	0.997
Male sex	255 (70)	157 (58)	0.002
MELD	13 (7)	7 (5)	< 0.001
Mixed cirrhosis etiology ^a	70 (19)	10 (4)	< 0.001
Ascites at presentation	203 (56)	56 (21)	< 0.001
Variceal bleeding at presentation	24 (7)	18 (7)	1.000
HCC at presentation	10 (4)	11 (7)	0.369

Data are expressed as means (SD) or number of patients (%) as appropriate.

ALD, alcoholic liver disease; HCC, hepatocellular carcinoma.

^aIn ALD cirrhosis: ALD and chronic hepatitis C, n = 66; primary biliary cirrhosis, n = 2; hemochromatosis, n = 1; hepatitis B, n = 1. In non-ALD cirrhosis: primary sclerosing cholangitis and autoimmune hepatitis, n = 5; primary biliary cirrhosis and primary sclerosing cholangitis, n = 1; primary biliary cirrhosis and autoimmune hepatitis, n = 1; chronic hepatitis C and autoimmune hepatitis, n = 1; chronic hepatitis C and α 1-antitrypsin insufficiency, n = 1; chronic hepatitis C and hepatitis B, n = 1.

Time trends in the occurrence and outcome of severe bacterial infections experienced by annual incident cohorts in 2001–2010

The frequency of severe infections increased significantly in patients diagnosed with incident cirrhosis later during the study period in both ALD and non-ALD cirrhosis (Fig. 1). This appeared to be related to an increase in HCA and HA infections, which was more evident in the ALD group (Fig. 1). Similarly, an increased frequency of sepsis, severe sepsis, infection-related ACLF, and infection-related in-hospital mortality was observed in both etiology groups, but reached statistical significance only in the group of ALD cirrhosis (Fig. 1). A trend toward decreasing MELD score values in patients diagnosed in the study years 2001–2010 was observed (from a mean score of 11 in 2001 to 10 in 2010, $P = 0.061$), suggesting that severity of cirrhosis at diagnosis is probably not a major predictor of the changes observed in the frequency of infections during the study period.

Occurrence and localization of severe bacterial infections

During a median follow-up of 36 months (range: 0.1–134 months), 164/363 (45%) patients with ALD and 77/270 (28%) patients with non-ALD cirrhosis experienced at least one severe infection episode. Overall, a total of 272 (median 1, range 1–8) and 126 (median 1, range 1–5) severe bacterial infection episodes occurred in each group, respectively. Severe infections occurred more frequently in ALD compared with non-ALD cirrhotic patients during follow-up (Fig. 2). ALD patients compared with those without continuous alcohol misuse following the diagnosis of cirrhosis in the medical records experienced severe infections more frequently (51 vs. 38%, $P = 0.025$). However, after adjusting for confounders (age and MELD score at cirrhosis diagnosis, decompensation at cirrhosis diagnosis or during follow-up [28]) by Cox regression analysis, alcoholic etiology was not found to be related independently to the occurrence of severe infections [hazard ratio (HR) 1.36, 95% confidence interval (CI) 0.99–1.87], irrespective of whether there was evidence of continuous alcohol misuse (HR 1.34, 95% CI 0.95–1.89) or not (HR 1.35, 95% CI 0.93–1.96) following the diagnosis

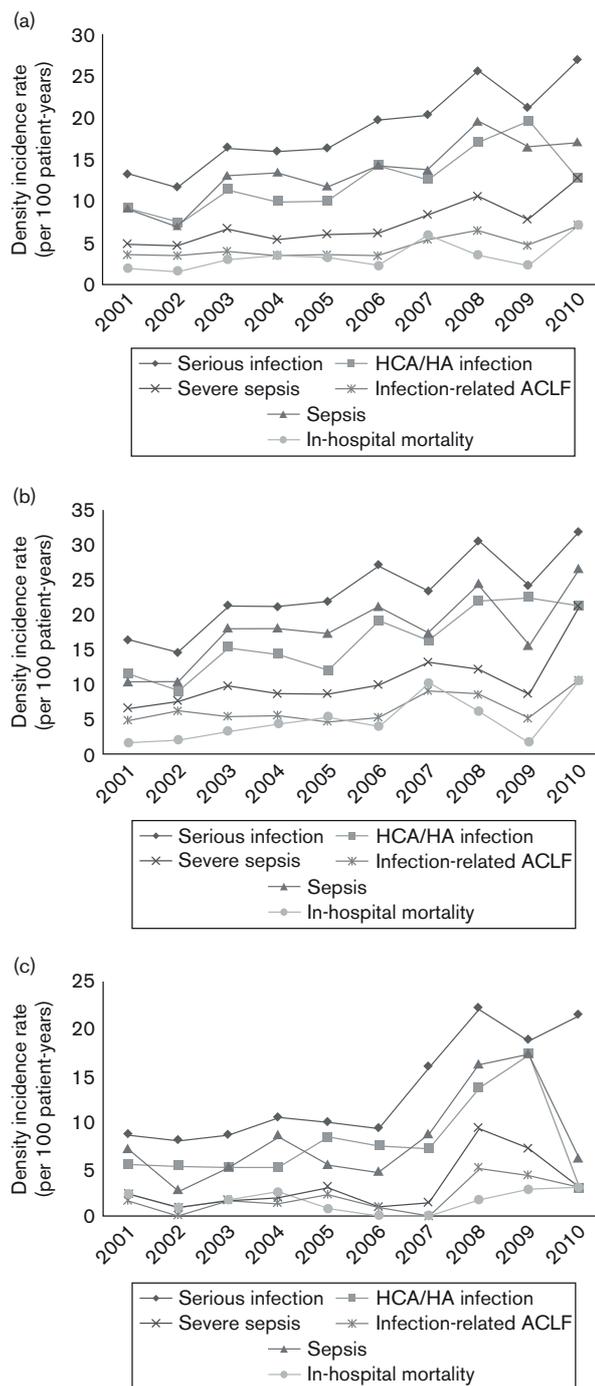


Fig. 1. Density incidence rates of severe bacterial infection episodes occurring in (a) all patients diagnosed with incident cirrhosis ($n = 633$), (b) patients diagnosed with incident ALD cirrhosis ($n = 363$), (c) patients diagnosed with incident non-ALD cirrhosis ($n = 270$) in the study period 2001–2010. Increasing density incidence rates were found for severe infections occurring in both ALD and non-ALD cirrhosis ($P = 0.001$). Density incidence rates for HCA/HA infection, sepsis, severe sepsis, infection-related ACLF, and in-hospital mortality increased in all patients ($P \leq 0.05$ for all) in patients with ALD cirrhosis ($P = 0.001, 0.012, 0.018, 0.055, 0.058$, respectively), but not in those with non-ALD cirrhosis ($P > 0.05$ for all). P -values are provided by the Pearson correlation test. ACLF, acute-on-chronic liver failure; ALD, alcoholic liver disease; HCA/HA, healthcare-associated/hospital-acquired.

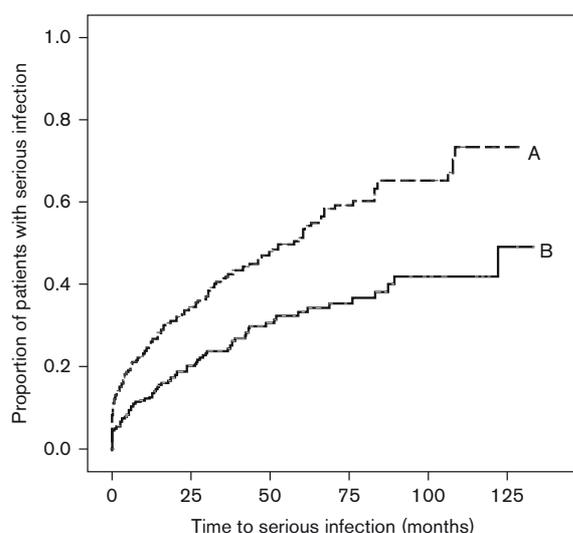


Fig. 2. Relationship between cirrhosis etiology and occurrence of infection. Proportion of patients with ALD cirrhosis (A) and non-ALD cirrhosis (B) with at least one severe bacterial infection during follow-up. The development of a severe bacterial infection was considered to be an event. Patients were censored at death, transplantation, or the end of follow-up. $P < 0.001$. ALD, alcoholic liver disease.

of cirrhosis. Among patients with decompensated disease upon cirrhosis diagnosis, patients with versus those without ALD cirrhosis did not differ significantly in the occurrence of severe bacterial infections ($P = 0.142$; data not shown).

Pneumonia occurred more often in ALD versus non-ALD patients (Table 2), which was confirmed in survival analysis (see figure, Supplemental digital content 2, <http://links.lww.com/EJGH/A29>). No significant differences were observed between the two etiology groups with respect to other infection sites (Table 2). After adjustment for confounders (C-reactive protein, age, MELD score, and encephalopathy at infection diagnosis, comorbidity, length of hospital stay, ICU care) by logistic regression analysis, ALD cirrhosis was still related independently to the occurrence of pneumonia [odds ratio (OR) 2.63, 95% CI 1.16–5.93]. Severe infection episodes because of pneumonia occurred more often in patients with versus without active alcohol misuse at infection diagnosis (19 vs. 10%, $P = 0.027$). Among patients with compensated disease upon diagnosis of infection, those with ALD experienced pneumonia more frequently compared with those with non-ALD cirrhosis (19 vs. 10%, $P = 0.189$). Similarly, among severe infections occurring in patients with decompensated status, pneumonia was more frequently experienced by patients with ALD versus non-ALD cirrhosis (16 vs. 6%, $P = 0.041$).

Bacterial infection outcome

Severe bacterial infection episodes in patients with ALD versus non-ALD cirrhosis were more often complicated by sepsis, severe sepsis, AKI, and ACLF (Table 2). As already described by our group, an independent relation appears to exist between infection-related ACLF and active alcoholism [24]. After adjustment for confounders (sex, infection site, HCA/HA infection, diabetes, MELD score, albumin, sodium, leukocyte count, C-reactive protein, and

Table 2. Characteristics of severe bacterial infection episodes occurring in cirrhotic patients with alcoholic liver disease and nonalcoholic liver disease

	ALD cirrhosis (n = 272)	Non-ALD cirrhosis (n = 126)	P-value
Age at infection diagnosis (years)	62 (10)	65 (12)	0.002
Male sex	85 (31)	47 (37)	0.253
MELD score at infection diagnosis	18 (8)	14 (8)	<0.001
Decompensated status at infection diagnosis ^a	220 (81)	67 (53)	<0.001
Hepatic encephalopathy at infection diagnosis	86 (32)	29 (23)	0.096
HCC	11 (4)	4 (3)	0.784
Active alcohol misuse at infection diagnosis	172 (63)		
Charlson comorbidity index > 1 ^b	65 (24)	48 (38)	0.004
HCA/HA infection	188 (69)	81 (64)	0.358
Mean arterial pressure (mm/Hg)	72 (33)	83 (28)	0.002
White cell count ($\times 10^9/l$)	11 (9)	11 (6)	0.299
CRP (mg/l)	80 (80)	78 (75)	0.816
Localization of infection			
Urinary tract infection	50 (18)	26 (21)	0.587
Pneumonia	45 (17)	10 (8)	0.020
Skin/soft-tissue infection	34 (13)	17 (13)	0.872
Spontaneous Bacterial peritonitis	43 (16)	18 (14)	0.766
Spontaneous bacteremia	36 (13)	12 (9)	0.325
Mixed	8 (3)	11 (9)	0.020
Other	55 (20)	33 (26)	0.195
Infection-related morbidity and outcome			
Sepsis	213 (78)	83 (66)	0.010
Severe sepsis	119 (44)	32 (25)	0.001
Infection-related AKI	150 (57)	49 (40)	0.002
Infection-related ACLF	75 (28)	20 (16)	0.011
Length of stay > 18 days ^c	98 (36)	24 (19)	0.001
ICU care	15 (5)	9 (7)	0.506
In-hospital mortality	58 (21)	18 (14)	0.102

Data are expressed as means (SD) or number of patients (%) as appropriate.

ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; ALD, alcoholic liver disease; CRP, C-reactive protein; HCA/HA, healthcare-associated/hospital-acquired; HCC, hepatocellular carcinoma.

^aDecompensated status was defined as the occurrence of ascites and/or variceal bleeding [28].

^bA score of 1 was the median value of the Charlson comorbidity index (excluding liver disease).

^c18 days was the mean value of length of hospital stay in all infections during the study period.

mean arterial pressure at infection diagnosis), by logistic regression analysis, however, ALD cirrhosis (with or without active alcohol misuse) was not found to be an independent predictor of either sepsis, severe sepsis, or infection-related AKI ($P > 0.05$ for all). Prolonged length of hospital stay (>18 days) was significantly more frequent in hospital episodes because of or with bacterial infections in patients with ALD versus non-ALD cirrhosis, and this relationship remained after adjustment for confounders (infection site, ongoing antibiotic use at infection diagnosis, HCA/HA infection, gastrointestinal bleeding during the same hospitalization with infection, sepsis, severe sepsis, infection-related ACLF, infection-related AKI, albumin, sodium, MELD score, leukocyte count, mean arterial pressure at infection diagnosis) by logistic regression analysis (OR 2.11, 95% CI 1.09–4.09).

When survival analysis was carried out among patients with at least one severe infection during follow-up, no significant difference was observed between ALD and non-ALD cirrhotics with respect to infection-related mortality

(Fig. 3). When ALD patients with active alcohol misuse following the diagnosis of cirrhosis and at least one severe bacterial infection during follow-up were compared with abstinent ALD patients with respect to infection-related in-hospital mortality, no significant difference was observed (38 vs. 25%, $P=0.120$).

Bacterial resistance patterns in culture-positive severe bacterial infections

In all, 261/398 of all severe bacterial infections had a positive bacterial culture [169/272 (62%) in ALD patients vs. 92/126 (73%) in non-ALD patients, $P=0.041$]. The proportion of infections caused by gram-positive strains was significantly higher in ALD compared with non-ALD patients (Table 3). Resistance to piperacillin–tazobactam, carbapenems, and third-generation cephalosporins was more common in severe infections occurring in ALD versus non-ALD patients (Table 3).

After adjustment for confounders (infection site, comorbidity, HCA/HA infection, second infection, ongoing antibiotic use, MELD score at infection diagnosis, performance of an invasive procedure within 24 h before infection diagnosis) by logistic regression analysis, alcoholic cirrhosis was an independent predictor of the occurrence of infections caused by Gram-positive bacteria (OR 2.20, 95% CI 1.24–3.93), and *Enterococcus* (OR 2.66, 95% CI 1.11–6.40) in particular, but not resistance to piperacillin–tazobactam (OR 1.66, 95% CI 0.41–6.76) or carbapenems (OR 6.04, 95% CI 0.88–41.45). Among patients with decompensated status upon diagnosis of infection, severe infections caused by Gram-positive bacteria (68 vs. 37%, $P=0.007$) or *Enterococcus* (19 vs. 5%, $P=0.077$) occurred more frequently in those with ALD versus non-ALD cirrhosis. Similarly, among patients with decompensated disease upon diagnosis of infection, those with ALD more often experienced severe infections caused

by Gram-positive bacteria (53 vs. 41%, $P=0.188$) or *Enterococcus* (22 vs. 12%, $P=0.143$) compared with those with non-ALD cirrhosis, although these differences did not reach statistical significance.

Discussion

In our population-based 10-year cirrhotic cohort, we found an increasing occurrence of bacterial infections over time in both ALD and non-ALD cirrhosis. The severity of infections and infection-related in-hospital mortality also seems to be increasing, mainly in alcoholic cirrhotics. Our results suggest that the observed increased occurrence of severe bacterial infections in ALD compared with non-ALD cirrhosis is related to increased liver disease severity observed in the former and not cirrhosis etiology *per se*. However, pneumonia is associated independently with ALD cirrhosis and this is also true for infections caused by Gram-positive bacteria, such as *Enterococcus*.

Our findings are consistent with those of a recent study from the USA showing increasing occurrence of bacterial infections among hospitalized cirrhotics [2]. One explanation for this may be the observed increasing frequency of HCA infections in these patients. An increase in this type of infections, possibly as a result of the frequent performance of invasive procedures, has also been suggested in previous reports [13,27]. Our results further indicate that this increase is more evident in ALD compared with non-ALD cirrhosis. In contrast with published data from the USA [2], our study shows an increase in infection-related in-hospital mortality in patients diagnosed with cirrhosis during the later years, particularly in patients with ALD cirrhosis. In turn, this could be related to the increasing rates of sepsis, severe sepsis, and, particularly, infection-related ACLF, which is known to be associated with high mortality rates [17]. Interestingly, this increase in infection morbidity and mortality occurred in our cohort despite a trend for decreasing disease severity at diagnosis of cirrhosis during the study period.

Alcohol-induced alterations in both innate and adaptive immune cell functions result in impaired host defense to invading pathogens [4]. Furthermore, acquired C3 deficiency, impaired function of macrophage receptors, and neutrophil dysfunction have been shown to predispose ALD cirrhotics to infections [29–31]. In our study, although severe infections were experienced more frequently by ALD compared with non-ALD patients, no independent relation was found between cirrhosis etiology and the occurrence of bacterial infections following adjustment for severity of cirrhosis. Our findings indicate that susceptibility to bacterial infections is associated more with liver disease severity than etiology, in agreement with previously published data [3,9,11–13].

Our findings that ALD cirrhosis is an independent predictor of pneumonia are novel and in line with data on the impact of alcohol on pulmonary immunity and its role in predisposing noncirrhotic individuals to bacterial pulmonary infections [32]. Our results suggest that physicians should maintain a high level of clinical suspicion of pneumonia in hospitalized cirrhotics with ALD and they underline the need for vaccination of these patients against pneumococcus. However, data on smoking history of the patients in our cohort were not available. There is a high

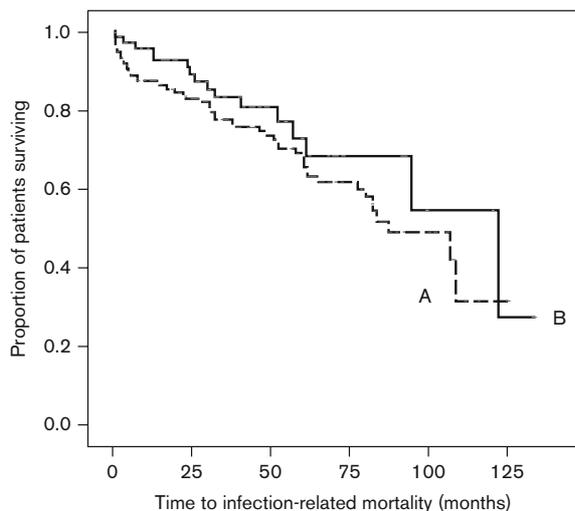


Fig. 3. Relationship between etiology of cirrhosis and infection-related mortality. Survival of patients with ALD (A) and non-ALD cirrhosis (B) with at least one severe bacterial infection during follow-up. Infection-related death was considered to be an event. Patients were censored at noninfection-related death, transplantation, or the end of follow-up. $P=0.177$. ALD, alcoholic liver disease.

Table 3. Microbiologic findings in culture-positive severe bacterial infection episodes occurring in cirrhotic patients with alcoholic liver disease and nonalcoholic liver disease

	ALD cirrhosis (n = 168)	Non-ALD cirrhosis (n = 92)	P-value
Gram-negative bacilli	63 (37)	51 (55)	0.006
Enterobacteriaceae resistant to at least one β -lactam	8 (5)	3 (3)	0.752
QRGNR	1 (1)	10 (11)	< 0.001
Gram-positive cocci	94 (56)	36 (39)	0.014
<i>Enterococcus</i>	36 (21)	8 (9)	0.009
Ampicillin-resistant <i>Enterococcus</i>	17 (10)	2 (2)	0.023
Gram-negative and Gram-positive strains	11 (6)	5 (5)	0.794
Third-generation cephalosporin resistant strains	60 (36)	25 (27)	0.167
Carbapenem-resistant strains	20 (12)	2 (2)	0.009
Piperacillin-tazobactam resistant strains	22 (13)	5 (5)	0.057
Quinolone-resistant strains	44 (33)	27 (33)	1.000
Bacteria causing antibiotic-resistant infections ^a	22 (13)	14 (15)	0.708

Data presented as n (%).

ALD, alcoholic liver disease; QRGNR, quinolone-resistant Gram-negative rods.

^aSee the Materials and methods section for definition.

rate of co-occurrence between smoking and alcohol use [33] and smoking is a well-recognized risk factor for the development of pneumonia [34]. Thus, it is conceivable that this may have confounded our findings.

Previous studies in noncirrhotic individuals with pneumonia have reported a more severe course in patients abusing alcohol [32]. Although two studies in cirrhosis have shown poor outcome in patients with ALD and bacteremia [7] or meningitis [8], most existing data show no difference with respect to infection-related morbidity and mortality between infected patients with ALD and non-ALD cirrhosis [5,6,19,20,35]. Similarly, we did not find any relation between bacterial infection severity features and cirrhosis etiology in our cohort. Nevertheless, length of hospital stay was more often prolonged in bacterial infections occurring in patients with ALD cirrhosis in our cohort, which indicates that bacterial infections in these patients may cause increased utilization of hospital resources [2].

Interestingly, infections caused by Gram-positive bacteria were more common in patients with ALD cirrhosis, which can probably be explained by the higher occurrence of pneumonia and enterococcal infections in these patients. In turn, the more frequent occurrence of *Enterococcus* in culture-positive infections in ALD cirrhosis could explain the higher rates of bacterial resistance to commonly used antibiotics in these infections. Previous studies have established an association between alcoholism and bacterial resistance to antibiotics in noncirrhotic individuals [14,15] and alcohol misuse has been defined as a risk factor for drug-resistant *Streptococcus pneumoniae* infection [16]. Previous data derived from small cirrhotic cohorts have reported higher rates of resistant bacteria and *Enterococcus* in spontaneous bacterial peritonitis occurring in ALD compared with non-ALD patients [36,37]. Taken together, our and previous findings suggest the need for a heightened suspicion of Gram-positive and potentially resistant organisms in patients with ALD cirrhosis hospitalized with bacterial infections.

The present study has certain limitations, mostly related to its retrospective nature. Although the proportion of patients with ALD cirrhosis in our cohort is similar to that in previous Scandinavian studies [22], it is conceivable that some patients may have concealed their alcohol consumption, leading to misclassification. Similarly, patients considered to be abstinent

following the diagnosis of cirrhosis, may have had ongoing alcohol consumption not captured by laboratory tests performed at the discretion of the caring physician or at other healthcare episodes during follow-up. The occurrence of infection-related AKI and ACLF in the current report may be underestimated, although they were similar to those reported in previous prospective studies [11,17,19]. Moreover, specimens were sent for culture according to the clinical judgment of the team caring for the patients enrolled, which may have influenced bacterial resistance rates. Nevertheless, the proportion of culture-positive infections (~66%) in the present study was not lower than that reported in previous prospective studies [13,27]. Further large, prospective studies are needed to confirm our findings and to fully delineate the potential impact of alcoholic etiology of cirrhosis on the occurrence, localization, outcome, and resistance patterns of bacterial infections.

In conclusion, bacterial infections are more common in ALD cirrhotics, but this appears to be related to the increased cirrhosis severity in these patients. However, ALD cirrhosis is related to the development of pneumonia as well as infections caused by gram-positive bacteria with more challenging resistance patterns. Although the etiology of cirrhosis *per se* does not appear to have any major impact on infection outcome, infections in ALD cirrhosis may lead to prolonged length of hospital stay, which in turn could lead to increased resource utilization [2]. This is of particular concern in the light of the observed increasing burden of bacterial infections in cirrhosis over time, particularly in ALD patients.

Acknowledgements

This study was supported by a grant from Region Skåne (Government), the Swedish Society of Medicine and Royal Physiographic Society in Lund, Region Skåne.

Conflicts of interest

There are no conflicts of interest.

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Paper IV

Dysfunction of Circulating Polymorphonuclear Leukocytes and Monocytes in Ambulatory Cirrhotics Predicts Patient Outcome

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Received: 11 December 2015 / Accepted: 11 March 2016
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Abstract

Background Cirrhosis represents a state of functional immune paresis with increased infection risk.

Aims To investigate polymorphonuclear (PMN) leukocyte and monocyte function in ambulatory cirrhotics, and their potential relation with cirrhosis etiology or patient outcome.

Methods Consecutive ambulatory cirrhotics without current or recent (<1 month) infection or acute decompensation were prospectively enrolled in 2013 and followed for a median time of 20 months until death, transplant or end of 2014. Oxidative burst and phagocytosis of circulating PMNs and monocytes were investigated at baseline and after *in vitro* *Escherichia coli* stimulation. Seventeen healthy blood donors served as controls. Baseline clinical and laboratory data as well as follow-up data on the

development of cirrhosis complications, including acute-on-chronic liver failure (ACLF), and bacterial infections were collected.

Results Sixty patients were included (70 % male, median age 63 years, 52 % with alcoholic cirrhosis). Compared to controls, cirrhotics showed increased resting and stimulated burst as well as reduced phagocytosis of PMNs, and increased stimulated monocyte burst ($p < 0.05$ for all). Alcoholic etiology was not related to PMN or monocyte dysfunction ($p > 0.05$ for all). In Cox regression analysis, increased stimulated monocyte and PMN burst were independent predictors of sepsis, severe sepsis and ACLF occurrence. Also, increased stimulated monocyte burst was associated with worse transplant-free survival ($p < 0.05$ for all).

Conclusions Stimulated PMN and monocyte oxidative burst are increased in ambulatory cirrhotics without acute decompensation. In turn, these changes are associated to sepsis and ACLF occurrence.

Electronic supplementary material The online version of this article (doi:10.1007/s10620-016-4132-3) contains supplementary material, which is available to authorized users.

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Keywords Liver cirrhosis · Bacterial infection · Polymorphonuclear leucocytes · Monocytes · Outcome

Abbreviations

ACLF	Acute-on-chronic liver failure
ALD	Alcoholic liver disease
AUDIT	Alcohol use disorders identification test
AUROC	Area under the ROC curve
CI	Confidence interval
HR	Hazard ratio
MFI	Median fluorescence intensity
PMA	Phorbol-12-myristate-13-acetate
PMN	Polymorphonuclear leukocytes
ROC	Receiver operating characteristics
ROS	Reactive oxygen species
TNF α	Tumor necrosis alpha

Introduction

Cirrhotic patients are prone to bacterial infections, which are associated with significant morbidity and mortality [1]. The increased risk of infections appears to be secondary, at least in part, to impairment of several host defense mechanisms [2]. Polymorphonuclear leukocytes (PMNs) are recruited to inflammatory sites and, upon activation by pro-inflammatory mediators, they produce reactive oxygen species (ROS) to eradicate pathogens. Whilst derangements in migration and phagocytosis of PMNs have been demonstrated in decompensated patients admitted due to cirrhosis complications [3–5], particularly in patients with alcoholic hepatitis [4], existing data on defects in innate immunity in ambulatory cirrhotics without acute decompensation are not unanimous. Some studies report high resting oxidative burst and/or reduced phagocytic capacity of PMNs [5–8], whereas others show normal PMN function [9]. Impaired phagocytosis and high resting burst of PMNs have been demonstrated to be associated with increased risk of infection and mortality in hospitalized cirrhotics with alcoholic hepatitis [4], as well as in a cohort comprising both patients with stable cirrhosis and acute-on-chronic liver failure (ACLF) [5]. However, the potential relationship between patient outcome and deranged PMN function has been poorly investigated in exclusively ambulatory cirrhotic patients without acute decompensation.

Recently, monocytes have been shown to be spontaneously activated in hospitalized Child–Pugh C cirrhotics with ascites, producing tumor necrosis factor alpha (TNF α) [10]. In patients with ACLF, monocytes have been reported to be functionally deactivated which may be associated with adverse outcome [11]. However, data on potential abnormalities in monocyte function in ambulatory cirrhotic

patients without acute decompensation are very limited and derive mainly from old reports [12], while their prognostic significance in this group remains unknown.

The primary aim of our study was to investigate PMN and monocyte function in ambulatory cirrhotic patients without acute decompensation, in relation to clinical characteristics such as liver disease etiology and pro-inflammatory cytokine levels. Furthermore, we aimed to study the potential relation of PMN and monocyte dysfunction with the occurrence of bacterial infections and mortality in these patients.

Patients and Methods

Patient Selection

Consecutive, ambulatory patients with cirrhosis recruited from the liver outpatient clinic at Skåne University Hospital, Sweden, between April and October 2013 were included in a prospective longitudinal cohort study. The diagnosis of cirrhosis was established histologically or based on a combination of at least 2 of the following: clinical, biochemical, and imaging data. Only ambulatory patients without acute decompensation in the last 30 days were included. Thus, exclusion criteria were age <18 or >75 years, severe complications of cirrhosis such as gastrointestinal bleeding, hepatorenal syndrome or hepatic encephalopathy (West-haven grade >1) in ≤ 30 days, bacterial infection or surgery in ≤ 30 days, and severe trauma or blood transfusion in ≤ 10 days. Patients treated with systemic antibiotics (including those on prophylactic antibiotic therapy), steroids or any other immunosuppressive medications or those with hepatic or extrahepatic malignancy, or severe systemic diseases (such as severe chronic heart failure) were also excluded. Ongoing infection was excluded upon enrollment by means of clinical examination, laboratory investigations and, if necessary, by imaging tests (such as chest X-ray). All patients were examined with abdominal ultrasound and, if ascites was detected, diagnostic paracentesis was performed to rule out spontaneous bacterial peritonitis. The study was approved by the local ethics committee and all patients gave written informed consent.

Data Collection

On the day of inclusion in the study, data on demographics, comorbid illness, etiology and history of complications of cirrhosis were collected. If there were a history of ascites, encephalopathy, variceal bleeding or jaundice, patients were considered to have decompensated disease. Drinking habits were assessed by means of the alcohol use disorders

identification test (AUDIT) [13]. An AUDIT score ≥ 8 was considered to be in keeping with alcoholic liver disease (ALD). All patients with ALD-cirrhosis in our cohort had had a long history of heavy alcohol consumption (≥ 60 g ethanol per day for male patients and ≥ 20 g for female patients) for ≥ 10 years.

PMN and Monocyte Function

At the time of inclusion in the study, peripheral blood was collected in vacutainer tubes containing sodium heparin (Becton–Dickinson, New York, USA), for immediate assessment of phagocytosis and ROS production by peripheral blood PMNs and monocytes, using the Phagotest and Phagoburst assays, respectively (Glycotope Biotechnology, Germany) [4, 8]. The tests were performed on whole blood and evaluated using flow cytometry (Navios, with the CXP Software for acquisition, and the Kaluza software for analysis; Beckman Coulter, Brea, CA, USA). ROS production was investigated without stimulus at 37 °C (resting oxidative burst) and after activation with phorbol 12-myristate 13-acetate (PMA) or opsonised *Escherichia coli*. In the last step, red blood cells were lysed and the remaining cells were fixed. Finally, propidium iodide was added to facilitate single cell analysis. The collection gate was set to 15,000 cells. No patients were found to have ROS deficiency. Oxidative burst was determined by the percentage of cells producing ROS. Median values of fluorescence intensity (MFI) of the ROS-producing cells were used to quantify oxidative burst activity. Phagocytic activity was calculated from the percentage of cells undergoing phagocytosis. Phagocytic capacity was assessed by quantifying the number of *E. coli* bacteria engulfed per individual cell, expressed as the MFI. The collection gate was set to 10,000 cells. Data on phagocytosis were available in 46/60 (76 %) patients. Seventeen healthy blood donors served as controls. They had no liver disease or any other acute or chronic condition or acute alcohol intake, as per blood donation requirements in Sweden.

Cytokine Analyses

At the time of inclusion in the study, peripheral blood was collected in 5-ml EDTA or SST tubes to obtain plasma or serum, respectively. Before storage at -80 °C, the samples were centrifuged at 2200g for 10 min. Levels of the pro-inflammatory cytokines TNF α , IL-6, IL-8 were determined in serum (TNF α and IL-6) or plasma (IL-8) at an accredited laboratory (Clinical Immunology and Transfusion Medicine, Lund, Sweden) using the IMMULITE[®] 1000 system (Siemens, Germany). Limits of normal (based on analysis of 50 healthy blood donors, 25 females and 25 males) were

set as follows; IL-6 < 8 ng/L; IL-8 < 60 ng/L and TNF- α < 15 ng/L.

Follow-Up

Patients were followed prospectively until transplant, death or the end of 2014. First-time serious bacterial infection (i.e. infection resulting in or occurring during an inpatient hospital episode), first-time sepsis, severe sepsis, and ACLF during follow-up were recorded. Infections, sepsis and severe sepsis were defined according to conventional criteria [14]. ACLF was defined according to the criteria established by the CANONIC study [15].

Statistics

Data are expressed as median and interquartile range or number of patients and percentage, as appropriate. Group comparisons were performed using the Fisher exact test for categorical and the Mann–Whitney *U* test for continuous variables. The Spearman's rank correlation coefficient rho (*r*) was calculated for correlation analysis. The relation between PMN and monocyte function, on the one hand, and patient outcome, on the other hand, was assessed with Cox regression analysis. Adjustment for potential confounders (i.e. age, gender and MELD score) was performed by further Cox regression analysis in the cases of variables of cell function that were univariately related to patient outcome at $p < 0.05$. The utility of *E. coli*-stimulated burst in predicting the occurrence of outcome variables within the first year after recruitment was assessed by means of receiver operating characteristics (ROC) analysis. Survival analysis was performed using the Kaplan–Meier method and groups were compared with the log-rank test. All tests were 2-tailed and were conducted at a 5 % significance level.

Results

Patient Population

A total of 60 outpatients were included. The majority had Child–Pugh class A cirrhosis (83 %) and about half had ALD cirrhosis (Table 1). None of the patients had been hospitalized due to a liver-related reason within 3 months prior to inclusion in the study.

Increased Oxidative Burst and Reduced Phagocytosis in Cirrhotic Patients Compared to Controls

Compared to healthy controls, cirrhotics showed increased resting and *E. coli*-stimulated PMN burst activity (Fig. 1a)

as well as reduced phagocytosis of opsonized *E. coli* (Table 2). A similar pattern was observed in monocytes, with an increased *E. coli*-stimulated burst (Fig. 1b) and a tendency towards decreased phagocytic capacity (Table 2).

Relationship of PMN and Monocyte Function with Severity and Alcoholic Etiology of Cirrhosis

Both resting and PMA-stimulated monocyte burst was higher in patients with prior or current decompensation versus those without (Table 2). Phagocytic capacity of PMNs and monocytes did not differ significantly between the two groups (Table 2). Since the group of patients with prior or current decompensation was heterogeneous (MELD scores ranging from 6 to 17), analyses were repeated between patients with MELD score ≤ 6 versus MELD score >6 (MELD score = 6 was the median MELD value at study inclusion) showing lower phagocytic capacity of both PMNs and monocytes in the latter group, although results did not reach statistical significance [MFI 58.2 (39.7–67.5) versus 49.9 (40.3–79.8), $p = 0.816$ and 31.7 (18.3–39.1) versus 27.7 (16.2–43.2), $p = 0.965$, respectively]. Furthermore, the MELD and Child–Pugh scores correlated positively with the percentage of resting monocytes producing ROS ($r = 0.220$, $p = 0.097$ and $r = 0.390$, $p = 0.002$ respectively), and the latter

Table 1 Baseline characteristics of cirrhotic patients included in the study ($n = 60$)

Male gender	42 (70)
Age, years	63 (57–69)
Diabetes	21 (35)
Other comorbidity ^a	35 (58)
Etiology of cirrhosis	
ALD (or mixed)	31 (52)
Viral	7 (12)
Cryptogenic/NASH	14 (23)
Cholestatic	4 (7)
Other	4 (7)
Decompensation	23 (38)
Prior to study inclusion	10 (17)
Current	13 (21)
MELD score	6 (6–8)
Child–Pugh class A/B/C	50 (83)/8 (13)/2 (4)

Data are expressed as n (%) or median (interquartile range) as appropriate

ALD alcoholic liver disease, NASH nonalcoholic steatohepatitis, MELD model for end-stage liver disease

^a The most common comorbid conditions other than diabetes were: arterial hypertension ($n = 19$), cardiovascular disease ($n = 9$), and chronic obstructive pulmonary disease ($n = 4$)

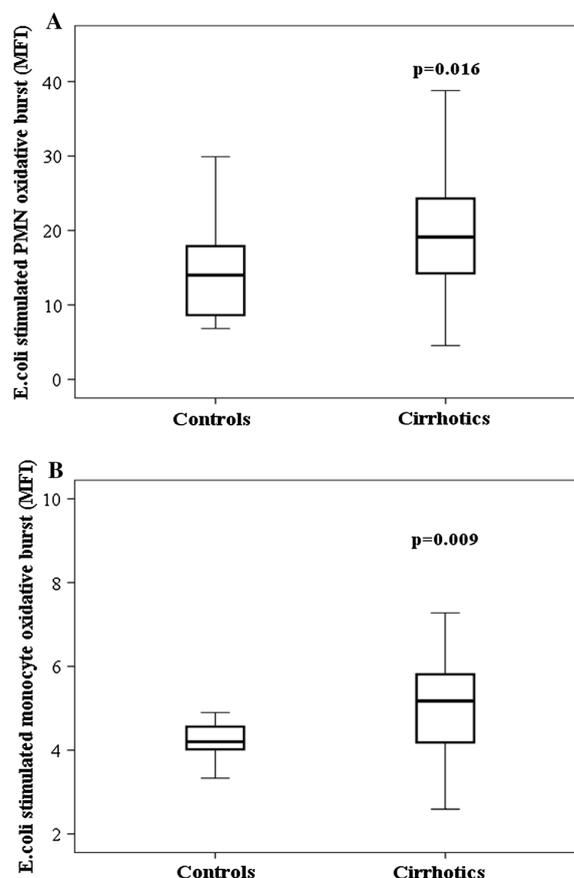


Fig. 1 *E. coli*-stimulated PMN (a) and monocyte (b) oxidative burst in cirrhotics ($n = 60$) and controls ($n = 17$). Cirrhotics showed increased *E. coli*-stimulated PMN and monocyte oxidative burst, as measured by MFI, compared to controls (19.1 vs. 14.0 and 5.2 vs. 4.2, respectively). PMN polymorphonuclear leukocyte, MFI median fluorescence intensity

correlated also with the percentage of resting PMNs producing ROS ($r = 0.391$, $p = 0.002$). No differences in PMN function were observed in patients with versus without prior or current decompensation (Table 2).

Alcoholic etiology was not significantly related to PMN or monocyte cell function (see Supplementary file).

Relation of PMN and Monocyte Function with Pro-Inflammatory Cytokine Levels

The percentage of resting PMNs producing ROS were correlated with IL-6 ($r = 0.289$, $p = 0.032$) and TNF α levels ($r = 0.346$, $p = 0.009$). Moreover, impaired phagocytic capacity of PMNs correlated with increased levels of TNF α ($r = -0.333$, $p = 0.024$). In the case of monocytes, the observed correlations did not reach statistical significance (see Supplementary file).

Table 2 Polymorphonuclear leukocyte and monocyte function in patients with cirrhosis, with or without decompensation, and in healthy controls

	Cirrhotics (<i>n</i> = 60)	No decompensation (<i>n</i> = 37)	Prior or current decompensation (<i>n</i> = 23)	Controls (<i>n</i> = 17)
MELD score		6 (6–7)	7 (6–12)	
ALD cirrhosis		14 (38)	17 (74) ^d	
PMN function				
Resting oxidative burst (%) ¹	0.5 (0.3–0.9)	0.4 (0.2–0.6)	0.6 (0.4–1.1)	0.2 (0.1–0.5) ^{a,c}
Resting oxidative burst (MFI)	3.4 (3.0–3.8)	3.3 (3.0–3.8)	3.4 (3.0–3.9)	3.3 (3.0–3.7)
<i>E. coli</i> oxidative burst (MFI)	19.1 (14.0–24.3)	18.4 (13.5–26.3)	19.6 (15.9–24.3)	14.0 (8.3–19.3) ^{a,b,c}
PMA oxidative burst (MFI)	16.5 (16.8–30.2)	16.5 (8.9–16.5)	19.4 (8.9–19.4)	19.9 (11.1–31.2)
Phagocytic activity (%) ²	95.9 (87.6–97.8)	95.9 (88.9–98.1)	95.6 (86.7–97.7)	98.0 (97.5–99.0) ^{a,b,c}
Phagocytic capacity (MFI)	51.9 (39.6–51.0)	48.3 (42.4–69.6)	64.1 (35.1–71.5)	69.6 (61.2–83.9) ^{a,b,c}
Monocyte function				
Resting oxidative burst (%) ¹	0.7 (0.3–1.9)	0.6 (0.1–1.4)	1.2 (0.4–3.9) ^d	1.0 (0.4–2.6)
Resting oxidative burst (MFI)	2.9 (2.5–3.2)	2.8 (2.5–3.1)	3.1 (2.8–3.1) ^d	2.5 (1.9–3.3)
<i>E. coli</i> oxidative burst (MFI)	5.2 (4.1–5.7)	5.0 (3.9–5.5)	5.2 (4.6–5.8)	4.2 (3.9–4.6) ^{a,b,c}
PMA oxidative burst (MFI)	4.3 (3.3–6.2)	3.8 (3.3–5.4)	4.9 (3.6–6.9) ^d	4.0 (3.5–4.6) ^c
Phagocytic activity (%) ²	89.3 (74.1–94.9)	89.2 (76.6–94.2)	88.8 (72.3–95.4)	90.0 (81.0–93.0)
Phagocytic capacity (MFI)	30.7 (17.1–39.7)	31.1 (19.7–31.5)	29.1 (15.2–29.1)	34.8 (31.1–40.2)
Cytokines				
S-IL-6 (ng/L)		5 (3–11)	9 (7–12) ^d	
P-IL-8 (ng/L)		6 (5–16)	7 (5–17)	
S-TNF α (ng/L)		13 (11–16)	15 (12–16)	

Data are expressed as *n* (%) or median (interquartile range) as appropriate. Cytokine levels were not measured in controls and data on phagocytosis were available in 23/31 patients with ALD or mixed, 6/7 with viral, 11/14 with cryptogenic/NASH, 4/4 with cholestatic and 2/4 with other cirrhosis (46/60, 76 % of all cirrhotics)

ALD alcoholic liver disease, MELD model for end-stage liver disease, PMN polymorphonuclear leukocyte, MFI median fluorescence intensity, PMA phorbol-12-myristate-13-acetate, IL interleukin, TNF tumor necrosis factor

^a *p* < 0.05 in cirrhotics versus controls

^b In patients without decompensation versus controls

^c In patients with prior or current decompensation versus controls

^d In patients with versus those without prior or current decompensation

¹ Percent of bursting cells

² Percent of cells undergoing phagocytosis

Relation of PMN and Monocyte Function with Patient Outcome

During a median follow-up of 20 months (range 3–21), 15 (25 %) patients experienced at least one serious bacterial infection (see Supplementary file for infection sites), 9 (15 %) sepsis, 5 (8 %) severe sepsis, 7 (12 %) ACLF (*n* = 4 infection-related ACLF; *n* = 3 ACLF due to gastrointestinal bleeding) and 8 (13 %) died or received a liver transplant. Neither PMN nor monocyte resting burst could predict the occurrence of serious bacterial infection during

follow-up in univariate Cox regression analysis (*p* > 0.05; data not shown).

PMN Function and Outcome

In Cox regression analysis, however, *E. coli*-stimulated PMN burst (MFI) was related to the occurrence of ACLF and severe sepsis. These associations persisted after adjustment for confounders [hazard ratio (HR) 1.15, 95 % confidence interval (CI) 1.04–1.28 and HR 1.12, 95 % CI 1.03–1.22, respectively]. Similarly, in ROC analysis,

E. coli-stimulated PMN burst ≥ 21.5 *E. coli* (MFI) could predict the occurrence of ACLF [area under the ROC (AUROC) curve 0.87, 95 % CI 0.72–1.00] and severe sepsis (AUROC 0.89, 95 % CI 0.77–1.00) within the first year following inclusion in the study, with a sensitivity of 100 % and a specificity of 69 % (Fig. 2a, b and Supplementary figure 1).

Monocyte Function and Outcome

In adjusted Cox regression analysis, *E. coli*-stimulated monocyte burst (MFI) was found to be related to the occurrence of sepsis (HR 1.23, 95 % CI 1.04–1.45), severe sepsis (HR 1.32, 95 % CI 1.08–1.62), and ACLF during follow-up (HR 1.49, 95 % CI 1.16–1.89). Similarly, increasing *E. coli*-stimulated monocyte burst and lower monocyte phagocytic activity were related to worse transplant-free survival even after adjustment for confounders (HR 1.35, 95 % CI 1.08–1.68 and HR 0.92, 95 % CI 0.84–0.99, respectively). In ROC analysis, *E. coli*-stimulated monocyte burst (MFI) could predict the occurrence of severe sepsis (AUROC 0.89, 95 % CI 0.76–1.00), ACLF (AUROC 0.88, 95 % CI 0.74–1.00) and transplant or death (AUROC 0.88, 95 % CI 0.74–1.00) within a year following inclusion in the study (see Supplementary figure 2). A cut-off of ≥ 5.58 in *E. coli*-stimulated monocyte burst (MFI) demonstrated the best sensitivity 100 % and specificity 74 % to predict 1-year mortality or transplant and the occurrence of ACLF. However, in survival analysis (extending over 1 year), the differences between groups did not reach statistical significance (Fig. 3a, b). The same cut-off was used to predict the occurrence of severe sepsis (sensitivity 100 % and specificity 74 %) within a year (Fig. 3c).

Discussion

The findings of this prospective longitudinal cohort study indicate that circulating PMN function is impaired in ambulatory patients with cirrhosis without acute decompensation, as evidenced by reduced phagocytosis. Monocyte- and PMN-stimulated oxidative burst are enhanced with the former being further increased following decompensation. The degree of functional impairment of PMNs seems to correlate with pro-inflammatory cytokines. Functional impairment of PMNs and monocytes predicts patient outcome in terms of sepsis development but also ACLF and mortality.

Resting and stimulated oxidative burst of circulating PMNs have previously been shown to be increased in hospitalized cirrhotics with complications of cirrhosis [4, 16]. In ambulatory cirrhotic patients without acute

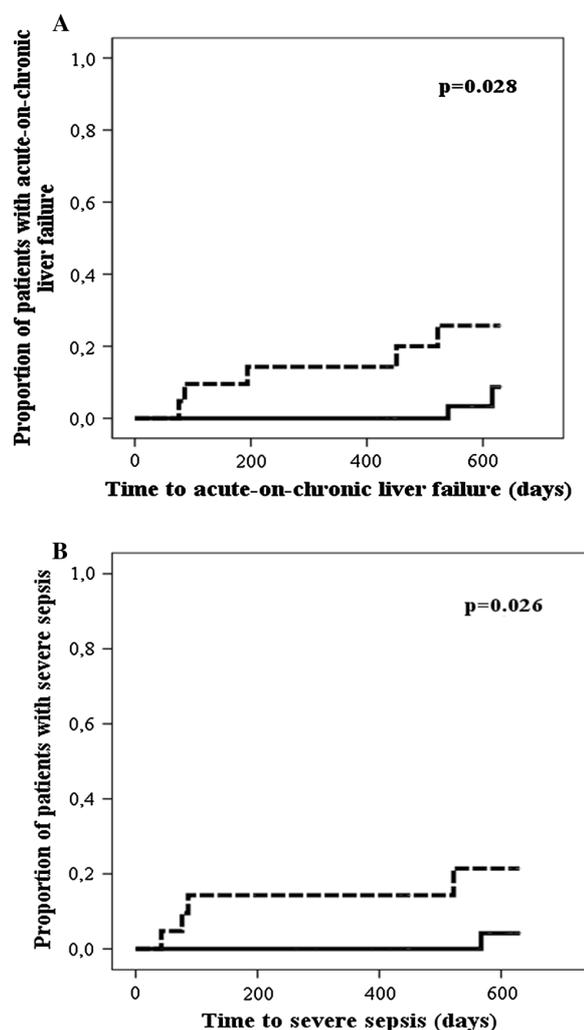
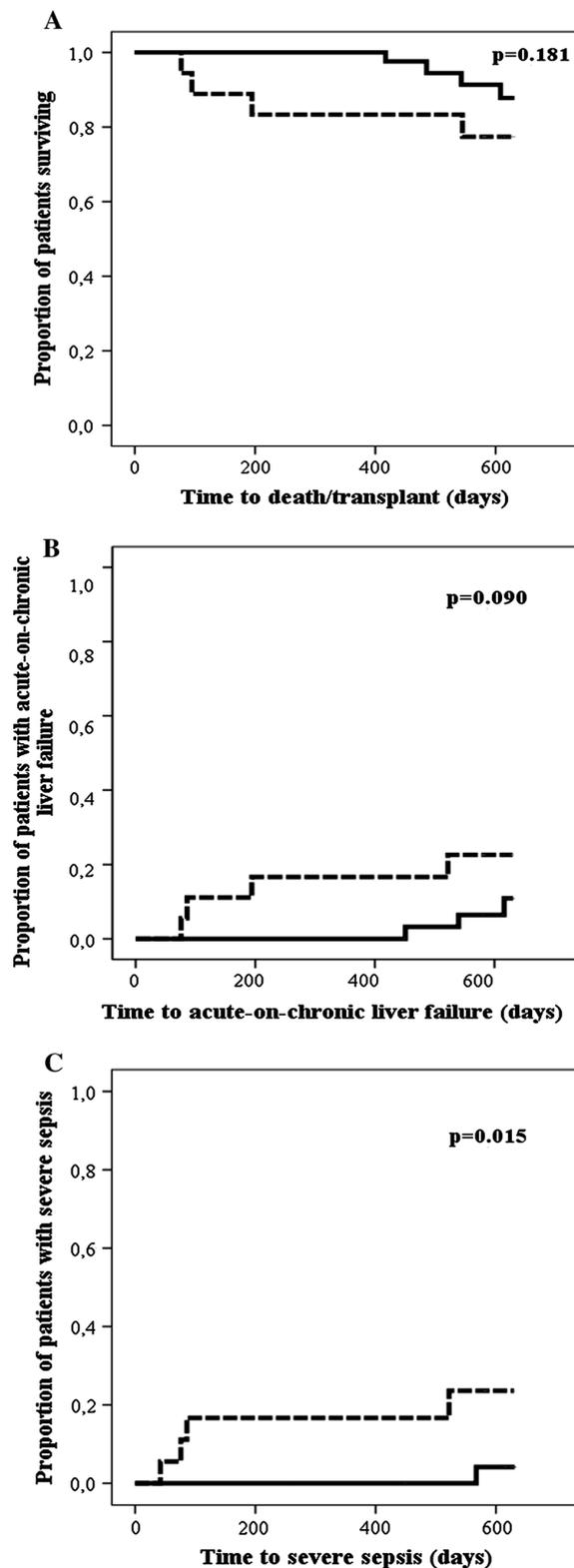


Fig. 2 Relationship between *E. coli*-stimulated PMN burst and patient outcome. Development of acute-on-chronic liver failure (a) and severe sepsis (b) during follow-up in patients with *E. coli*-stimulated PMN burst (MFI) ≥ 21.5 (dashed line) or < 21.5 (continuous line) at inclusion in the study. Patients were censored at death, transplant or end of follow-up. PMN polymorphonuclear leukocyte, MFI median fluorescence intensity

decompensation, however, published data are not unanimous, with some studies reporting normal [9], and others increased PMN oxidative burst [8]. In our cohort of ambulatory cirrhotics without evidence of acute decompensation, both resting and *E. coli*-stimulated oxidative burst of PMNs were increased compared with healthy controls. Similarly, circulating monocytes showed increased *E. coli*-stimulated oxidative burst in line with a previous study on hospitalized cirrhotics [16]. Phagocytic function was impaired both in PMNs and monocytes, but this reached statistical significance only in the former.



◀ **Fig. 3** Relationship between *E. coli*-stimulated monocyte burst and patient outcome. Transplant-free survival (a), development of acute-on-chronic liver failure (b), and severe sepsis (c) during follow-up in patients with *E. coli*-stimulated monocyte burst (MFI) ≥ 5.58 (dashed line) versus < 5.58 (continuous line) at inclusion in the study. Death or transplant was considered event in (a) and patients were censored at the end of follow-up. In (b, c), patients were censored at death, transplant or end of follow-up. MFI median fluorescence intensity

Although PMN phagocytosis has been reported to be normal or even increased in some studies [9, 17, 18], our findings are in keeping with two recent reports in which PMNs showed impaired phagocytosis [5, 8]. Differences among published studies regarding PMN phagocytosis in cirrhosis may be attributed to the complexity of neutrophil function that may be influenced by several factors [19], which warrant further investigation.

In our study, we observed a higher resting and PMA-stimulated monocyte oxidative burst in patients compared to those without prior or current decompensation, which is a novel finding. Also, phagocytic capacity of PMNs was lower in patients with more severe liver disease which, although it did not reach statistical significance, is in keeping with previously reported data [3, 5, 6, 8]. The lack of statistical significance of the latter finding could, possibly, be explained by the fact that patients in our cohort had predominantly Child–Pugh A cirrhosis and low MELD scores, compared to the wider distribution of patients across the cirrhosis severity spectrum in previous reports [5, 8]. Our finding that both increased resting oxidative burst and impaired phagocytic capacity of PMNs correlated with increasing pro-inflammatory cytokine levels is in accordance with previous observations [8]. In our study, however, the observed correlations between monocyte function and pro-inflammatory cytokine levels were weak and it remains unclear to what extent monocyte functional impairment in cirrhosis may be linked to inflammation (which is characteristic of cirrhosis). Recently, inflammation in cirrhosis has been shown to be associated with higher PMN expression of toll-like receptors, due to binding of ligands such as endotoxin or other pathogens, present in cirrhotic plasma, which in turn enhances the production of pro-inflammatory cytokines [4, 8]. Further studies are warranted to fully delineate the pathophysiology of monocyte impairment in cirrhosis.

Chronic alcohol intake may have an impact on PMN [20] and monocyte function [21] in non-cirrhotic individuals. However, circulating PMN function has recently been reported to be similar in ALD and non-ALD cirrhosis [8]. Similarly, in the current study, we did not find any impact of cirrhosis etiology (ALD vs. non-ALD) on circulating PMN or monocyte function. However, our study was not designed to address this question and the ALD and non-

ALD groups were not matched. In addition, the heterogeneity of patients with non-ALD cirrhosis may have also impacted our findings.

Defects in PMN function have been shown to be associated with a significantly greater risk of infection and mortality in hospitalized cirrhotics with alcoholic hepatitis [4]. Also, increased resting oxidative burst has been reported to be related to worse mortality in cirrhosis in a previous study including both stable cirrhotics and patients with ACLF [5]. Our data extend these findings to only ambulatory cirrhotics without acute decompensation by showing that stimulated PMN oxidative burst predicts the occurrence of severe sepsis and ACLF. The products of oxidative burst, though effective in first line defense against infection, may lead to bystander tissue damage and inflammation. Excessive activation of neutrophils has been shown to play a key role in the pathogenesis of organ failure in severe sepsis in non-cirrhotic patients [22]. We therefore hypothesize that increased circulating ROS in cirrhosis may lead to considerable damage to the endothelium which, in turn, may be responsible for secondary organ impairment. Furthermore, impaired monocyte function has been shown to be associated with poor clinical outcome and may account for the predisposition to infectious complications in both acute liver failure [23] and ACLF [11, 24]. Our findings that increased stimulated oxidative burst and reduced phagocytosis of monocytes are predictors of severe sepsis, ACLF, and mortality indicate a potential role for monocyte function as a prognostic biomarker in ambulatory cirrhotic patients without acute decompensation. To our knowledge, this is the first study assessing the impact of monocyte dysfunction on patient outcome in this patient group.

Certain study limitations need to be acknowledged. First, the majority of patients included were classified as Child–Pugh A. Although this makes it possible to draw conclusions regarding ambulatory patients without acute decompensation and less severe cirrhosis, it limits our results to this group. Also, analyses on phagocytosis were performed in a subgroup of patients (46/60, 76 %), which may also have influenced our findings. Pro-inflammatory cytokines were not measured in controls and, thus, it may be difficult to interpret the observed correlation between their levels and PMN function. Nevertheless, these findings are consistent with previous data in cirrhosis [8]. The groups of controls and cirrhotics were not matched for age, sex and bone mass index and, thus, we cannot exclude age-, sex- or bone mass index-related differences in impairment of PMN or monocyte function between these groups. However, our findings on impaired phagocytosis and increased oxidative burst of PMNs in patients with cirrhosis are in keeping with recent reports [5, 8, 16]. Hence, larger prospective studies are warranted in order to

elucidate the pathophysiology of PMN and monocyte dysfunction in cirrhosis.

In conclusion, the function of both PMNs and monocytes is altered in ambulatory cirrhotic patients without acute decompensation. Our results extend previous findings on PMN function [4, 5] suggesting that defects not only in PMN but also in monocyte function may be predictors of the development of severe bacterial infections and of poor prognosis in ambulatory patients with cirrhosis. It is conceivable that the evaluation of treatment strategies aiming to identify and treat patients with PMN and/or monocyte impairment early in the course of cirrhosis may help reduce the occurrence of serious bacterial infections and mortality in these patients.

Financial support This study was supported by a grant from the Region Skåne (Government), the Swedish Society of Medicine, and the Royal Physiographic Society in Lund, Sweden. The work was independent of these grants.

Compliance with ethical standards

Conflict of interest None.

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Lund University, Faculty of Medicine
Doctoral Dissertation Series 2016:75
ISBN 978-91-7619-302-0
ISSN 1652-8220

