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END-STAGE LIVER DISEASE: THE HIDDEN IMMUNOSUPPRESSIVE CONDITION FROM AN EPIDEMIOLOGICAL UPDATE TO THERAPEUTIC MANAGEMENT MODELS

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ABSTRACT

The objective of this work was to describe the current epidemiology, evaluate the short and long-term prognosis and test novel strategies to treat infections in cirrhotic patients

Methods

We conducted four studies using two different cohort of cirrhotic patients collected prospectively and developing bloodstream infections (BSIs) at 19 centers from September-2014 to December-2015 (BICHROME) and all consecutive cirrhotic patients admitted for an episode of acute decompensation from January-2014 to March-2016 at S.Orsola-Malpighi Hospital, Bologna and at the “Infermi” Hospital, Rimini(BIC).

Results

The BICHROME study included 312 patients. Gram-negative bacteria, Gram-positive cocci (GPC) and *Candida* spp. caused 53%, 47% and 7% of episodes, respectively. At multivariate analysis factors independently associated to GPC isolation were alcoholic cirrhosis (p=0.03), device-related infection (p=0.007), pneumonia (p=0.02), previous hepatorenal syndrome (p=0.03) and diabetes with organ damage (p=0.008)

The 30-day mortality rate was 25% and best predicted by the CLIF-SOFA score (aROC 0.82). In a Cox-regression model, delayed (>24h) antibiotic treatment (p<0.001), inadequate empirical therapy(p<0.001) and CLIF-SOFA(p<0.001) were predictors of 30-day mortality. Among patients receiving adequate treatment with piperacillin-tazobactam or carbapenems, those receiving continuous or extended infusion of such antibiotics showed a significantly lower mortality rate even after adjusting for cofounding factors(p=0.04).

In the BIC study, among the 516 patients enrolled, 32% presented an infection. Multivariate logistic regression showed that MELD-Na (p=0.001), QuickSOFA (p=0.004), bacteremia (p=0.004) and isolation MDR pathogens (p=0.048) were independent predictors of acute-on-chronic liver failure(ACLF). Kaplan-Meyers curves showed that 1-year survival was similar in infected and non-infected patients without ACLF (71% vs 67%,p=0.337). As expected, 1-year survival was worsened by the presence of ACLF.

Conclusion

With this work we explored the current epidemiology of bacterial infection in cirrhotic patients, we identified risk factors for MDR pathogens and for BSI caused by GPC. We also assessed long-term prognosis of infection complicated or non-complicated by ACLF.

SUMMARY

INTRODUCTION

Liver cirrhosis is the 10th most common cause of death in Western world (1). Among the complications of the end-stage liver disease (ESLD), infection represents the leading cause of acute decompensation (2, 3) and is associated with a high mortality ranging from 12% to 52% (4, 5).

Despite these patients are particularly prone to develop bacterial and fungal infections(6), the cirrhosis of the liver is not commonly considered a major immunodepressive condition. However, patients with ESLD exhibit an important impairment of immune system. This condition, called cirrhosis-associated immune dysfunction(CAID) summarizes both local and systemic immune system alterations in liver cirrhosis that play a pivotal role in determining both the high incidence of infections and the ominous infections related mortality in this patient population (7, 8). Overall mortality of infected cirrhotic patients in around 30% at 1 month and more than 50% at 12 months (8). The high mortality rate of infections in cirrhotic patients is related not only to the direct effects of infections but, above all, to their pivotal role in triggering the condition of acute-on-chronic liver failure (ACLF). For this reason, infection is considered an important prognostic marker in patients with ESLD.

Another concerning feature of infections in cirrhotic patients is the growing prevalence of multidrug-resistant (MDR) or extensively drug-resistant (XDR) pathogens, which are associated with higher mortality, increased length of in-hospital stay and higher healthcare related costs if compared with infection caused by susceptible strains (9-11). In addition to these clinical features, the threat of MDR/XDR pathogens relies on their ability to rapidly spread to patients in absence of contact precautions. As a consequence, an important transmission of MDR gram-negative bacilli between patients is observed during outbreaks(12).

In this setting a multifaced approach is needed to face all the management challenges offered by patients with ESLD with infection. This include the knowledge of contemporary epidemiology, the development of prognostic tools and the testing of novel therapeutic strategies.

Epidemiology of bacterial infection in patients with liver cirrhosis

In light of the emerging threat of multidrug-resistant organisms (MDRO), mainly related the ominous spread of extended-spectrum beta-lactamase producing (ESBL) and carbapenem-resistant Enterobacteriaceae (CRE) and carbapenem resistant non-fermenting bacilli in the last decade, an increasing number of epidemiological studies were recently published. To better understand the evolution of epidemiology of bacterial and fungal infections in this setting the most representative studies are summarized in the table 1.

Table 1. Summary of epidemiological studies on patients with liver cirrhosis. Only studies including all different source of infection are reported

Studies published in the 90'								
Author/year/ Geographic area (ref)	Population	Most representative source of infection				Etiology (prevalence of MDRO)		
		SBP	UTI	LRTI	Primary BSI	Gram-negative	Gram-positive	Fungi
Caly/1993/ Brazil(13)	All cirrhotics	31%	25%	25%	NR	72%(NR)	28%	NR
Toledo/1994/ Spain(14)	All cirrhotics	44%	26%	10%	5%	65% (61% E.coli)	39%	NR

Studies published from 2000 to 2015								
Author/year/ Geographic area (ref)	Population	Most representative source of infection				Etiology (prevalence of MDRO)		
		SBP	UTI	LRTI	Primary BSI	Gram-positive	Gram-negative	Fungi
Borzio/2001/ Italy (15)	All cirrhotics	23%	41%	17%	21%	46%	49%	4%
Rosa/2000/ Brazil (16)	All cirrhotics	54%	7%	18%	NR	NR	NR	NR
Fernandez/ 2002/Spain (17)	All cirrhotics	24%	19%	13%	5%	45%	47%	NR
Fernandez(2012)/Spain(4)/ first series	All cirrhotics	56%	43%	20%	13%	44% (MRSA 3% of all infections)	46% (ESBL 9% of all infections)	NR

Fernandez/2012/Spain(4)/ second series	All cirrhotics	20%	25%	13%	13%	MRSA 7% of all infections	ESBL 7% of all infections	NR
Studies published from 2015 to 2017								
Author/year/ Geographic area (ref)	Population	Most representative source of infection				Etiology (prevalence of MDRO)		
		SBP	UTI	LRTI	Primary BSI	Gram-positive	Gram-negative	Candida spp
Merli/2015/ Italy(9)	All cirrhotics	8%	61%	12%	6%	47%	47%	NR
Park/2015/ Korea (18)	Alcoholic liver disease	9%	4%	38%	4%	35% (MRSA 86%)	63% (ESBL in 42% of Enterobacteriaceae)	2%
Dionigi/2017 /England (19)	All cirrhotics	42%	19%	9%	28%	58% (MRSA 18%)	41% (ESBL 20% of GNB)	NR
Salerno/2017 Italy and England (20)	All cirrhotics	18%	43%	7%	17%	58% (MRSA 51%)	47% (44% ESBL production ,9% CR-GNB	3%
Piano/2017/ Italy(21)	All cirrhotics	33%	23%	14%	13%	46%	47%	7%

The wide variability in term of site of infection and causative pathogens is mainly related to several factors. First, with exception of SBP, there is no agreement for most of infection definitions and most studies did not adopted the widely agreed criteria for infection diagnosis used in non-cirrhotic population. Second, the epidemiology of infection is currently under constant evolution and may vary between centres. Third, similarly to the previous point, different study site may be characterized by different level of commitment in the management of cirrhotic patients. Thus, tertiary sites with dedicated liver unit and access to a transplantation program may exhibit a population with more advanced stage of liver disease if compared with urban hospitals. Despite inhomogeneity, these studies clearly show that the rate of MDRO has increased dramatically and the improvement of the management of liver cirrhosis may have changed also the characteristics of infection site. In fact, in the studies published in the 90' and in the first years of the 21th Century the diagnosis of SBP was prevalent (24-56% of case). Conversely latter studies report a lower

prevalence of SBP (8-18%, excluding one paper that included bacteriascites in the definition of SBP and reported 42% of such infection) and higher rate of bloodstream infection (6-28%) and pneumonia (7-38%).

Few studies reported to date differences in the kind of infection and in the causative pathogens in patients with alcoholic liver disease (ALD) and patients with other causes of liver cirrhosis. Previous studies on BSI including mainly patients with alcoholic cirrhosis report a higher prevalence of gram-positive cocci (GPC) among the different etiologies of BSI. However most of these studies are old or characterized by a single-center design(22, 23). In addition, infection in alcoholic cirrhosis seems to be characterized by higher frequency of ACLF, however conflicting results on the outcome are reported(3, 24)

Risk factors for multidrug-resistant pathogens

To date few studies evaluated risk factors for MDRO in the setting of cirrhosis (table 2).

Table 2 Risk factors for multidrug-resistant pathogens in patients with liver cirrhosis and infection

AUTHOR/YEAR/ GEOGRAPHC AREA (REF)	KIND OF INFECTIONS	PREVALENCE AND KIND OF MDRO	RISK FACTORS
Merli/2015/ Italy (9)	All bacterial infections	51%	• Antibiotic prophylaxis • HA or HCA infections
Kim/2013/ Korea(25)	Community-onset SBP	32% of FQ resistant *E. coli*	• FQ use (30dd) • Previous SBP episode • Third-generation cephalosporin resistance
Fernandez/2012/ Spain(4)/ first series	All bacterial infections		• Nosocomial origin of infection • Long-term norfloxacin prophylaxis • Recent infection by multi-resistant bacteria

			• Recent use of b-lactams
Chaulk/2013/ Canada(26)	SBP	19% third-generation cephalosporin resistance	• Nosocomial acquisition of infection
Song/2009/ Korea (27)	SBP	7% ESBL-Enterobacteriaceae	• Nosocomial acquisition • Previous SBP episode
Alexopolu/2012/ Greece(28)	SBP	24%	• MELD score • HCA • Quinolone prophylaxis
Ariza/2012/ Spain(29)	HA and HCA SBP	42% third generation cephalosporine resistance of HA SBP	• Diabetes mellitus • Upper GI bleeding • Hospital acquired • Previous 3rd Gen Cephalosporine use

Most of the reported studies focused on SBP whereas only 2 studies included all various sources of infection. The most reported risk factors for MDR were antibiotic exposure (antibiotic prophylaxis, use of third generation cephalosporines, fluoroquinolones or beta-lactams) and exposure to healthcare environment (i.e. hospital-acquired or healthcare associated infections, previous hospital admission).

Infection related ACLF and prognosis

As mentioned before the high mortality rate of infections in cirrhotic patients is related not only to the direct effects of infections but, above all, to their pivotal role in triggering the condition of acute-on-chronic liver failure. In a prospective multicenter study (CANONIC study), bacterial infection was found to be the precipitating event of ACLF in 32% of cases(30). A further analysis of the CANONIC study revealed that bloodstream infections, pneumonia and SBP are more likely to be associated with ACLF. In addition, in patients with ACLF grade I and II, the presence of bacterial or fungal infection was associated with worse outcome. Similarly, single-center study, among patients with ACLF, infection was a risk factor for 30-day. Despite these findings, a better understanding of the interaction between bacterial infection and ACLF is needed. In fact, the specific role of different kind of infections in determining ACLF and its risk factors are not clearly established.

Infection is considered an important prognostic marker in patients with ESLD. In a in a large multicenter cohort of patients with biopsy-proven compensated viral cirrhosis, the occurrence of a bacterial infection impaired survival both in patients HCV-infected (5-year survival: 60.2% vs 90.4%, $p<0.001$) and HBV-Infected (5-year survival: 69.2% vs 97.6%, $p<0.001$), representing the third cause of death (14.1%) after liver failure and liver cancer. Similarly, in a single-center study enrolling 501 patients, bacterial infection was independently associated to mortality. The authors concluded that bacterial infection represents a different stage of the disease, which affect survival, even after recovery form an infectious episode (19). Despite the finding that bacterial infection is a marker of poor prognosis seems clearly established, several aspects are worth to be deeply investigated. In fact, the role of different kind of infections are yet to be established.

Antimicrobial Pharmacokinetics/ Pharmacodynamic issues in liver cirrhosis

Ensuring a prompt and appropriate empirical antimicrobial treatment for infections in liver cirrhosis is essential in LC. (31, 32)

The concept of appropriateness for empirical and targeted antimicrobial treatment relies on a right antimicrobial coverage associated to an appropriate exposure consistent with the drugs' pharmacokinetic-pharmacodynamic (PK/PD) features. Pharmacokinetic variability is a major contributor to therapeutic failure: therefore to guarantee a correct exposure to antibiotics, timely administration of the right dose at the right schedule, according to the pathophysiological and immunological status of the patient, is required. (33)

Patient with LC have several unique pathophysiological characteristics that can alter the PK/PD behavior and the *in vivo* activity of antimicrobial agents. These characteristics include: i) hypoalbuminemia and reduction binding to proteins; ii) altered distribution; iii) altered clearance of the antimicrobial. (34)

The reduction of antimicrobial protein binding is a consequence of decreased albumin production and accumulation of antibiotic binding inhibitors (such as bilirubin or α-acid glycoprotein) in patients with LC.(34) Depending on the degree of antibiotic protein binding, patients with LC may have, both in plasma and tissues, a higher fraction of unbound drug. This is the microbiologically active drug, but also the fraction that is cleared more rapidly through renal or hepatic pathways. Hence, patients with hypoalbuminemia have a higher proportion of drug "escaping" from the bloodstream and distributing into tissues, translating to increased distribution volume (Vd) and reduced or sometimes sub-therapeutic bloodstream concentrations required to treat severe infection. (35, 36)

In patients with advanced LC, splanchnic congestion and fluid retention due to hypoalbuminemia and reduced renal blood flow can further increase the Vd for relatively hydrophilic antibiotics, such as beta-lactams, aminoglycosides, and vancomycin. As a result, most of the patients with ACLF presents with oedema, ascites and third space expansion resulting in inadequate blood levels of these antibiotics. (36, 37) Therefore larger loading and daily doses and are often required for hydrophilic antibiotics to achieve therapeutic blood levels.

On the other hand, increased Vd may also prolong the drug elimination irrespective of the clearance rates. (35) In some patients with LC, antibiotics half-life is increased, paradoxically causing drug accumulation and potential for toxicity. (38)

Finally, the PK of antibiotics can be affected by liver-disease related changes in renal function that are very common in this population. Renal failure in LC is mainly due to a reduced renal perfusion secondary to a vasodilatation in the splanchnic circulation without a compensation of cardiac output.(39) Although clearance of creatinine is widely accepted as a viable method for renal function assessment, several studies demonstrate that measured creatinine clearance from timed urine collection may overestimate the glomerular filtration rate in LC even in patients without hepatorenal syndrome. (40)

Unfortunately, antibiotic PK/PD is rarely studied in patients with liver dysfunction, especially in patients with advanced cirrhosis and ascites (i.e. Child-Pugh Class C). This kind of patients are commonly excluded form phase 1, phase 2 and phase 3 studies. Consequently, there is currently little or no scientific basis for antibiotic doses currently administered to treat life-threatening infections in patients with advanced cirrhosis. Given the unpredictable drug exposure, therapeutic drug monitoring (TDM) might play a pivotal role for individualizing doses, both in lowering exposure-dependent toxicity and in ensuring an optimal drug exposure, especially for the treatment of serious infections or MDR pathogens.

Beta-lactams are commonly used and represent the first-line therapy of most infection in patients with liver cirrhosis(41). Beta-lactams are time-depending drugs which ensure the best effectiveness with a prolonged time of exposure above the pathogen minimal inhibitory concentration (T>MIC)(42). Previous studies in general population indicate that continuous or extended infusion of beta-lactams is associated to better drug exposure and higher T>MIC and consequently better outcome for severe infection(43).

According with the aforementioned pathophysiological characteristics, the cirrhotic patient seems an important setting to test continuous infusion of beta-lactams for treating severe infections.

OBJECTIVES OF THIS THESIS

The present thesis comprises 4 different studies.

The objectives of the studies are hereby reported:

1. **Bloodstream infection in cirrhotic patients: an exploratory prospective multicenter study (study already published: Bartoletti M, Giannella M, Lewis R, Caraceni P, Tedeschi S, Paul M, et al. A prospective multicentre study of the epidemiology and outcomes of bloodstream infection in cirrhotic patients. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2017. Epub 2017/08/19.)**

    - Describe the current epidemiology of BSI in patients with liver cirrhosis in a large multicenter study
    - Assess the best predictors of 30-day mortality in cirrhotic patients with BSI
    - Find universal risk factors for MDRO in cirrhotic patients with BSI

2. **Continuous infusion of beta-lactam antibiotics in cirrhotic patients with bloodstream infection: results from a prospective multicenter observational study**

    - Assess the effectiveness of continuous or extended infusion of beta-lactams in cirrhotic patients with BSI

3. **Differences in the etiology and outcome of bloodstream infection in patients with alcohol-related liver cirrhosis and non-alcoholic liver cirrhosis: results form a prospective multicenter study.**
    - Describe differences in the severity, source and outcome in patients with alcoholic liver disease and cirrhosis secondary to other conditions
    - Find risk factors for BSI caused by GPC

4. **Epidemiology of acute-on-chronic liver failure associated with bacterial infection in patients with liver cirrhosis: risk factors and outcome**

- Find risk factors for ALCF in patients with any bacterial infection or fungal infection
- Describe the long-term outcome of patients with or without bacterial or fungal infection complicated or non-complicated with ACLF.

STUDY 1. BLOODSTREAM INFECTION IN CIRRHOTIC PATIENTS: AN EXPLORATORY PROSPECTIVE MULTICENTER STUDY

METHODS

Study design

We performed an exploratory prospective, multicentre, observational cohort study with the endorsement of European Study Group of Bloodstream infections and Sepsis (ESGBIS). The ESGBIS members who agreed to participate were asked to report all consecutive patients with liver cirrhosis who developed a BSI, from September 2014 to December 2015. The study was approved by all local institutional review board in participating hospitals. Written informed consent was obtained from patients or from legal surrogates before enrolment.

Population

All adult (>18 years) patients with liver cirrhosis developing BSI were included in the study. The diagnosis of liver cirrhosis was based on previous liver biopsy results or a composite of clinical signs and findings provided by laboratory test results, endoscopy and radiologic imaging.(3)

Bloodstream infection was defined the growth of a non-common skin contaminant from ≥ 1 blood culture (BC) and of a common skin contaminant such as diphtheroids, *Bacillus* species, *Propionibacterium* species, coagulase negative staphylococci (CoNS), or micrococci from ≥ 2 BCs drawn on separate sites and reporting the same antimicrobial susceptibility test profile.

Patients with previous liver transplantation were excluded. Patients with subsequent episodes of BSI with an interval between BSIs lower than 3 months were excluded. Patients were followed-up to 30 days after the BSI onset. This latter was set at the day of blood cultures collection.

Data collection

Data were collected using an electronic case report form available at the study web site. The integrity of data was systematically checked by an investigator before being entered into the database by a monthly assessment of data completeness and consistency. In case of inconsistent or missing data, queries were generated and distributed to the participating site's investigators for reconciliation. The following variables were collected at the moment of enrolment using patient's medical records: demographic variables (sex, age); the cause and severity of liver disease according with the baseline model for end-stage liver disease (MELD); presence of hepatocellular carcinoma (HCC); presence of other co-morbidities according with the Charlson score(44); invasive procedure performed within 30 days before BSI onset were collected: gastrointestinal endoscopy including esophagogastroduodenoscopy, colonoscopy, endoscopic retrograde cholangiopancreatography, endoscopic ultrasound; transjugular intrahepatic portosystemic shunt (TIPS) insertion; biliary procedures including biliary percutaneous drainage and/or stenting; HCC treatments; date, ward and cause of hospitalization; epidemiological classification of BSI; infection severity according with Bone's score(45), sequential organ failure assessment (SOFA), chronic liver failure-SOFA (CLIF-SOFA) and MELD scores calculated at the day of drawing the positive BCs; pathogens isolated and their susceptibility patterns; antibiotics administered as empirical therapy and that as definitive therapy. Outcome variables were collected after 30 days from BSI onset during either bed-side evaluation, outpatient visit, or telephone call. These included the need of intensive care unit (ICU) admission, length of in-hospital stay and 30-day transplant-free mortality.

Definitions

MELD was calculated at the time of index BC (BSI-MELD) as previously described.(46) Baseline MELD was defined as the most recent MELD within 2 weeks prior BC drawn. The patient had to be free from symptoms of infection and/or acute decompensation. Δ- MELD was defined as the difference between BSI-MELD and baseline MELD.

Primary BSI was defined as the laboratory confirmed BSI that is not secondary to an infection at another body site after comprehensive screening with clinical findings, laboratory test (e.g. urine analysis, peritoneal fluid cell count) and radiological imaging (chest X-rays or computed tomography scan, abdomen echography). Source of secondary BSI was

defined according with Centres for Diseases Control and Prevention criteria(47), in addition: spontaneous bacterial peritonitis (SBP) was deemed as the source of BSI when the same organism is isolated form BC and peritoneal fluid, in presence of ≥ 250 polymorphonuclear cells/ml of fluid without other evident infection sources;(48) TIPS related BSI was defined as persistent (positive BC after ≥ 7 days despite adequate antimicrobial treatment) or relapse (growth of the same organism as in the original BC after the end of therapy but before day 30) bacteraemia in a patient with TIPS and no other known source of infection after a comprehensive diagnostic work-up.(49)

Sepsis grading was assessed with SOFA(50) and Bone's criteria. Sepsis was defined by at least 2 of the following: temperature >38C or <36°C, respiratory rate >20 or PaCO2 <32 mmHg, heart rate >90, altered mental status, systolic blood pressure <90 mmHg, leukocyte count >12.000/mm3, or <4,000/mm3 or immature forms >10%. Severe sepsis was defined as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion; septic shock was defined as sepsis-induced hypotension persisting despite adequate fluid resuscitation(45). CLIF-SOFA was calculated as described by Moreau et al.(3) Organ failures were assessed according with the following criteria: liver failure was defined by a serum bilirubin level of ≥12.0 mg/dL; kidney failure was defined by a serum creatinine level of ≥ 2.0 mg/dL or the use of renal replacement therapy; cerebral failure was defined by grade III or IV hepatic encephalopathy, according to the West Haven classification; coagulation failure was defined by an international normalized ratio 2.5 and/or a platelet count of <20 X109/L; circulatory failure was defined by the use of dopamine, dobutamine, or terlipressin.

Acute-on-chronic liver failure (ACLF) was diagnosed and classified as previously described.(3) Patients were classified as having : i) hospital acquired BSI if infection signs/symptoms started >48 hours after hospital admission, or in less than 48 hours after hospital discharge; ii) healthcare-associated BSI if they acquired bacteraemia outside the hospital but fulfilled any of the following criteria: prior hospitalization for ≥2 days or surgery in the past 90 days, residence in a nursing home or long-term care facility, intravenous therapy, wound care or specialized nursing care at home in the past 30 days, chemotherapy in the past 30 days and chronic haemodialysis; iii) community acquired BSI was defined for any other case.(51)

Gram-negative were classified as multidrug-resistant (MDR) according to the European Society of Clinical Microbiology and Infectious Diseases consensus definitions.(52) As for

Gram-positive bacteria: methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant CoNS and *Enterococcus faecium* were classified as MDR.

Empirical therapy, defined as treatment administration before the susceptibility tests were available, was considered as adequate when at least one antibiotic was active in vitro against the isolated pathogen and was administered within 24 hours after index BCs, at recommended dosages according with pharmacokinetic / pharmacodynamic drug properties for non-cirrhotic patients.(33, 36) Definitive therapy, treatment administration according with the susceptibility results, was considered as adequate when an active antimicrobial regimen, adjusted according to microbiological results, was administered until the end of antibiotic course (for at least 48 h).

Microbiology

Before study onset, the use of standard diagnostic methods was required and agreed with all the participating centres. They include at least the use of an automated detector system, the performance of Gram stain and/or rapid test (such as MALDI-TOF, PNA FISH) with immediate communication of the preliminary information to the attending physicians, the use of an automated system (Vitek n=17, MicroScan n=2) for susceptibility testing. Breakpoints, screening and conformation of the main mechanisms of resistance were done according with EUCAST guidelines.(53)

Statistical analysis

Categorical variables were presented as absolute numbers and their relative frequencies and were compared using the *chi*-square test. Quantitative variables were presented as mean and standard deviation (SD) if normally distributed or as median and interquartile range (IQR) if non-normally distributed. Non-normally distributed continuous variables were compared using the Mann-Whitney U test, normally distributed continuous variables were compared using the *t* test.

Analysis of predictors of mortality

The discrimination of six established mortality risk scores for all-cause 30-day mortality were analyzed calculating the area under the receiver operator curve (aROC). Risk scores with

the highest aROC were then evaluated in a multivariate Cox-regression model to identify the risk score with the best overall fit (Akaike information criterion) and discrimination (Harrell's C statistic) for 30-day all-cause mortality. Finally, factors associated (P<.1) to 30-mortality in the univariate analysis entered in a Cox-regression model including the best risk score previously identified. All variables were explored for interaction and collinearity. The impact of infection-related or treatment variables was assessed at three levels of baseline risk (low, medium and high) determined by classification-regression-tree (CART) analysis. Martingale residuals from the Cox model were used to calculate Chi-square values at all score cutpoints at P<.05.

The strength of association between specific pathogen resistance profiles and the inadequate antimicrobial therapy were analyzed using the Spearman's rank correlation coefficient and plotted as weighted bubble plot by pathogen prevalence.

Analysis of risk factors for MDRO

To assess the independent risk factors for MDRO isolation, all the variables with a p value ≤.10 at the univariate analysis were included in a forward, conditional stepwise multivariate logistic regression model. The validity of the final model was assessed by estimating goodness-of-fit to the data with the Hosmer-Lemeshow test.

All analysis was performed with Stata IC 13 (Stata Corp, College Station, Texas).

RESULTS

Patients recruitment and baseline characteristics

Nineteen of the 25 invited centres agreed to participate. Participating hospitals were all tertiary teaching facilities from Italy (10 centres), Spain (5 centres), Germany (2 centres), Croatia (1 centre) and Israel (1 centre).

A total of 323 patients were enrolled in the study. Excluded patients had incomplete data (7 cases), had a single BSI caused by CoNS (2 cases) or were recipient of liver transplant (2 cases). Thus, 312 patients were analysed (fugure 1).

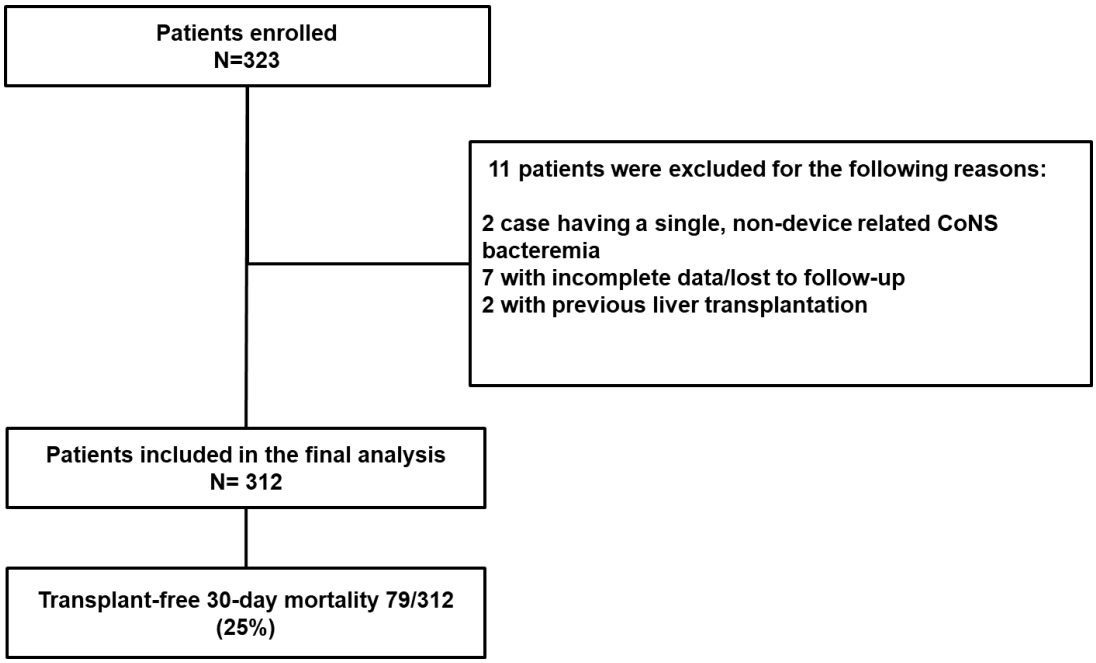

Figure 1 Study flow-chart

Overall, 204 (65%) were male, mean (±SD) age was 61(±12) years. Alcohol abuse and HCV infection were the cause of cirrhosis in the 40% and 36% of cases, respectively. The most common reasons for hospital admission were suspected bacterial infection (42%), acute decompensation non-related to bacterial infection (40%), or scheduled procedures (6%) (Table 3).

BSIs were classified as primary 99 (32%), catheter related 32 (10%) and secondary 181 (58%). Secondary BSIs included: intra-abdominal sources (99, 32%), mostly represented by SBP (50/99 50%) and cholangitis (26/99, 26%); urinary tract (35, 11%); lower respiratory tract (19, 6%); and others including endocarditis (11, 3%), skin and soft tissues (10, 3%),

TIPS (5, 2%), bone and joint infections (4, 1%) and surgical sites (4, 1%). Six patients had multiple sources reported for their BSI.

Thirty-day mortality

At the end of 30-day follow-up, 79/312 (25%) patients died. According with BSI source, SBP (18/50, 36%) and pneumonia (6/19, 31%), showed the highest mortality, together with primary BSI (30/99, 29%). Comparison of survivors and non-survivors showed that patients with worse outcome were more likely to be admitted for non-infectious causes, such as hepatic encephalopathy and hepato-renal syndrome. In addition, at BSI diagnosis non-survivors had more frequently ACLF, septic shock, higher CLIF-SOFA and higher Δ-MELD (table 3)

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Table 3. Characteristics of the entire cohort. Difference of underlying conditions, BSI data and therapeutic management between survivors and non-survivors.

	TOTAL, N=312 (100%)	**NON-SURVIVORS, N=79 (25%)**	**SURVIVORS N=233 (75%)**	***P***
Demographic data				
Age (years) [mean (± SD)]	61 (±12)	60 (±10)	62 (± 12)	.26
Male sex	204 (65)	52 (65)	152 (65)	.92
Liver disease [a]				
Hepatitis C	112 (36)	27 (34)	85 (36)	.71
Alcoholic	125 (40)	32 (41)	93 (40)	.96
Hepatocellular carcinoma	50 (16)	11 (14)	39 (16)	.72
Baseline MELD	15 (11-20)	18 (14-21)	14 (10-19)	.01
Admission diagnosis				
Ascitic decompensation	44 (14)	13 (16)	31 (13)	.48
Hepato-renal syndrome	14 (4)	7 (9)	7 (3)	.05
Hepatic encephalopathy	29 (9)	13 (16)	12 (5)	.01
Bacterial infection	131 (42)	20 (25)	111 (48)	.01
Co-morbidities				
Charlson index [mean (± SD)]	7 (±3)	7 (±3)	7 (±3)	.81
BSI data				
Site of infection acquisition				
Community-acquired BSI	60 (19)	12 (15)	48 (21)	.29
Hospital-acquired BSI	170 (54)	50(63)	120 (51)	.06
Healthcare associated	82 (26)	17 (21)	65 (28)	.30
Primary	99 (32)	30 (38)	70 (30)	.19
SBP	50 (16)	18 (23)	32 (13)	.05
Urinary tract	35 (11)	5 (6)	30 (13)	.09
Infection severity				
ACLF	113 (36)	59 (74)	54 (23)	<.001
CLIF-SOFA score [median (IQR)]	6 (4-9)	10 (7-15)	5 (3-7)	<.001
MELD at BSI [median (IQR)]	18 (12-24)	26 (21-31)	16 (12-20)	<.001
Δ MELD (at BSI - baseline) [median (IQR)]	2 (0-5)	5 (2-10)	1 (0-4)	<.001
Severe sepsis	45 (14)	16 (20)	29 (12)	.10
Septic shock	41 (13)	27 (35)	14 (6)	<.001
Therapeutic management				

Adequate empirical treatment	190 (61)	31 (39)	159 (68)	<.001
<6h	153 (49)	20 (25)	133 (57)	<.001
Between 6 and 24h	24 (8)	4 (5)	20 (9)	.31
>24h	13 (4)	7 (9)	6 (3)	.01
Inadequate	122 (39)	48 (61)	74 (32)	<.001
ICU admission	84 (27)	39 (50)	45 (19)	<.001

Among the different risk scores calculated at the day of BSI onset, CLIF-SOFA (aROC 0.82; 95% CI 0.78-0.86), SOFA (aROC 0.82¸95% CI 0.77-0.86); MELD (aROC 0.79; 95% CI 0.74-0.83) and delta MELD (aROC 0.76; 95% CI 0.70-0.84) showed roughly equivalent discriminative performance for 30-day all-cause mortality (Figure 2A). In contrast, 30-day mortality probability related to sepsis grading (aROC 0.58; 95% CI 0.53-0.64) and Charlson co-morbidity index (aROC 0.52; 0.44-0.61) did not reliably discriminate non-surviving versus surviving patients with liver cirrhosis and BSI at 30 days.

In the Cox-regression model, CLIF-SOFA was associated with the lowest Akaike information criterion (AIC) score (768.07) and Harrell's C index (0.80) followed by BSI-MELD (AIC 798; Harrell's C 0.76). Therefore, risk factors for poor survival identified in univariate analysis (*P*<.1) related to admission diagnosis, epidemiological classification of BSI, presence of sepsis or septic shock and timing and adequacy of antimicrobial therapy were entered into multivariate Cox-regression model with patients CLIF-SOFA score as a continuous variable. Severity of sepsis displayed a collinearity with CLIF-SOFA scores and was not retained in the final model. Delayed (>24h) empirical treatment [HR 7.58 (95% CI 3.29-18.67), *P*<.0001], inadequate empirical treatment [HR 3.14 (95% CI 1.93-5.12), P<.0001] and CLIF-SOFA [HR 1.35 (95% CI 1.28-1.43), *P*<.0001] were independently associated with increased 30-day mortality. Admission to the hospital with a diagnosis of bacterial infection was associated with a lower 30-day mortality [HR 0.58 (95% CI 0.35-0.97), *P*<.04]. Exclusion of admission diagnosis was associated with a modest reduction in the AIC (752 vs. 753). Therefore, the most parsimonious model for 30-day mortality included only CLIF-SOFA, inadequate empirical treatment and delayed empirical treatment > 24 hours. After stratification for CLIF-SOFA using CART analysis (Figure 2B), the impact of adequate empirical therapy administered within and after 24 hours was evident in patients with low, medium and high-baseline risk for mortality (Figure 2C and 2D).

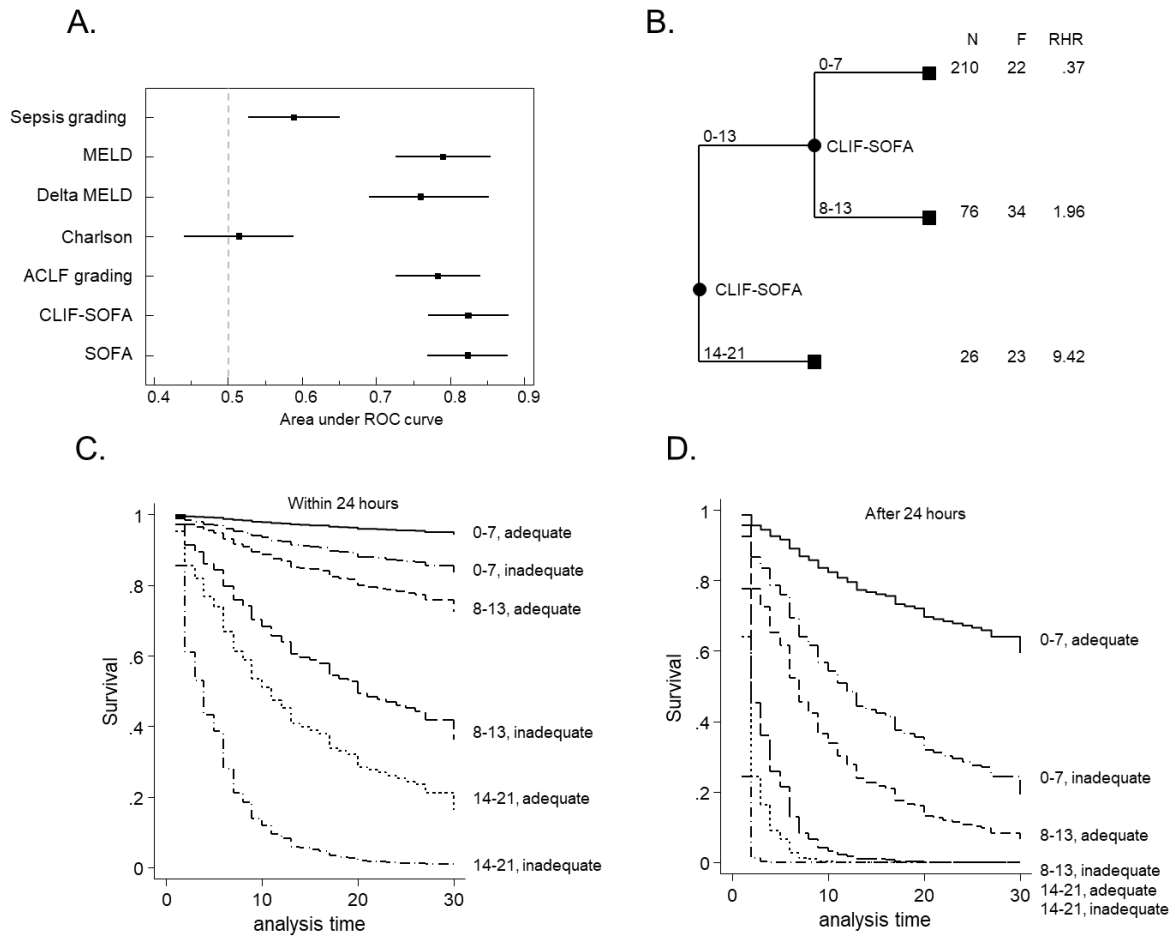

Figure 2. Relationship of mortality risk models and 30-day all-cause mortality. (a) comparison of risk score discrimination for 30-day mortality. (b) CART-defined cutpoints for CLIF-SOFA score analysis for failure time data. Martingale residuals of a Cox model were used to calculate chi square values for all possible cutpoints on all the CART covariates at P<.05, N=numbers of patients; F=numbers of failures, and RHR=relative hazard ratio; (c) Cox-regression survival estimates of patients receiving adequate empirical antimicrobial therapy within 24 hours stratified by CLIF-SOFA score; (d) Cox-regression survival estimates of patients who do not receive adequate empirical antimicrobial therapy within 24 hours stratified by CLIF-SOFA score. MELD model for end-stage liver disease, ACLF acute-on-chronic liver failure, CLIF-SOFA chronic liver failure-sequential organ failure assessment, SOFA sequential organ failure assessment

Etiology

Overall, a total of 337 isolates were identified from the 312 BSI episodes (Table 4). Gram-negative bacteria (GNB) accounted for 164/312 BSIs (53%). Most GNB were Enterobacteriaceae (136/164, 83%): *Escherichia coli* (82/164, 50%), *Klebsiella pneumoniae* (29/164,17%) and *Enterobacter* spp. (13/164, 8%). Fluoroquinolone-resistance, extended-spectrum β-lactamase (ESBL) and *K. pneumoniae* carbapenemase (KPC) production was identified in 38%, 33% and 7% of Enterobacteriaceae, respectively. Non-fermenting GNB were isolated in 9% of BSI episodes, most of which were *Pseudomonas* spp. (16/28, 60%). Gram-positive bacteria (GPB) were found in 146/312 (47%) BSI episodes. The most common isolated species were methicillin-sensitive *Staphylococcus aureus* (MSSA) (40/146, 27%), CoNS (25/146, 17%), *Streptococcus* spp. (24/146, 16%), *Enterococcus faecium* (22/146, 15%), *Enterococcus faecalis* (15/146, 10%) and methicillin-resistant *S. aureus* (MRSA) (12/146, 8%).

Candida species accounted for 7% (21/312) of BSIs. Among the 21 episodes of candidemia (9 primary BSI, 7 CR-BSI, 4 BSI secondary to SBP and 1 secondary to endocarditis), the majority were caused by *Candida albicans* (n=13), followed by *Candida glabrata* (n=3).

The main causative pathogens in polymicrobial infection were enterococci (46% of cases) Enterobacteriaceae (36% of cases, 45% of which MDR), *Candida* spp. (27% of cases) and non-fermenting bacilli (23% of cases).

Pathogens associated to the highest mortality rate were Carbapenem resistant-Enterobacteriaceae (4/9, 44%), *Candida* spp (9/21, 43%), *E. faecium* (9/22, 41%), ESBL-producing Enterobacteriaceae (16/45, 36%), *Streptococcus pneumoniae* (3/9, 33%) and MSSA (13/41, 32%).

Significant differences were found in the etiology distribution of BSI reported from the different countries (Table 4). We also found differences in community acquired, healthcare associated infections, as expected (table 5). Lastly etiology of BSI according with source of infection is presented in the table 6

Table 4. Etiology of 312 BSI in cirrhotic patients. Differences between countries.

	Total, n=312 episodes (100%)	***Italy n=149 episodes (47%)***	***Spain n=67 episodes (21%)***	***Germany n=57 episodes (18%)***	***Israel n=36 episodes (11%)***	***P***
Gram positive	146 (47)	60 (40)	34 (51)	36 (63)	14 (39)	.02*
Coagulase negative staphylococci	25 (8)	10 (7)	6 (9)	6 (10)	3 (8)	.88
Staphylococcus aureus (MSSA)	41 (13)	13 (9)	8 (12)	16 (26)	4 (11)	.02*
Staphylococcus aureus (MRSA)	12 (4)	7 (5)	4 (6)	0 (0)	1 (3)	.39
Streptococcus spp	24 (8)	7 (5)	9 (13)	3 (5)	3 (8)	.003
Enterococcus faecalis	15 (5)	7 (5)	1 (1)	4 (7)	3 (8)	.45
Enterococcus faecium	22 (7)	11 (7)	1 (1)	9 (16)	1 (3)	.02
Other Gram positive[a]	8 (3)	3(2)	1 (1)	2 (3)	1 (3)	.56
Gram negative	164 (53)	89 (60)	31 (46)	21 (37)	22 (61)	.01*
Enterobacteriaceae	136 (44)	75 (50)	26 (39)	19 (33)	15 (41)	.19
Escherichia coli	82 (26)	43 (29)	16 (24)	4 (7)	10 (28)	.70
Escherichia coli (FQR)	34 (11)	18 (21)	5 (7)	*5 (9)*	6 (27)	.56
Escherichia coli (ESBL)	23 (7)	15 (10)	0 (0)	3 (5)	5 (14)	.04*§
Klebsiella pneumoniae	29 (9)	19 (13)	4 (6)	2 (3)	3 (8)	.10
Klebsiella pneumoniae (FQR)	14 (4)	10 (7)	1 (1)	1 (2)	1 (3)	.03
Klebsiella pneumoniae (ESBL)	15 (5)	10 (7)	2(3)	1 (2)	2 (6)	.04
Klebsiella pneumoniae (CR)	9 (3)	8 (5)	1 (1)	0 (0)	0 (0)	.15
Enterobacter spp	13 (4)	5 (3)	5 (7)	2 (3)	1 (3)	.61
Other enterobactericeae [b]	15 (5)	10 (6)	1 (2)	2 (3)	2 (6)	.48
Non-fermenters	28 (9)	15 (10)	5 (7)	2 (3)	6 (17)	.22
Pseudomonas aeruginosa	16 (5)	9 (6)	4 (6)	0 (0)	3 (8)	
Acinetobacter baumanii	3 (1)	3 (2)	1 (2)	0 (0)	0 (0)	
Stenotrophomonas maltophilia	4 (1)	3 (2)	0 (0)	0 (0)	1 (3)	
Other non-fermenters[c]	5 (2)	0 (0)	1 (1)	2 (3)	2 (6)	
Other gram negative[d]	2 (1)	1 (1)	1 (1)	0 (0)	0 (0)	
Anaerobes	6 (2)	3 (2)	1 (1)	2 (3)	0 (0)	
Fungi						
Candida species	21 (7)	11 (7)	4 (6)	5 (9)	1 (3)	.76
Mixed infections	30 (10)	16 (11)	3 (10)	10 (17)	1 (3)	.07

BSI bloodstream infection, MSSA methicillin-susceptible *S.aureus*, MRSA methicillin-resistant *S. aureus* ; FQR, fluoroquinolone-resistant; ESBL, extended-spectrum beta-lactamase, CR carbapenem-resistant

[#]3 cases of BSI enrolled in Croatia are included only in the summary column (1 case of FQR-*Klebsiella pneumoniae*, 2 cases of *Streptococcus* spp BSI)

**P*<.01 between Italy and Germany

[§]*P*<.01 between Italy and Spain

[a]*Enterococcus raffinosus* (n=4), *Listeria monocytogenes* (n=3), *Corynebacterium striatum* (n=1)

[b]*Morganella morganii* (n=4), *Citrobacter* spp (n=3), *Klebsiella oxytoca* (n=3), *Roultella planticola* (n=2), *Proteus mirabilis* (n=1*), Pantoea agglomerans* (n=1)

[c]*Acinetobacter lwoffii* (n=1), *Aeromonas veronii* (n=1), *Pseudomonas alcaligenes* (n=1), *Pseudomonas stutzeri* (n=1), *Pseudomonas oryzihabitans* (n=1)

[d]*Moraxella catarrhalis* (n=1), *Haemophilus influenzae* (n=1)

Table 5. Etiology of 60 community acquired bloodstream infection, 82 healthcare associated bloodstream infection, 170 hospital acquired bloodstream infections.

	Community acquired BSI N= 60 (19%)	***Healthcare associated BSI N= 82 (26%)***	***Hospital-acquired BSI N= 170 (54%)***	**P**
Gram-positive	26 (43)	36 (44)	84 (49)	.59
Coagulase negative staphylococci	2 (3)	6 (7)	17 (10)	.25
Staphylococcus aureus (MSSA)	7 (12)	9 (11)	25 (15)	.25
Staphylococcus aureus (MRSA)	1 (2)	4 (5)	7 (4)	.59
Streptococcus spp	13 (22)	4 (5)	7 (4)	<.001*[#]
Enterococcus faecalis	1 (1)	5 (6)	9 (5)	.45
Enterococcus faecium	1 (1)	6 (7)	15 (9)	.33
Other Gram-positive[a]	1 (1)	2 (2)	5 (3)	.56
Gram-negative	35 (58)	48 (58)	81 (48)	.16
Enterobacteriaceae	32 (53)	39 (48)	65 (38)	.09
Escherichia coli	26 (43)	27 (33)	29 (17)	<.001*[§]
Klebsiella pneumoniae	3 (5)	8 (10)	18 (12)	.43
Enterobacter spp	0 (0)	2 (2)	11 (6)	.06
Other Enterobactericeae[b]	3 (5)	3 (4)	9 (5)	.48
FQR-Enterobacteriaceae	5 (8)	21 (26)	26 (15)	0.01[#]
ESBL-producing Enterobacteriaceae	2 (3)	13 (16)	30 (18)	0.02*
Carbapenem-resistant Enterobacteriaceae	0 (0)	1 (1)	8 (5)	0.10
Non-fermenters	3 (10)	9 (11)	16 (10)	.44
Pseudomonas aeruginosa	2 (3)	6 (7)	8 (5)	
Acinetobacter baumannii	0 (0)	0 (0)	3 (2)	
Stenotrophomonas maltophilia	0 (0)	1 (1)	3 (2)	
Other non-fermenters	1 (1)	2 (2)	2 (3)	
Other Gram-negative[d]	1 (2)	1 (1)	0 (0)	
Anaerobes	3 (2)	1 (1)	2 (3)	
Fungi				
Candida species	1 (2)	2 (2)	19 (11)	.76
Mixed infections	3 (5)	5 (6)	22 (13)	.09

BSI bloodstream infection, MSSA methicillin-susceptible *S.aureus*, MRSA methicillin-resistant *S. aureus* ; FQR, fluoroquinolone-resistant; ESBL, extended-spectrum beta-lactamase, CR carbapenem-resistant

Table 6 . Pathogen distribution according with source of bloodstream infection

	PRIMARY BSI N=99 (32%)	**UTI N= 35 (11%)**	**PNEUMONIA N=19 (6%)**	**DEVICE-RELATED INFECTION* N = 37 (12%)**	**NON-SBP IAI§ N=49 (16%)**	**SBP N= 50 (16%)**	**OTHER# N=28 (6%)**
Gram-positive	49 (49)	6 (17)	13 (68)	26 (70)	18 (37)	17 (34)	20 (71)
Coagulase-negative staphylococci	6 (6)	0 (0)	0(0)	13 (35)	2 (4)	2 (4)	2 (7)
MRSA	4 (4)	1 (3)	2 (10)	2 (5)	1 (2)	0 (0)	2 (7)
MSSA	13 (13)	1 (3)	5 (26)	6 (16)	3 (6)	4 (8)	10 (36)
Streptococcus spp	8 (8)	1 (3)	5 (26)	1 (3)	1 (2)	5 (10)	3 (11)
Enterococcus faecalis	6(6)	2 (6)	0 (0)	2 (3)	1 (2)	3 (6)	1 (4)
Enterococcus faecium	11 (11)	2 (6)	0(0)	1 (3)	6 (12)	2 (4)	1 (3)
Gram-negative	49 (49)	29 (83)	6 (31)	12 (32)	35 (71)	31 (62)	7 (25)
Enterobacteriaceae	39 (39)	27 (77)	2 (10)	9 (24)	33 (67)	25 (50)	4 (14)
Escherichia coli	21 (21)	22 (63)	2 (10)	1 (3)	17 (35)	18 (36)	1 (4)
Klebsiella pneumoniae	10 (10	3 (9)	0 (0)	5 (13)	7 (14)	4 (8)	1 (4)
Enterobacter spp	3 (3)	1 (3)	0	2(5)	4 (8)	3 (6)	1 (4)
FQR-Enterobacteriaceae	14 (14)	13 (37)	1 (5)	14 (28)	4 (11)	6 (12)	1 (4)
ESBL-producing Enterobacteriaceae	14 (14)	5 (14)	1 (5)	6 (16)	13 (26)	4 (8)	3 (11)
CR-Enterobacteriaceae	1 (1)	1 (3)	0 (0)	2 (5)	5 (10)	1 (2)	0 (0)
Non-fermenters	9 (9)	3 (9)	3 (16)	4 (11)	2 (4)	5 (10)	3 (11)
P. aeruginosa	5 (5)	3 (9)	2 (10)	2 (5)	2 (4)	1 (2)	1 (4)
Fungi							
Candida spp	7 (7)	0 (0)	0(0)	7 (19)	1(2)	4 (8)	1 (4)
Mixed	14 (14)	1 (3)	0 (0)	9 (24)	4 (8)	4 (8)	0 (0)

BSI bloodstream infection, MSSA methicillin-susceptible S. aureus, MRSA methicillin-resistant S. aureus; FQR, fluoroquinolone-resistant; ESBL, extended-spectrum beta-lactamase, CR carbapenem-resistant; SBP spontaneous bacterial peritonitis

* Device-related infection included catheter-related BSI (n=32) and TIPS related BSI (n=5)

§Non-SBP IAI included all intrabdominal infections and biliary tract infections with exception of SBP

Other infection included endocarditis (n=11), skin and soft tissues (n=10) bone and joint infections (n=4) and surgical sites (n=4). One patients were classified having both bone and joint and surgical site infection.

Prevalence and risk factor for MDRO

Overall, 98 (31%) BSIs were caused by MDRO. Distribution of MDRO according with epidemiological classification was 7% for community acquired, 22% for healthcare associated episodes and 70% for hospital acquired BSI (*P*<.001). As shown in Figures 3A and 3B, identification of MDRO or *Candida* spp was strongly associated with receipt of inappropriate antimicrobial or antifungal treatment in the first 24 hours.

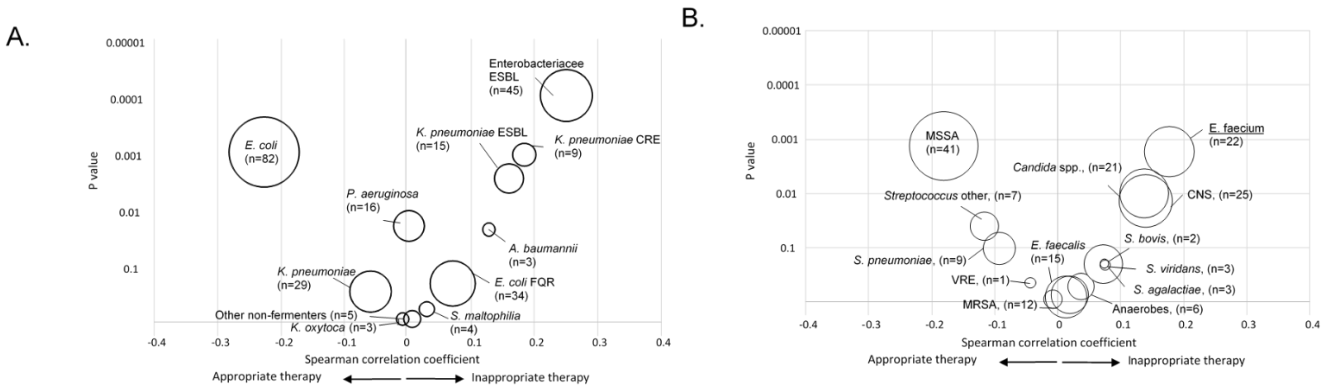

Figure 3 Correlation of isolated bloodstream pathogen with probability of inappropriate antibiotic therapy. A spearman rank coefficient and associated P value were calculated for most common isolated pathogens including a) Gram-negative bacilli and b) Gram-positive cocci plus Candida spp. The size of the bubble is relative to the number of isolates.

In order to assess risk factors for MDRO, patients with and without MDRO bacteria were compared (Table 7). At multivariate analysis, adjusted for clinical severity (CLIF-SOFA) and length of in-hospital stay before the onset of BSI, independent factors associated to MDRO isolation were previous (<30 days) antimicrobial exposure [OR 2.91 (95% CI 1.73-4.88), *P*<.001] and previous (<30 days) invasive procedures [OR 2.51 (95% CI 1.48-4.24), *P*=.001], whereas SBP source of BSI was associated with a lower risk of MDRO [OR 0.30 (95%CI 0.12-0.73), *P*=.008) (Table 7).

Table 7. Demographics, underlying disease and comorbidities associated with multidrug-resistant pathogens isolation among 312 cirrhotic patients with bloodstream infection.

	Patients with MDRO n=98 (31%)	**Patients without MDRO BSI n=214 (62%)**	***P***	**Multivariate analysis*, OR (95% CI), p**
Demographic data				
Age (years) [mean (± SD)]	62 (±12)	61 (± 12)	.87	
Male sex	59 (60)	145 (67)	.19	
Comorbidities				
COPD	15 (15)	25 (11)	.37	
Diabetes (any stage)	39 (40)	86 (40)	.92	
Chronic kidney disease	16(16)	39 (18)	.68	
Hemodilysis	2 (1)	1 (1)	.87	
Liver disease[a]				
Hepatitis C	37 (38)	75 (35)	.64	
Hepatits B	6 (6)	14 (6)	1	
Alcoholic	38 (39)	87 (41)	.80	
Hepatocellular carcinoma	15 (15)	35 (16)	.81	
Baseline MELD [b] [median (IQR)]	15 (11-19)	14 (11-20)	.46	
Previous hospitalization	66/96 (67)	111/210	.009	
Previous ICU admission	13/96 (13)	(51)	.10	
Gastrointestinal bleeding	23/96 (24)	16/210 (8)	<.001	
Previous hepatorenal	18 (18)	16 (7)	.02	
syndrome	27 (28)	19 (9)	.14	
Previous hepatic		42 (20)		
encephalopathy episode				
Invasive procedures	**49 (50)**	**55 (25)**	**<.001**	**2.51 (1.48-4.24), .001**

Endoscopy	39 (40)	47 (22)	.002	
Surgery	11 (11)	15/213 (8)	.19	
TIPS	13 (13)	6 (3)	<.001	
Antimicrobial exposure [c]	**56 (57)**	**64 (30)**	**<.001**	**2.91 (1.73-4.88), <.001**
Antibiotic prophylaxis	20 (20)	37 (17)	.53	
Quinolone prophylaxis	6 (6)	6 (3)	.20	
Fluoroquinolones	22 (22)	21 (10)	.004	
Third generation cephalosporine	24 (24)	23 (11)	.007	
BL/BLIs	25 (25)	23 (11)	.001	
Time between hospital admission and BSI onset (days) [median (IQR)]	7 (1-21)	1 (0-8)	<.001	
BSI severity				
MELD at BSI [median (IQR)]	19 (14-24)	17 (12-25)	.38	
CLIF-SOFA [median (IQR)]	6 (4-9)	6 (4-8)	.08	
Δ- MELD	3 (0-5)	2 (0-4)	.09	
BSI classification				
Community acquired	7 (7)	53 (24)	<.001	
Healthcare associated	22 (22)	60 (28)	.33	
Hospital acquired	69 (70)	101 (47)	<.001	
BSI Source				
Primary	31 (31)	69 (32)	1	
Catheter-related	13 (13)	20 (9)	.32	
Biliary tract	14 (14)	12 (6)	.01	
Intrabdominal	10 (8)	13 (7)	.65	
Urinary	9 (7)	26 (13)	.13	
Pneumonia	5 (4)	14 (6)	.33	
SBP	**7 (7)**	**43 (20)**	**.004**	**0.30 (0.12-0.73), .008**

MDR multidrug-resistant organism, BSI bloodstream infection, CI confidence interval, SD standard deviation, COPD chronic obstructive pulmonary disease, MELD model for end-stage liver disease, IQR interquartile range, ICU intensive care unit, TIPS transjugular intrahepatic portosystemic shunt, BL/BLI beta-lactam/beta-lactamase inhibitor, CLIF-SOFA chronic liver failure-sequential organ failure assessment, SBP spontaneous bacterial peritonitis.

[a]37 patients had multiple cause of liver cirrhosis

b baseline MELD was available in 231 patients

c defined as exposure to one or more antibiotic drugs (≥ 3 days) in the previous 30 days

The accuracy of the predictive model was assessed across different countries considering the different prevalence of MDRO in Italy, Germany, Spain and Israel which was assessed as 37.5%, 26.3%, 22.4% and 30.6% of BSI, respectively (Table 8).

Table 8. Sensitivity, specificity, area under receiver operating characteristic curve (aROC), positive predictive value (PPV) and negative predictive value (NPV) of the prediction model for MDRO (for variables included in the model see text and table 7) according to the prevalence of MDRO in cirrhotic patients with BSI per country.

Country	Prevalence (%)	Sensitivity (%)	Specificity (%)	aROC	PPV (%)	NPV (%)
Italy	37.5	19.6	92.5	0.71	61.1	65.5
Germany	26.3	60.0	85.7	0.71	60.0	85.7
Spain	22.4	33.3	96.2	0.70	71.4	83.3
Israel	30.6	27.3	95.8	0.70	75.0	74.2

DISCUSSION

The main finding of this study is that MDRO account for nearly one-third of BSI in patients with liver cirrhosis and are frequently associated with delays in effective treatment or inadequate empirical therapy, which are independent risk factors for death following a positive blood culture in both low and high-risk patients. Among commonly used mortality risk scores, we found that CLIF-SOFA and SOFA best discriminated non-surviving from surviving cirrhotic patients. Finally, we found that previous antimicrobial exposure, invasive procedures and source of infection play a role in determining the presence of MDRO. Similar to earlier studies, we found that alcohol abuse and HCV infection are still the main causes of underlying disease.(4) Most BSI episodes are acquired while in hospital or following frequent exposures to the healthcare environment.(9)

The mortality rate of BSI was 25%, which appears significantly higher than that associated with BSI in general population and with other bacterial infections in patients with liver cirrhosis.(4, 54, 55) However we found that common sepsis criteria used to discriminate infection severity were less predictive of outcomes in patients with liver cirrhosis. Similar findings were reported in a recent analysis of over 100,000 patients with infection and organ failure, where few patients with ESLD fulfill sepsis criteria consistent with their disease outcome.(56) Severity assessments based on parameters of organ failure may be more accurate in predicting outcome in cirrhotic patients with BSI.(50) The CLIF-SOFA score, an *ad hoc* adjustment of SOFA criteria, was the best predictor of 30-day mortality in our study cohort. In addition, in our series a cut-off of 7 points of CLIF-SOFA showed a good accuracy in distinguishing patients with higher risk of mortality. Is worth to be noticed that altogether high, medium and low risk patients had a benefit, with a different degree, of adequate empirical treatment (figure 2c and 2d).

The other major finding of this study was that besides underlying condition and infection severity, timely appropriate antimicrobial therapy has a major impact on the outcome of BSI, confirming prior results.(31, 57) The main predictor of inappropriate therapy in our patient cohort was isolation of a MDRO or *Candida* spp.

The heavy prevalence of MDRO in this series is notable. We observed a substantial rate of MDRO in several countries, especially Italy, confirming previous single-centre studies.(9, 11, 58) The prevalence of MDRO among BSI in cirrhotic patients was 37% in Italy, 30% in

Israel, 26% in Germany and 22% in Spain. It is well known that patients with liver cirrhosis undergo recurrent hospitalizations, invasive procedures and/or antimicrobial treatment, or prophylaxis, which subsequently increase the risk of acquiring a MDRO.

The problem of MDROs and the related ineffectiveness of empirical treatment is a growing topic of importance in the management of liver cirrhosis, although most studies have focused solely on the role of healthcare associated infections (HAI). However, defining HAI only on the basis of current items, in the liver cirrhosis populations is not straightforward. For example, in our cohort less than 1% of patients were undergoing chronic haemodialysis. Similarly, the rate of residents in nursing home with liver cirrhosis reported in large surveys in US seems negligible.(59) As a result, common criteria for HAI were not associated with isolation of MDRO in our cohort, but the low sensitivity of the current definition could be misleading. In our study, risk factors independently associated to MDRO were antimicrobial exposure or undergoing invasive procedures in the previous 30 days of infection onset. By contrast, having a SBP as a source of infection was associated with lower risk of MDRO. This latter factor may seem unexpected. However, in the study of Fernandez et al,(4) among all infection caused by MDRO, only 9% were represented by SBP. This finding requires further studies for confirmation.

Our study has some limitations including the heterogeneity of data due to the different epidemiology and different practice patterns over the different centres. This latter however is in line with the exploratory and observational design of the study. Another important limitation is that the prevalence of MDRO may be influenced by local or national ongoing outbreaks. To minimize the potential of bias, the study was conducted only in centres where an infection control programme was present.

Notwithstanding these limitations, we believe that this study could give substantial, generalizable information on the epidemiology of BSI in liver cirrhosis and provide the basis for further interventional studies on the management of BSI in this setting.

STUDY 2. CONTINUOUS INFUSION OF BETA-LACTAM ANTIBIOTICS IN CIRRHOTIC PATIENTS WITH BLOODSTREAM INFECTION: RESULTS FROM A PROSPECTIVE MULTICENTRE OBSERVATIONAL STUDY

METHODS

The BICHROME study was a prospective, multicenter study enrolling cirrhotic patients with BSI. Details of methods used in the study are presented in the previous section.

Population

All adult (>18 years) patients with liver cirrhosis who developed BSI at the participating centres were included in the study. The diagnosis of liver cirrhosis was based on previous liver biopsy results or a composite of clinical signs and findings provided by laboratory test results, endoscopy and radiologic imaging. Patients with previous liver transplantation were excluded. Patients with subsequent episodes of BSI were excluded. Patients were followed-up to 30 days after the BSI onset defined by the first positive blood culture. Of the patients initially included in the BICHROME cohort we selected patients using the following inclusion criteria: i) receipt adequate empirical and definitive treatment; ii) treatment with empirical (for at least 48h) or definite antibiotic treatment (for at least 7 days in survivors) with either piperacillin-tazobactam (TZP) or a carbapenem (CAR).

We included into the continuous/extended infusion group patients who received a TZP loading dose of 4.5-9 g followed by 18g (or less in case of renal function impairment) per 24h by continuous infusion, or a meropenem (MER) dose of 1-2 g followed by 2-6 g (or less in case of renal function impairment) per 24h of meropenem divided in 3-4 infusions of at least 4 hours each, or a loading dose of 1 g imipenem and cilastatin followed by 2-3 g/ of imipenem-cilastatin per 24 h as a continuous infusion adjusted for renal function.

Bloodstream infection was defined the growth of a non-common skin contaminant from ≥ 1 blood culture (BC) or of a common skin contaminant such as diphtheroids, Bacillus species, Propionibacterium species, coagulase negative staphylococci (CoNS), or micrococci from ≥ 2 BCs drawn on separate sites and reporting the same antimicrobial susceptibility test profile.

Statistical analysis

Categorical variables were analyzed as absolute numbers and their relative frequencies. Continuous variables were analyzed as mean and standard deviation (SD) if normally distributed, or as median and interquartile range (IQR) if non-normally distributed. categorical variables were compared using the χ^2 test, whereas continuous variables were compared using the Mann-Whitney U or two-tailed Student's T- test, when appropriate. Survival after 30 days from BSI diagnosis in patients receiving intermittent vs extended infusion of beta-lactams was assessed by Kaplan-Meier curves.

Factors associated with 30-day mortality at univariate analysis were included in a Cox regression multivariable model to assess factors independently associated to 30-day mortality.

RESULTS

During the study period, 323 patients with BSI were enrolled. Excluded patients had incomplete data (7 cases), had a single BSI caused by CoNS (2 cases) or were recipient of liver transplant (2 cases). Thus, 312 unique patients were analysed. Among these, 190 patients received adequate empirical antibiotic treatment and 123 of 190 received TZP or CAR as empiric and/or definitive therapy. Of these 123 patients, 118 (96%) received empiric TZP or carbapenem and 91 (70%) of them received an intermittent administration of the same drug, whereas 37 patients received a continuous or extended infusion. Fifty-two patients received definitive therapy with TZP or CAR, 23 treated with intermittent administration and 27 with continuous infusion of antibiotic (figure 3).

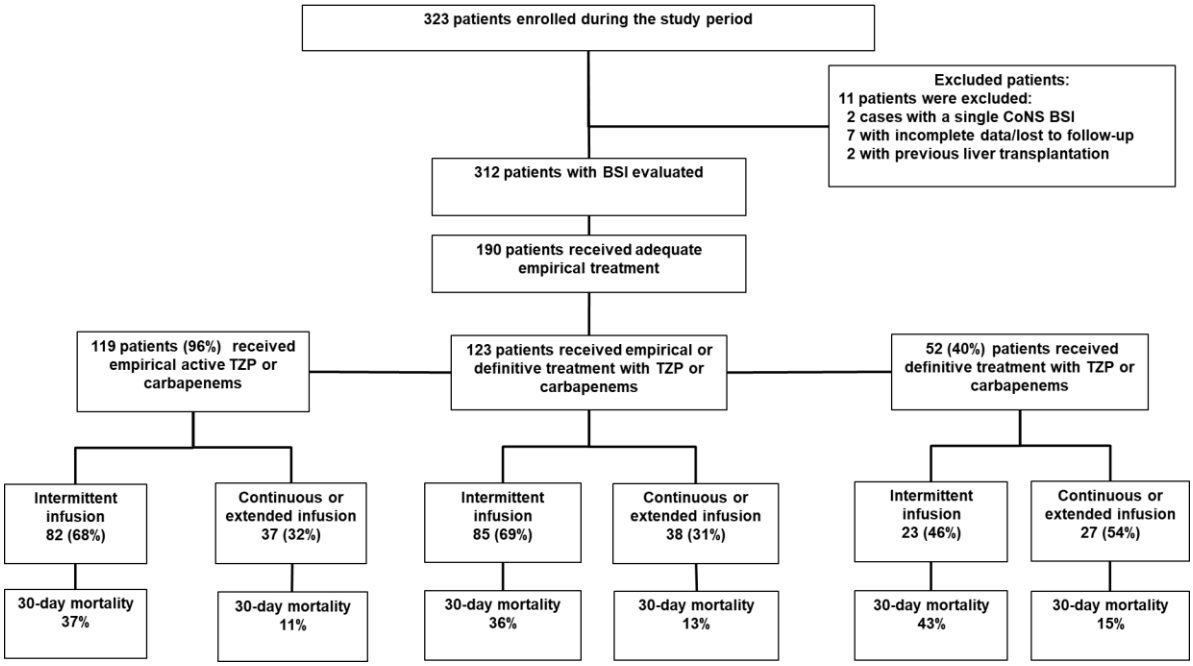

Figure 3. Study flow-chart. Abbreviations: TZP piperacillin-tazobactam; CAR carbapenems

Characteristics of patients included in the study

Overall, the entire cohort of 123 patients were characterized as follows. Mean age was 61 (±12) years and 83 (67%) of patients were male. The main causes of liver cirrhosis were viral in 43 (36%) subjects (36 cases of hepatitis C infection, 7 cases of hepatitis B infection), alcoholic in 32 (26%) and cryptogenic in 20 (16%) cases. In 21 cases (16%) cirrhosis was complicated by HCC. The median (IQR) Charlson comorbidity index were 7 (5-8). Comparing patients receiving intermittent administration with patients treated with continuous infusion of TZP or carbapenems no differences were found in demographics, cirrhosis characteristics and cause of hospital admission (table1). On the other hand, when analysing BSI characteristics, patients treated with continuous infusion of TZP or carbapenems were more likely to have hospital acquired infections (68% vs 43%, p=0.01), intra-abdominal infections (other than SBP) (34% vs 16%, p=0.03). No differences were found analysing severity of the infection.

Table 9. Differences in demographics, underlying disease, comorbidities and characteristics of infection among patients receiving intermittent administration and patients receiving continuous infusion piperacillin-tazobactam or carbapenems

	TOTAL, N=123 (100%)	**INTERMITTENT INFUSION, N=85 (69%)**	**CONTINUOUS/ EXTENDED INFUSION N= 38 (31%)**	***P***
Demographic data				
Age (years) [mean (± SD)]	61 (±12)	60 (±12)	63 (± 9)	0.19
Male sex	83 (67)	57 (67)	26 (68)	0.82
Liver disease [a]				
Viral cirrhosis	43 (36)	31 (35)	12 (32)	0.80
Alcoholic cirrhosis	32 (26)	23 (27)	9 (24)	0.82
NAFLD	13 (11)	8 (9)	5 (13)	0.53
Cryptogenic	20 (16)	12 (14)	8 (21)	0.33
Alcoholic + viral cirrhosis	11 (9)	8 (9)	3 (8)	1
Hepatocellular carcinoma	21 (16)	7 (18)	14 (16)	0.79
Admission diagnosis				
Ascitic decompensation	17 (14)	14 (17)	3 (8)	0.26
Acute kidney injury	5 (4)	0 (0)	5 (4)	0.17
Worsening of liver disease	11 (9)	8 (10)	3 (8)	0.75

Hepatic encephalopathy	11 (9)	6 (7)	5 (13)	0.49
Suspected bacterial infection	53 (44)	38 (47)	15 (39)	0.46
Co-morbidities				
Charlson index [median (IQR)]	7 (5-8)	7 (5-9)	6 (4-8)	0.85
Previous (<90 days) hospital admission	77 (64)	55 (67)	22 (58)	0.43
Previous (<90 days) ICU admission	11 (9)	10 (12)	1 (3)	0.17
BSI data				
Site of infection acquisition				
Community-acquired BSI	22 (18)	17 (20)	5 (13)	0.36
Hospital-acquired BSI	63 (52)	37 (43)	26 (68)	0.01
Healthcare associated	38 (30)	31 (35)	7 (18)	0.09
Primary	39 (32)	28 (33)	11 (29)	0.66
Pneumonia	11 (9)	9 (11)	2 (5)	0.50
SBP	21 (16)	17 (19)	4 (10)	0.23
Intra-abdominal (other than SBP)	27 (23)	14 (16)	13 (34)	0.03
Urinary tract	17 (14)	13 (15)	4 (10)	0.58
Infection severity				
ACLF				0.30
Grade 1	18 (15)	15 (18)	3 (8)	
Grade 2	18 (15)	14 (16)	4 (10)	
Grade 3	14 (11)	10 (12)	4 (10)	
CLIF-SOFA score [median (IQR)]	7 (4-10)	6 (3-9)	7 (5-9)	0.90
SOFA score [median (IQR)]	6 (4-9)	6 (3-8)	6 (4-9)	0.88
MELD at BSI [median (IQR)]	19 (11-25)	17 (12-19)	19 (13-24)	0.85
Sepsis	95 (77)	63 (71)	32 (84)	0.28
Septic shock	22 (13)	18 (21)	4 (10)	0.20
Empiric treatment				
Piperacillin-tazobactam	81 (66)	52 (61)	29 (76)	0.10
Meropenem	26 (21)	20 (23)	6 (16)	0.33
Imipenem	6 (5)	6 (7)	0 (0)	0.17
Ertapenem				
Definitive treatment				
Piperacillin-tazobactam	24 (19)	9 (10)	15 (39)	<0.001
Meropenem	12 (10)	7 (8)	5 (13)	0.39
Imipenem	4 (3)	2 (2)	2 (2)	0.4
Ertapenem	3 (2)	3 (3)	0 (0)	0.52

Microbiology

Detailed pathogens distribution is showed in table 2. Patients receiving continuous infusion of TZP or carbapenems had higher prevalence of Gram-negative infection (82% vs 58%, p=0.01), including non-*Escherichia coli* non-*Klebsiella pneumoniae* Enterobacteriaceae (21% vs 7%, p=0.02), non-fermenting bacilli (21% vs 8%, p=0.04). We also found a trend toward higher incidence of carbapenem-resistant(CR)-Enterobacteriaceae (5% vs 0%) and extended spectrum beta-lactamase(ESBL)-producing Enterobacteriaceae (24% vs 14%) among patients receiving TZP or carbapenems in continuous/extended infusion with a significant difference in terms of any MDR-gram-negatives (32% vs 16%, p=0.05).

Table 10. Causative pathogen distribution among patients treated with piperacillin/tazobactam or carbapenem. Differences of isolates among patients receiving intermittent administration and among patient treated with continuous/extended infusion of antimicrobial.

	TOTAL, N=123 (100%)	**INTERMITTENT INFUSION, N=85 (69%)**	**CONTINUOUS/ EXTENDED INFUSION N= 38 (31%)**	***P***
Gram-positive	42 (37)	34 (40)	8 (21)	0.04
Methicillin susceptible-*Staphylococcus aureus*	22 (18)	18 (21)	4 (10)	0.20
Streptococcus spp	8 (6)	8 (9)	0 (0)	0.10
Enterococcus spp	9 (14)	10 (14)	11 (10)	0.31
Other gram-positive[a]	4 (3)	4 (5)	0(0)	0.31
Gram-negative	80 (65)	49 (58)	31 (82)	0.01
Enterobacteriaceae	65 (52)	42 (49)	23 (60)	0.25
Escherichia coli	40 (32)	31 (36)	9 (23)	0.16
Klebsiella pneumoniae	11(9)	5 (6)	6 (16)	0.09
Other Enterobacteriaceae[b]	14 (11)	6 (7)	8 (21)	0.02
ESBL-Enterobacteriaceae	21 (14)	12 (14)	9 (24)	0.19
CR-Enterobacteriaceae	2 (2)	0 (0)	2 (5)	0.09
Non-fermenters	15 (12)	7 (8)	8 (21)	0.04
Pseudomonas aeruginosa	11 (7)	5 (6)	6 (16)	0.09
Other non-fermenters	4 (3)	2 (2)	2 (5)	0.58
MDR-Gram-negative	26 (21)	14 (16)	12 (32)	0.05
Anaerobes	4 (3)	3 (3)	1 (3)	1

[a] 3 cases of methicillin susceptible coagulase-negative staphylococci, 1 case of *Listeria monocytogens* BSI

[b] 5 cases of *Enterobacter* spp, 3 cases of *Klebsiella oxytoca*, 2 cases of Citrobacter spp, 1 case of *Proteus mirabilis*, 1 case of *Escherichia hermannii*, 1 case of *Morganella morganii*

Outcome

At the end of 30-day follow up 31 out of 123 patients (25%) died with a median (IQR) time to death of 9 (2-20) from index BSI. Kaplan-Meier curves indicate that patients receiving continuous or extended infusion of TZP or carbapenems had a significantly lower mortality rate (16% vs 36%, log-rank P=0.045) (figure 4a). Similar results were obtained analysing separately patients receiving empiric treatment with CI beta-lactams vs intermittent administration (P=0.019) (figure 4b) or definitive treatment (P=0.034) (figure 4c).

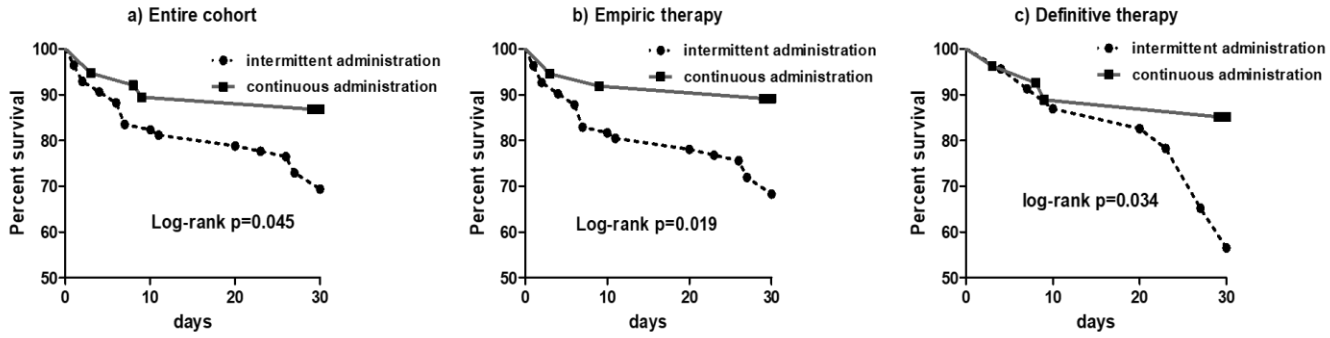

Figure 4. Kaplan-Meier curves for 30-day mortality. Comparison of outcome in patients receiving continuous/extended versus intermittent infusion of piperacillin-tazobactam or carbapenems in patients with liver cirrhosis and bloodstream infection. Results in the entire cohort (a) or in patients receiving empiric (b) or definitive (c) treatment with piperacillin-tazobactam or carbapenems.

Similarly, the mortality rate was lower in patients treated empirically with continuous or extended infusion of TZP or carbapenems (11% vs 3% p=0.019). At multivariate analysis using a Cox regression model, after adjusting for infection severity, using CLIF-SOFA and source of infection, receipt of empiric continuous or extended infusion of TZP or carbapenem was associated with significative lower mortality [HR 0.34 (95% CI 0.11-0.93), p=0.036] (table 11)

Table 11. Multivariable Cox regression model for 30-day mortality

Model Covariate	**Hazard ratio**	**95% CI**	***P***
CLIF-SOFA	1.37	1.24-1.51	<0.0001
SBP as source of BSI	2.35	1.11-4.89	0.03
Empiric extended infusion piperacillin-tazobactam or carbapenem	0.32	0.11-0.93	0.04

Abbreviations: CLIF-SOFA chronic liver failure-sequential organ failure assessment, SBP spontaneous bacterial peritonitis; BSI bloodstream infection CI confidence interval

DISCUSSION

In this prospective multicentre study of cirrhotic patients with BSI administration of CI of TZP and MER was associated with improved survival. To date no studies were performed to assess efficacy of CI of beta-lactams in patients with liver cirrhosis. Previous studies on different patient population showed a significative advantage in CI over IA of beta-lactams.
Beta-lactams show a time-dependent bactericidal effect. Therefore, they achieve the best bacterial killing when the time that serum concentrations remain above the minimal inhibitory concentration (MIC) is prolonged (t>MIC). This important pharmacokinetic parameter is usually achieved during continuous infusion of such drugs.
An important aspect of our study is that the best effectiveness of treatment in terms of outcome was achieved when CI of beta-lactams was employed in the early phase of infection. In fact, empiric CI infusion of beta-lactam was an independent factor related to lower odds of mortality (table 11). Previous studies showed that continuous infusion of beta-lactams when compared with bolus administration, has shown significantly higher serum and interstitial concentration of antibiotic in critically-ill patients during the first two days of treatment. (60) This aspect may be of particular interest as during the early phase of sepsis insufficient dose of beta-lactam antibiotics are often observed with conventional dosages.(61) In patients with liver cirrhosis an increased volume of distribution due to oedemas and ascites and lower protein binding, may be correlated with lower circulating drug and resulting with insufficient drug serum concentration during the first days of antimicrobial treatment. (34)
Continuous infusion of beta-lactams may be also necessary dealing with difficult-to-treat MDR pathogens. In fact, earlier studies suggested that pathogens with higher MIC can be adequately treated when CI of beta-lactams is employed. This aspect is of interest in the field of cirrhotic patients as this setting is particularly involved by the spread of MDRs(11). In our study, 20% of isolates where classified as MDR Gram-negatives and the prevalence was higher in the group of patients receiving CI of TZP or MER.
Beyond the major prevalence of MDR pathogens, other significative differences were found in patients treated with CI of TZP and MER when compared with patients receiving IA of the same drugs. In fact, the former group had higher prevalence of hospital acquired infections and IAI infections. All of these factors were previously associated with poor outcome in both cirrhotic and non-cirrhotic population.(23, 62-64) In addition in patients with IAI poor penetration of antibiotics in the abdominal district is common(63).

Our study has several limitations. First, the core BICHROME study was designed to explore the contemporary epidemiology of BSI in patients with liver cirrhosis. Thus, we did not collect several important variables, including serum trough levels of beta-lactams, that would furtherly illustrate the results of this study. Second, as the use of CI or IA was not dictated by study protocol an inter-centre heterogeneously may have been occurred.

Despite these limitations, our results are consistent with previous report in non-cirrhotic population and come from a prospective multicentre study. This latter aspect represents the main strength of our report.

In conclusion, CI of beta-lactams to treat BSI in cirrhotic patients is associated to improved outcome and achieve the best performance when used as empirical treatment in the early phase of infection.

STUDY 3 - DIFFERENCES IN THE ETIOLOGY AND OUTCOME OF BLOODSTREAM INFECTION IN PATIENTS WITH ALCOHOL-RELATED LIVER CIRRHOSIS AND NON-ALCOHOLIC LIVER CIRRHOSIS: RESULTS FORM A PROSPECTIVE MULTICENTRE STUDY.

METHODS

The BICHROME was a prospective multicenter study conducted in Nineteen tertiary centres from Italy (10 centres), Spain (5 centres), Germany (2 centres), Croatia (1 centre) and Israel (1 centre). Details on the methods, patients' recruitment and definitions used in the study are extensively described elsewhere(10).

Population

All adult (>18 years) patients with liver cirrhosis who developed BSI at the participating centres were included in the study. The diagnosis of liver cirrhosis and related was based on previous liver biopsy results or a composite of clinical signs and findings provided by laboratory test results, endoscopy and radiologic imaging.

Bloodstream infection was defined the growth of a non-common skin contaminant from ≥ 1 blood culture (BC) or of a common skin contaminant such as diphtheroids, Bacillus species, Propionibacterium species, coagulase negative staphylococci (CoNS), or micrococci from ≥ 2 BCs drawn on separate sites and reporting the same antimicrobial susceptibility test profile.

Patients with previous liver transplantation were excluded. Patients with subsequent episodes of BSI were excluded. Patients were followed-up to 30 days after the BSI onset defined by the first positive blood culture.

Data collection and definitions

Data was collected using an electronic case report form available at the study web site. The integrity of data was systematically checked, and queries were generated in case of inconsistent or missing data for reconciliation. The following variables were collected at the

moment of enrolment: demographic variables (sex, age); the cause and severity of liver disease according with model for end-stage liver disease (MELD) collected at baseline and BSI onset presence of hepatocellular carcinoma (HCC); presence of other co-morbidities according with the Charlson score(44). BSI were classified as hospital acquired, healthcare associated, or community acquired according with Friedman criteria. Infection severity was assessed according with sepsis criteria, sequential organ failure assessment (SOFA), chronic liver failure-SOFA (CLIF-SOFA)(21, 65). We also collected cases and grade of acute-on-chronic liver failure (ACLF), as described by Moreau et al.(3). Outcome variables were collected at day 7 and 30 after BSI onset by either bed-side evaluation, outpatient visit, or telephone call. These included the need of intensive care unit (ICU) admission, length of hospital stay and 7-day and 30-day transplant-free mortality.

Microbiology

Before study onset, the use of standard diagnostic methods was required and agreed with all the participating centres. This included the use of an automated blood culture detector system, the performance of Gram stain and/or rapid test (such as MALDI-TOF, PNA FISH) with immediate communication of the preliminary information to the attending physicians, the use of an automated system (Vitek n=17, MicroScan n=2) for susceptibility testing. Breakpoints, screening and conformation of the main mechanisms of resistance were done according with EUCAST guidelines.(53) Pathogens were classified as multidrug-resistant according with previous criteria.(52)

Statistical analysis

Categorical variables were presented as absolute numbers and their relative frequencies and were compared using the *chi*-square test or Fisher exact test when appropriate. Quantitative variables were presented as mean and standard deviation (SD) if normally distributed or as median and interquartile range (IQR) if non-normally distributed. Non-normally distributed continuous variables were compared using the Mann-Whitney U test, normally distributed continuous variables were compared using the *t* test. The analysis of

variance (ANOVA) followed by the Bonferroni post-hoc test, or the Kruskal-Wallis followed by the Dunnett post-hoc test was performed when three or more groups were compared.
To assess risk factors for isolation of GPC, variables associated ($p<0.1$) to GPC at univariate analysis were entered in multivariate logistic regression model. The calibration of the model was assessed by Hosmer-Lemeshow goodness-of-fit test and discrimination was assessed by the analysis of area under the receiver-operator curve (ROC). All variables were explored for interaction or collinearity. Difference in outcome in patients with and without alcohol related cirrhosis were compared using Kaplan-Meier survival curves.

RESULTS

During the study period 323 patients with BSI were evaluated for inclusion in the study. Study flow chart is shown in Figure 1: 11 patients met at least one exclusion criterium and 25 patients were excluded because presented multiple cause of liver disease (24 patients with both viral and alcoholic cirrhosis and 1 patient with both autoimmune and alcoholic cirrhosis). Thus, 287 patients were analysed in this study. Overall, 185 (64%) were male and mean (±SD) age was 61(±12) years. Distribution of causes of liver cirrhosis were as follows: 109 (38%) patients had viral cirrhosis (89 cases of HCV infection, 17 cases of HBV infection, 3 cases of mixed HBV-HCV infection), 99 (34%) patients had alcoholic liver disease (ALD) and 79 (28%) patients had non-viral non-alcoholic cirrhosis including 44 cryptogenic cirrhosis, 24 cases of cirrhosis due to non-alcoholic fatty liver disease (NAFLD), 6 autoimmune hepatitis, 5 primary biliary cirrhosis (figure 1).

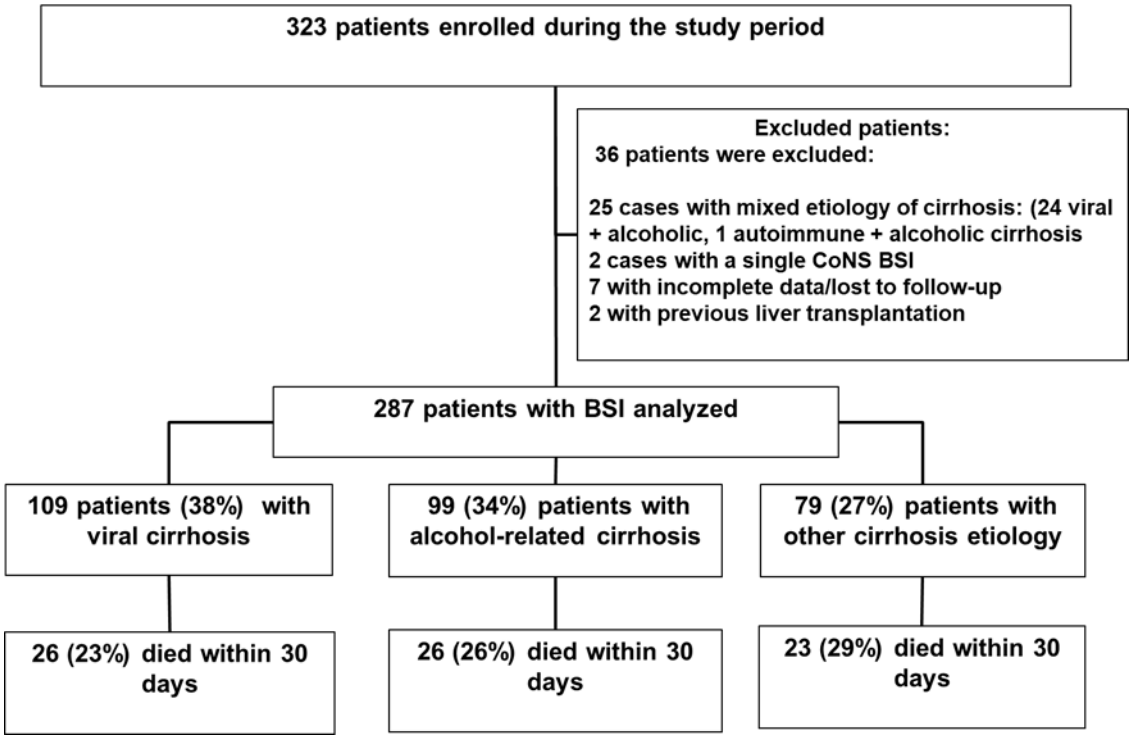

Figure 5 Study Flow-chart

Bloodstream infection in alcoholic cirrhosis compared with other causes of liver disease.
Comparison of BSI in patients with ALD, viral cirrhosis and non-alcoholic non-viral cirrhosis is shown in table 12. Briefly, patients with ALD were younger (mean age 57±10, $p<0.001$) and mostly male ($p=0.02$) and presented a lower number of comorbidities when compared

with both patients with viral cirrhosis or non-viral non-alcoholic cirrhosis. At BSI diagnosis, patients with ALD appeared more severe than those reported in other groups, as showed by higher SOFA (p=0.03) or CLIF-SOFA (p=0.03) and higher frequency of septic shock (22%, p=0.002).

Table 12 Difference in demographic characteristics and source and severity of bloodstream infection in 287 patients with liver cirrhosis according with cause of liver disease

	TOTAL, N=287 (100%)	**PATIENTS WITH ALCOHOLIC CIRRHOSIS =99 (34%)**	**PATIENTS WITH VIRAL CIRRHOSIS N=109 (38%)**	**PATIENTS WITH OTHER CIRRHOSIS AETIOLOGY N=105 (34%)**	***P***
Demographic data					
Age (years) [mean (± SD)]	61 (±12)	57 (±10)	64 (± 13)	63 (± 12)	0.26
Male sex	185 (64)	76 (77)	66 (61)	43 (54)	0.02*§
Co-morbidities					
Charlson index [median (IQR)]	7 (5-9)	5 (3-6)	7 (4-9)	7 (5-9)	<0.001*§
Hepatocellular carcinoma	47 (16)	12 (12)	25 (23)	10 (13)	0.06*
Baseline MELD [median (IQR))	15 (10-19)	16 (11-21)	15 (13-17)	14 (9-19)	0.42
Admission diagnosis					
Ascitic decompensation	40/281 (14)	20 (20)	11 (10)	9 (12)	0.10
GI-bleeding	15/281 (5)	7 (7)	4 (4)	4 (5)	0.57
Hepatic encephalopathy	28/281 (10)	10 (10)	14 (13)	4 (5)	0.28
Suspected bacterial infection	122/281 (42)	32 (32)	50 (47)	40 (53)	0.015*§
BSI data					
Site of infection acquisition					
Community-acquired BSI	53 (19)	23 (23)	17 (16)	13 (16)	0.31
Hospital-acquired BSI	161 (54)	58 (59)	57 (52)	46 (58)	0.59
Healthcare associated	73 (25)	18 (18)	35 (32)	20 (25)	0.07
Primary	94 (33)	37 (38)	28 (26)	29 (37)	0.14
SBP	50 (16)	15 (15)	16 (15)	19 (18)	0.79
Intra-abdominal (other than SBP)	48 (17)	9 (9)	22 (20)	17 (21)	0.04*§

Urinary tract	32 (11)	7 (7)	16 (15)	9 (11)	0.22
Pneumonia	18 (6)	10 (10)	6 (5)	2 (2)	0.10
Infection severity					
ACLF	105 (37)	45 (45)	36 (32)	25 (32)	0.09
Grade 1 ACLF	44 (15)	14 (14)	18 (16)	12 (15)	0.04
Grade 2 ACLF	36 (12)	16 (16)	13 (12)	7 (9)	
Grade 3 ACLF	26 (9)	16 (16)	5 (5)	5 (6)	
SOFA score [median (IQR)]	6 (3-9)	6 (4-8)	6 (4-7)	5 (4-7)	0.03*§
MELD at BSI [median (IQR)]	18 (12-24)	18 (12-26)	19 (14-24)	17 (12-23)	0.35
Septic shock	38 (13)	22 (22)	6 (5)	10 (13)	0.002*
Outcome					
ICU admission	77 (27)	38 (38)	18 (17)	21 (27)	0.002*
Need for mechanical ventilation	46 (16)	21 (21)	10 (9)	15 (20)	0.05
In-hospital mortality	87 (31)	32 (33)	31 (29)	24 (31)	0.89
7-day mortality	33 (11)	17 (17)	8 (7)	6 (8)	0.04

Microbiology

Significant differences in BSI causative pathogens were found comparing patients with different cirrhosis etiologies (Table 13). Indeed, GPC-BSI (0.002), including *Streptococcus* spp BSI (p=0.03) were found more frequently in patients with alcohol related cirrhosis. In contrast, GNB were detected less frequently in this group of patients if compared with those with viral cirrhosis and non-viral non-alcoholic cirrhosis (p<0.001) with significant differences in the prevalence of Enterobacteriaceae (p=0.004), including *Escherichia coli* (p=0.02), and different frequency of non-fermenting bacilli, such as Pseudomonas aeruginosa (p=0.05), among the groups. Conversely, we did not find differences in the prevalence of MDR pathogens.

Table 13. Causative pathogen distribution of 287 BSI in cirrhotic patients and differences between patients with alcoholic liver disease (ALD), viral cirrhosis and other causes of liver disease.

	TOTAL, N=287 EPISODES (100%)	**ALCOHOLIC CIRRHOSIS N=99 BSI EPISODES (32%)**	**VIRAL CIRRHOSIS N=109 BSI EPISODES (35%)**	**PATIENTS WITH OTHER CONDITIONS N=76 (34%)**	***P***
Gram positive	133 (46)	58 (59)	38 (35)	37 (47)	0.003*§
Coagulase negative staphylococci	23 (8)	12 (12)	8 (7)	3 (4)	0.12
Staphylococcus aureus	47 (17)	21 (21)	12 (11)	14 (17)	0.13
Staphylococcus aureus (MSSA)	38 (13)	17 (17)	9 (8)	12 (15)	0.13
Staphylococcus aureus (MRSA)	9 (3)	4 (4)	3 (3)	2 (2)	0.81
Streptococcus spp	21 (7)	13 (13)	5 (5)	3 (4)	0.02*§
Enterococcus spp	39 (14)	14 (14)	11 (10)	14 (18)	0.31
Gram negative	152 (53)	36 (36)	70 (64)	46 (58)	<0.001*§
Enterobacteriaceae	127 (44)	31 (32)	59 (54)	37 (47)	0.004*
Escherichia coli	76 (26)	17 (17)	37 (34)	22 (28)	0.02*
Klebsiella pneumoniae	29 (10)	7 (7)	13 (12)	9 (11)	0.46
ESBL-Enterobacteriaceae	42 (15)	10 (10)	21 (19)	11 (14)	0.17
CR-Enterobacteriaceae	9 (3)	1 (1)	4 (4)	4 (5)	0.28
Non-fermenters	25 (9)	4 (4)	11 (10)	10 (13)	0.10
Pseudomonas aeruginosa	15 (5)	1 (1)	7 (6)	7 (9)	0.05*
Fungi					

Candida species	22 (7)	8 (8)	7 (6)	7 (7)	0.87
Mixed infections	28 (10)	9 (9)	6 (5)	13 (16)	0.04
MDR pathogen	98 (31)	28 (29)	35 (32)	35 (33)	0.75

Outcome of patients with alcoholic cirrhosis compared with other conditions

During the study period, 77 (26%) patients needed ICU admission and 46 (16%) were mechanically ventilated.

Patients with ALD were admitted in ICU (p=0.002), underwent to mechanical ventilation (P=0.05) more frequently when compared with both patients with viral cirrhosis or non-viral non-alcoholic cirrhosis.

Overall, 87/287 (30%) patients died during the in-hospital stay. Thirty-day mortality rate did not differ among the groups (p=0.91) Conversely, Kaplan-Meier survival curves showed worse 7-day survival of patients with ALD if compared with patients with cirrhosis due to other causes (p=0.04) (figure 6a and 6b).

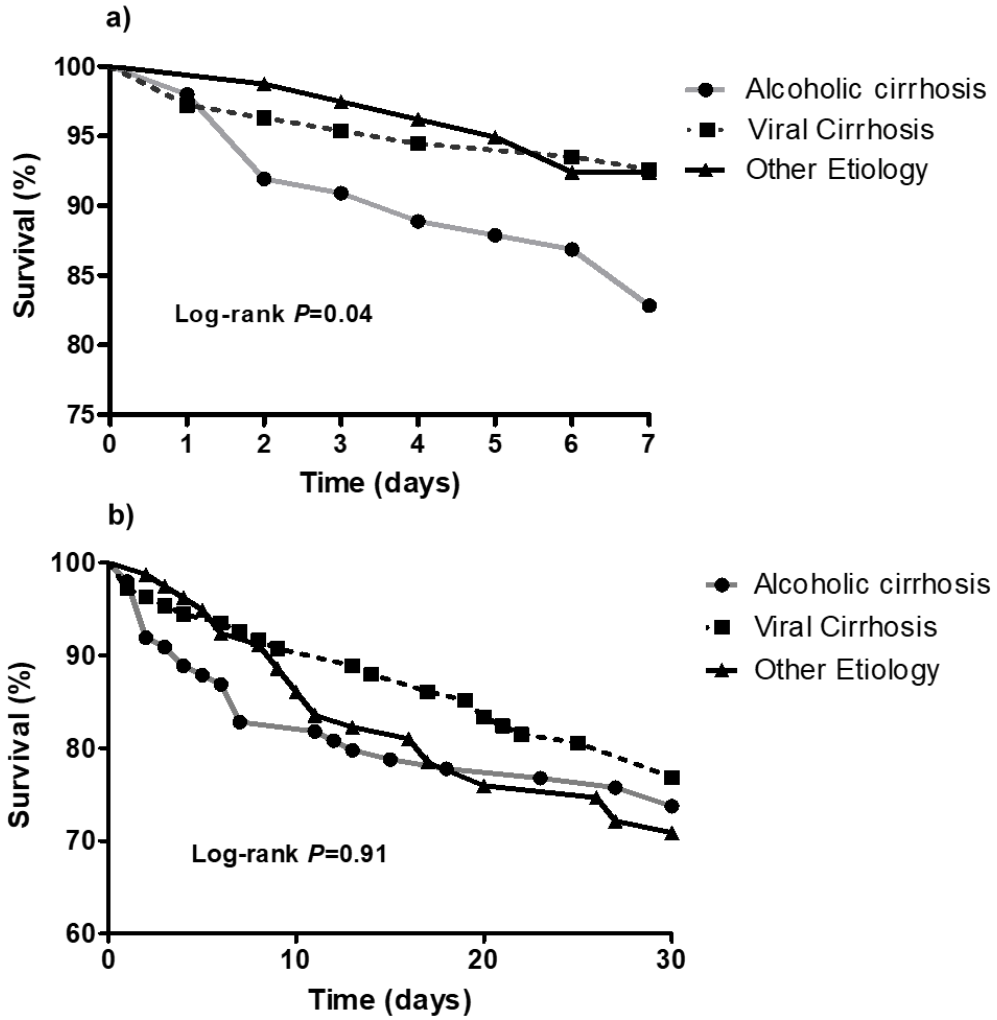

Figure 6. Differences in 7-day (a) and 30-day (b) mortality in patients with alcoholic liver disease, viral cirrhosis and other conditions.

Risk factors for BSI caused by Gram-positive cocci

After excluding 21 patients with mixed infections with isolation of both GPC and GNB and patients with fungal BSI, 112 patients with monomicrobial BSI caused by GPC were compared with 140 patients with monomicrobial BSI caused by Gram-negative bacilli (GNB) (table 14).

Table 14. Differences in demographics, underlying disease, source and severity of infection in patients with monomicrobial bloodstream infection caused by Gram-positive cocci and monomicrobial bloodstream infection caused by Gram-negatives

	PATIENTS WITH ISOLATION OF GRAM-POSITIVE COCCI N=112 (45%)	**PATIENTS WITHOUT ISOLATION OF GRAM-POSITIVE COCCI N= 140 (55%)**	***P***
Demographic data			
Age (years) [mean (± SD)]	61 (±10)	62 (± 14)	0.16
Male sex	77 (68)	85 (61)	0.18
Liver disease			
Viral cirrhosis	33 (29)	66 (47)	0.004
Alcoholic	53 (47)	34 (24)	<0.001
Cryptogenic	18 (13)	26 (17)	0.43
Non-alcoholic fatty liver disease	15 (11)	9 (6)	0.13
Autoimmune	4 (3)	2 (2)	0.51
Hepatocellular carcinoma	15 (13)	28 (20)	0.16
Baseline MELD [Median (IQR)]	15 (10-19)	14 (9-29)	0.56
Comorbidities			
Chronic renal failure	27 (24)	20 (14)	0.05
Diabetes	54 (48)	50 (37)	0.04
Diabetes with organ damage	28 (25)	17 (12)	0.008
Charlson score	6 (4-8)	7 (5-9)	0.56
Previous (<90 days) hospital admission	60 (54)	81 (59)	0.42
Previous (<90 days) ICU admission	14 (13)	7 (5)	0.03
Admission diagnosis			
Ascitic decompensation	19 (17)	15 (11)	0.50
Acute kidney injury	4 (4)	3 (2)	0.81
Worsening of liver function	11 (10)	9 (7)	0.33
Hepatic encephalopathy	5 (5)	19 (14)	0.02
Scheduled procedure	5 (3)	14 (9)	0.06

Complications before BSI (<30gg)			
Invasive procedure	51 (33)	48 (37)	0.53
Hepatorenal syndrome	20 (15)	12 (8)	0.05
SBP episode	11 (8)	7 (4)	0.19
GI bleeding	22 (16)	14 (9)	0.048
Surgery	12 (9)	11 (7)	0.61
Antibiotic exposure (previous 30 days)	37 (33)	55 (39)	0.30
Antibiotic prophylaxis	17 (15)	29 (21)	0.32
Quinolone prophylaxis	3 (3)	6 (4)	0.49
BSI data			
Site of infection acquisition			
Community-acquired BSI	20 (18)	31 (22)	0.40
Hospital-acquired BSI	64 (57)	68 (49)	0.17
Healthcare associated	28 (25)	41 (29)	0.44
Source			
Primary	37 (33)	43 (31)	0.69
SBP	12 (11)	27 (20)	0.06
Urinary tract	5 (4)	26 (19)	0.001
Pneumonia	13 (12)	5 (4)	0.01
SSTI	6 (5)	1 (1)	0.03
Intra-abdominal (other than SBP)	13 (12)	31 (22)	0.02
Device-related infection	17 (15)	7 (5)	0.006
Infection severity			
ACLF	45 (40)	47 (33)	0.28
ACLF grade			0.61
Grade 1	21 (19)	19 (14)	
Grade 2	13 (12)	19 (14)	
Grade 3	11 (10)	11 (8)	
SOFA score [median (IQR)]	5 (3-8)	6 (4-8)	0.30
Septic shock	19 (17)	14 (10)	0.10

Compared with patients with GNB, patients with GPC isolated in the BC had more frequently ALD [53 (47%) vs. 34 (24%), p<0.001], chronic renal failure [27 (24%) vs. 20 (14%), p=0.05], diabetes with organ damage [28 (25%) vs. 17 (12%), p=0.008], were admitted at ICU more frequently in the 90 days before the index BSI [14 (13) vs. 7 (5), p=0.03] and had a previous (<30 days) episode of hepatorenal syndrome [20 (15) vs 12 (8), P=0.05] or gastrointestinal

bleeding [22 (16%) vs. 14 (9), P=0.048] more frequently. Moreover, source of infection differed significantly among the patients with and without BSI caused by GPC. Indeed, individuals with GPC were had more frequently pneumonia [13 (12%) vs. 5 (4%), P=0.01] skin and soft tissue infection (SSTI) [6 (5%) vs 1(1%) and device related infection [17 (15%) vs 7 (5%), P=0.006] than patients with GNB. At multivariate analysis factors independently associated to isolation of GPC were alcoholic cirrhosis [OR 2.02 (95% CI 1,18-3.48), p=0.03], device-related infection [OR 3.08 (95 % CI 1.35-6.69), p=0.007], pneumonia as source of BSI [OR 3.81 (95% CI 1.23-11.79), p=0.02], previous hepatorenal syndrome [OR 2.54 (95% CI 1.11-5.85), p=0.03] and diabetes with organ damage [OR 2.52 (95% CI 1.27-4.99), P=0.008] (table 15)

Table 15. Logistic regression model assessing independent risk factors for isolation of Gram-positive in cirrhotic patients with bloodstream infection.

Model Covariate	**Odds ratio**	**95% CI**	***P***
Pneumonia	3.81	1.23-11.79	0.02
Device-related infection	3.08	1.35-6.69	0.007
Alcohol-related cirrhosis	2.02	1.18-3.48	0.01
Previous hepatorenal syndrome	2.54	1.11-5.85	0.03
Diabetes with organ damage	2.52	1.27-4.99	0.008

DISCUSSION

The main results of this study are that patients with alcoholic cirrhosis developing an episode of BSI are significantly younger and present a lower number of comorbidities when compared with the other groups. Conversely, they presented more severe infection with a significant higher unadjusted 7-day mortality. In addition, GPC are the main cause of BSI in this setting.

Previous studies demonstrated that alcohol abuser may acquire a dysregulation of immune system that include both impairment of its function and hyperexpression of inflammatory markers. Additionally, a higher incidence of ACLF in cirrhotic patients admitted for an episode of acute decompensation was observed in alcohol abuser in a large multicenter study (the CANONIC study)(3). An additional sub-analysis of the CANONIC study found a higher level of pro-inflammatory mediators in patients with ACLF caused by alcohol abuse when compared with patients with ACLF precipitated by other factors.(66). These studies may partially explain the higher severity of infection shown by patients with alcoholic cirrhosis in our study.

Alcohol abuse was previously found as a predictor of mortality in patients with pneumonia or invasive pneumococcal disease in studies performed in general population(67, 68). However, there is a lack of studies evaluating the impact of different causes of cirrhosis on infection-related mortality. In a previous retrospective study including episodes of bacterial infection among patients with liver cirrhosis, a non-statistically significant trend toward a higher in-hospital mortality was observed among patients with alcoholic liver disease compared with patients with non-alcoholic liver disease (21% versus 15%, P=0.102)(22). A similar non-statistical trend toward a higher incidence of pneumonia in patients with ALD was observed also our study and pneumonia, in turn was previously associated to higher mortality when compared with other source of infection in patients with liver cirrhosis and in general hospital population(69, 70). This finding may further explain the worse outcome of BSI observed in patients with ALD.

Another interesting finding of this study is the different distribution of pathogens according with the underlying cause of ESLD. In fact, the prevalence of GPC BSI in patients with alcoholic cirrhosis versus viral cirrhosis was significantly higher, [ALD (59%) vs others (40%) vs 40%, p=0.003)]. In addition, alcoholic cirrhosis was found as an independent risk factor for infection caused by GPC in our cohort. Indeed, alcohol abuse is a well-known risk factor for pneumonia, invasive pneumococcal disease and skin and soft tissue infection were GPC are important causative pathogens (71-75). Some previous single-centre studies reported

a growing incidence of infections caused by GPC in patients with alcoholic liver disease. In a study including 117 cases of BSI in cirrhotic patients a trend toward a higher incidence of GPC among patients with alcoholic cirrhosis was observed(76). In another report, Campillo et al found a prevalence of 70% of infections caused by GPC in a series of 200 cirrhotic patients, 175 of whom had alcohol-associated cirrhosis. Similarly, in a study enrolling all cases of positive-culture SBP in three different Danish hospitals, GPC were the main causative pathogens. In such series patients with underlying alcohol-related cirrhosis was 76%.(77) Finally, our findings are consistent with the study of Sargenti et al which found higher prevalence of GPC and pneumonia among patients with alcoholic liver disease when compared with patients with non-alcoholic cirrhosis. (58) If this finding will be further confirmed, the pathogenesis of infection in patients with liver cirrhosis may need to be revisited with respect to the underlying cause of the liver disease. In fact, in add

Unlike previous studies we did not find any association between quinolone prophylaxis and prevalence of BSI caused by GPC(78). However, the rate of patients receiving quinolone prophylaxis was very low in our series (10 patients, 3%).

According with our results some important indication may be drawn. In cirrhotic patients with pneumonia, device-related infection, the empirical antibiotic treatment should comprise anti-Gram-positive spectrum. In addition, in cirrhotic patients with ALD and non-urinary tract infection an empirical anti-Gram-positive coverage should be considered. Furtherly, the choice of anti-Gram-positive drug should be based on site of infection and local prevalence of penicillin-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci.

Our study has some limitations including the heterogeneity of data due to the different epidemiology and different practice patterns over the different centers. Also, the number of patients with alcoholic cirrhosis was different among center sited in Italy and other countries such as Germany and this may have had an impact in determining the different distribution of pathogens among patients with different underlying cause of cirrhosis. However, after entering the country of enrollment into the multivariable model we did not find any differences in either model results or model calibration and discrimination.

Another important limitation is that the core BICHROME study did not aim specifically to find differences in BSI etiology in patients with alcoholic cirrhosis. Thus, we did not collect some important variables such as recent active alcohol consumption or diagnosis of severe alcoholic hepatitis treated with steroids, which could had help us to better characterize our cohort(79). Notwithstanding these limitations we believe that this study provides some

important and novel information about the epidemiology and outcome of BSI in patients with alcoholic cirrhosis.

In conclusion, alcoholic liver disease is common in cirrhotic patients who develop an episode of BSI. Among patients, with ALD a different causative pathogen distribution is found with higher prevalence of gram-positive cocci if compared with patients with viral cirrhosis or other cause of cirrhosis. In addition, despite they present a lower number of comorbidities and are younger, BSI in patients with ALD is more severe. Therefore, this group of patients need ICU admission and mechanical ventilation more frequently and present a lower 7-day survival rate if compared with patients with other underlying liver disease.

STUDY 4. EPIDEMIOLOGY OF ACUTE-ON-CHRONIC LIVER FAILURE ASSOCIATED WITH BACTERIAL INFECTION IN PATIENTS WITH LIVER CIRRHOSIS: RISK FACTORS AND OUTCOME

METHODS

Study design

We conducted a prospective observational study from January 2014 to March 2016 at the S. Orsola-Malpighi University Hospital, Bologna and at the “Infermi” Hospital of Rimini, a tertiary teaching-hospital and a community hospital, respectively. The study was approved by the local institutional review boards. Written informed consent was obtained from patients or from legal surrogates before enrolment according to the 1975 Declaration of Helsinki.

Population

Consecutive patients with liver cirrhosis and acute decompensation (AD) admitted to the hospital were included in the study. The diagnosis of LC was based on previous liver biopsy findings or a composite of clinical signs and findings provided by laboratory test, endoscopy, and radiologic imaging. Exclusion criteria were: i) admission for a scheduled procedure; ii) hepatocellular carcinoma (HCC) beyond the Milan criteria [1]; iii) metastatic extrahepatic malignancy; iv) previous liver transplantation.

Patients recruitment and management

In any participating center, a sub-investigator was in charge to screen al potential cirrhotic patients with acute decompensation through careful evaluation of any newly admitted or transferred patient. All patients were enrolled at admission and daily evaluated during the entire in-hospital stay for potential development of ACLF and/or bacterial infection. All patients were managed according with international and local guidelines. Medical treatments and management of AD or ALCF, empirical and definitive antibiotic treatment was not dictated by study protocol.

Data collection

Data were collected using an online electronic case report form shared in the study website. The integrity of data was systematically checked by an investigator before being entered

into the database and by monthly assessment of data completeness and consistency. In case of inconsistent or missing data, queries were generated and distributed to the participating site investigators for reconciliation. Every case of infection was managed and reviewed by an infectious disease specialist and by a hepatologist. The following data were collected at the time of enrolment: demographic characteristics; etiology of cirrhosis, laboratory data and clinical data including the presence of hepatocellular carcinoma (HCC) and presence of other co-morbidities according with Charlson score (44). Basing on the collected data the Model for End-stage Liver Disease (MELD), Child-Turcotte-Pugh, CLIF-OF, CLIF-AD and CLIF-ACLF scores were calculated for each patient when appropriate (30, 80).

During hospitalization additional information were collected in case of invasive diagnostic or therapeutic procedures including esophagogastroduodenoscopy, colonoscopy, endoscopic retrograde cholangiopancreatography, endoscopic ultrasound, TIPS insertion, biliary procedures including biliary percutaneous drainage and/or stenting, HCC treatments.

For patients admitted due to a bacterial infection or who developed a bacterial infection during the hospital stay the following data were also collected: epidemiological classification of infection; severity of infection assessed with SOFA, CLIF-SOFA. Similarly, infection site, microbiology culture and their susceptibility data, and antibiotics administered as empirical or definitive therapy based on susceptibility reports were recorded. For patients with multiple admissions during the study period only the first admission was included in the analysis.

Patients were actively followed up for transplant status and survival during hospitalization and, after discharge, up to 1-year.

Definitions

Acute decompensation was defined by i) acute onset of grade 2 or grade 3 ascites, according to the International Ascites Club Classification (81); ii) new episode of hepatic encephalopathy in patient with previous normal consciousness and no evidence of an acute neurologic disease; iii) upper or lower gastrointestinal bleeding; iv) bacterial infection. The CLIF-SOFA score was calculated as described by Moreau et al. and ACLF was diagnosed and classified accordingly (30). Organ failures were assessed according the following criteria: liver failure was defined by a serum bilirubin level of ≥12.0 mg/dL; kidney failure was defined by a serum creatinine level of ≥ 2.0 mg/dL or the use of renal replacement therapy; cerebral failure was defined by grade III or IV hepatic encephalopathy, according to the West Haven classification; coagulation failure was defined by an international normalized ratio 2.5

and/or a platelet count of <20 X10^9/L; circulatory failure was defined by the use of dopamine, dobutamine, or terlipressin.. Any case of ACLF diagnosed simultaneously to bacterial or fungal infection or occurring within 28 days from the diagnosis of bacterial or fungal infection in patients without documentation of any other common precipitating event of ACLF, were defined as infection-related ACLF. Any case of bacterial infection diagnosed after ACLF was not included in this group.

Severity of infection was assessed with sepsis 3 criteria, sequential organ failure assessment (SOFA), quick-SOFA (qSOFA) and CLIF-SOFA. Pneumonia was defined as the radiologic evidence of a new, or progression of a previous, pulmonary infiltrate plus at least two of the following criteria: fever >38ºC, cough, purulent sputum, dyspnea or >20 bpm, pleuritic chest pain, and a leucocyte count of >10,000/mm^3 or <4,000/mm^3; urinary tract infection (UTI) was diagnosed in the presence of either one of the following criteria: i)flank pain, which must have onset or worsened within 7 day, ii) costovertebral angle tenderness on examination, iii) dysuria, urgency, frequency, and/or suprapubic pain plus at least one of the following: i) fever > 38°C; ii) nausea and vomiting. Uncomplicated lower urinary tract infections were excluded. Spontaneous bacterial peritonitis (SBP) was defined, in presence of ≥ 250 polymorphonuclear cells/ml in ascitic fluid examination. Intrabdominal infection (IAI) (other than PBS) was defined by new onset of fever and/or abdominal pain plus new or worsening radiological images of abscess, bowel perforation, appendicitis, diverticulitis and post-surgical effusion with or without peritonitis(82). Cholangitis or biliary tract infections were diagnosed as defined elsewhere(83). Skin and soft tissue infection (SSTI) was diagnosed in presence of purulent infections (cutaneous abscesses, furuncles, carbuncles) or in case of non-purulent infections (cellulitis, erysipelas or necrotizing infections). Bloodstream infection (BSI) were defined as true, clinically significant episode of bloodstream infection diagnosed during the study period. Episodes in which a potential contaminant (e.g., coagulase-negative staphylococci) was isolated only in one set of blood cultures without clinical evidence of infection were excluded. All BSI that were not secondary to an infection at another body site were defined primary BSI. Bacteremic infections included both primary BSI and any infection to another body site with positive blood cultures.

Patients were classified as having: i) hospital acquired infection if infection signs/symptoms started >48 hours after hospital admission, or in less than 48 hours after hospital discharge; healthcare associated infections according with standard criteria; community acquired infections in all other cases (51). Empiric antibiotic therapy was defined as the antibiotic administration before susceptibility report was available. For culture-positive infection

empiric antibiotic therapy was considered as appropriate when at least one *in vitro* active antibiotic (according with the susceptibility pattern of the isolate) was administered within 24 hours after drawing samples. In case of culture-negative infection appropriate empiric therapy was defined in base of infection site according with a recent international consensus paper on cirrhotic patients(41). Delayed or no antibiotic administration within this time frame is considered as inappropriate empiric therapy.

Statistical analysis

Categorical data were presented as absolute number and frequency while continuous data were reported as mean and standard deviation (SD), if normally distributed, or as median and interquartile range (IQR) if non-normally distributed. The Kolmogorov-Smirnov test and the Levene test were used to assess the normality of distribution and homogeneity of variances, afterward the unpaired student t-test or the Mann-Whitney U test were used to compare differences between groups when appropriate. The analysis of variance (ANOVA) followed by the Bonferroni post-oc test, or the Kruskal-Wallis followed by the Dunnett post-oc test was performed when three or more groups were compared.

To identify the risk factors for ACLF in patents with bacterial infection patients with bacterial infection developing ACLF (infection-related ACLF) were compared with patients with uncomplicated bacterial infection. Following univariate analysis, factors associated with ACLF (p<0.10) at were included in a multivariate logistic regression model. The calibration of the model was assessed by Hosmer-Lemeshow goodness-of-fit test and discrimination was assessed by the analysis of area under the receiver-operator curve (ROC). All variables were explored for interaction or collinearity.

To explore the relationship between bacterial infections and 1-year mortality rates survival curves were plotted according the Kaplan Meier method. Differences in survival rates were evaluated by means of Log-Rank test. Because long term survival may be influenced by a great number of underlying conditions, factors associated to 1-year mortality identified in univariate analysis (P<0.10) were used to develop a Cox regression model. Because several prognostic scores (i.e. MELD, MELD-Na, CLIFc-AD score) displayed collinearity, the performance of every single score was evaluated by area under the receiver operating characteristics (AUROC) curve. Therefore, we introduced only the best performing score(s) as continuous variable in the final Cox regression model. The impact of bacterial infection and ACLF was evaluated by entering these variables manually into the baseline Cox regression mortality risk model to estimate their effect on mortality. Al test were two-sided

and values of $p < 0.05$ were considered statistically significant. The analysis was performed by the Statistical Package for Social Sciences (SPSS v.21) software (IBM corp.)

RESULTS

Study population

During the study period 1140 consecutive hospital admissions to regular wards involving 916 cirrhotic patients were recorded. Of the 916 patients, 399 were electively admitted for scheduled diagnostic or invasive procedure and were excluded from the current analysis. Thus, the study cohort comprises 516 patients consecutively admitted for an episode of AD of the disease (Figure 1).

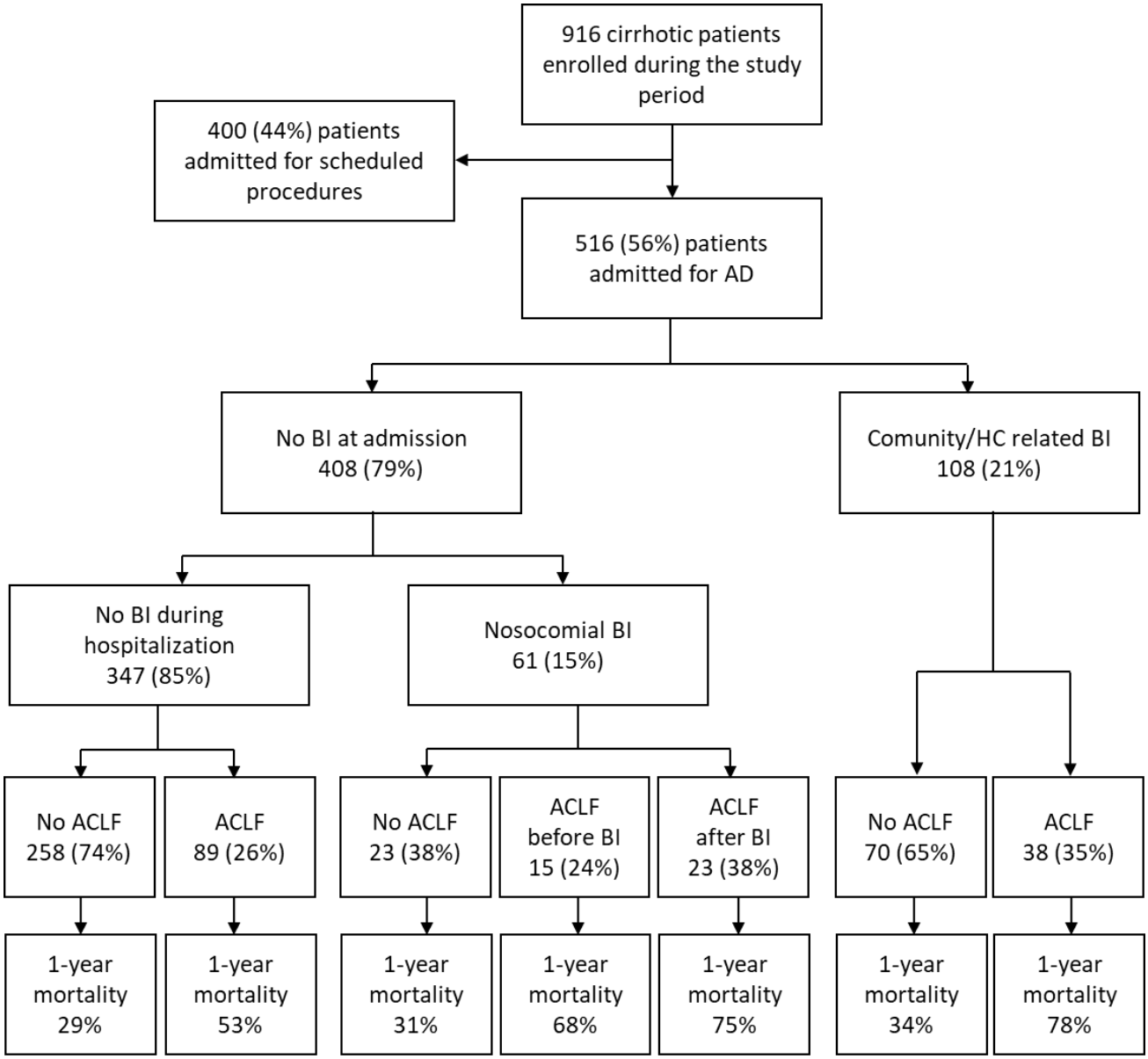

Figure 7. Details on patient's disposition. During the study period 916 cirrhotic patients were enrolled. Four hundred patients were admitted for scheduled diagnostic or therapeutic procedures and were excluded from the analysis. Therefore, the study population comprises 516 consecutive patients admitted for acute decompensation (AD) of the disease. A bacterial infection was diagnosed at admission or during the hospitalization in 169 patients,

among those, 76 developed Acute-on-Chronic Liver Failure (ACLF). Among patients without bacterial infection ACLF occurred in 89 subjects.

Comparison of patients with and without infections

One hundred sixty-nine (33%) patients presented at least an episode of bacterial or fungal infection. Of these, 108 (21%) cases were diagnosed at the time of admission whereas other 61 (12%) were classified as hospital-acquired infections. In the remaining 347 (67%) subjects, bacterial infection was not present neither at admission nor during hospitalization *(Figure 7)*.

Demographic and clinical data at admission of patients presenting or not an infection at admission or during hospitalization were reported in Table 16. The groups did not differ in terms of age, sex, etiology of cirrhosis, and clinical complications. Higher CRP levels were observed in all infected, whereas WBC count was higher at admission inly in patients with CA or HCA infections. In terms of prognostic scores, MELD-Na was higher in all infected patients at admission. On the other hand MELD was higher only in patients developing HA infections. Similarly, the latter group presented more frequently ACLF at hospital admission. Interestingly, treatment with rifaximin was significantly more frequent in patients without bacterial infection, while no differences were seen regarding the assumption of proton pump inhibitors, beta-blockers, and quinolones. Finally, co-morbidities and Charlson score were similar between the three groups. During the in-hospital stay both patients with CA/HCA and patients with HA infection developed ACLF more frequently than non-infected patients

Table 16. Clinical characteristics of the 516 patients with liver cirrhosis admitted for acute decompensation. Patients were divided according to the presence or not of a bacterial infection at the time of admission or the development of a nosocomial bacterial infection. Data are presented as frequencies [n(%)] or mean (±SD)/median (IQR) according to their distribution.

	NO BACTERIAL INFECTION	**BACTERIAL INFECTION AT ADMISSION**	**BACTERIAL INFECTION DURING HOSPITALIZATION**	***P***
n	347	108	61	
Anthropometric data				
Age (years)	61 (51-72)	64 (53-74)	61 (52-72)	0.470
Male sex	209 (60)	68 (62)	43 (73)	0.180
Etiology of cirrhosis[1]				
Viral	142 (41)	51 (47)	24 (39)	0.461
Alcohol	71 (21)	22 (20)	18 (29)	0.270
NASH	19 (6)	8 (7)	5 (8)	0.606
Viral and alcohol	45 (13)	7 (6)	3 (5)	0.049
Viral and NASH	11 (3)	1 (1)	1 (2)	0.385
Alcohol and NASH	6 (2)	1 (1)	3 (5)	0.173
Other	58 (17)	19 (18)	10 (16)	0.972
AD at admission				
Ascites	143 (41)	45 (42)	34 (56)	0.102
HE	107 (31)	22 (20)	11 (18)	0.024
Liver failure	47 (13)	9 (8)	12 (20)	0.105
Renal failure	25 (7)	4 (4)	8 (13)	0.075
GI bleeding	28 (8)	2 (2)	6 (10)	0.056
Biochemical and hemodynamic data				
WBC (10^9/L)	5.2 (3.5-7.4)	7.8 (5.0-10.9) §	5.6 (3.6-9.2)	<0.001
CRP (mg/dL)	0.91 (0.33-1.70)	3.94 (1.66-8.30) §	2.62 (0.58-5.40) §	<0.001
Platelets (10^9/L)	89 (55-139)	96 (61-176)	74 (56-123) #	0.188
Sodium (mmol/L)	137 (135-140)	136 (133-138) *	135 (132-139) *	<0.001

Bilirubin (mg/dL)	2.07 (1.07-4.24)	2.49 (1.06-4.43)	2.83 (1.50-10.30) *	0.012
Creatinine (mg/dL)	0.90 (0.74-1.30)	1.03 (0.78-1.44)	1.25 (0.88-1.85) §	0.001
Albumin (mg/dL)	3.2 (2.8-3.6)	3.0 (2.7-3.4)	3.1 (2.7-3.5)	0.081
INR	1.40 (1.22-1.59)	1.38 (1.25-1.68)	1.46 (1.28-1.73)	0.072
MAP (mmHg)	87 (78-93)	83 (77-96)	82 (75-90)	0.085
HR (bpm)	74 (65-83)	80 (70-90) *	80 (70-88) *	<0.001
Clinical data				
Child-Pugh score	8 (7-10)	8 (7-10)	9 (8-11) §	0.028
Child-Pugh Class				
Class A	84 (24)	25 (23)	7 (12)	0.088
Class B	159 (46)	55 (51)	30 (49)	0.619
Class C	104 (30)	28 (26)	24 (39)	0.186
MELD	15 (11-20)	16 (11-20)	21 (14-27) §	0.001
MELD-Na	16 (12-22)	19 (14-23) §	21 (17-30) §	<0.001
CLIF-C-AD^3	50 (45-57)	54 (50-64) *	54 (49-59) *	<0.001
ACLF at admission	67 (19)	21 (19)	21 (34)	0.025
Grade 1	29 (43)	12 (57)	6 (29)	0.174
Grade 2	35 (52)	6 (30)	13 (62)	0.075
Grade 3	3 (4)	3 (14)	2 (9)	0.295
ACLF during hospitalization	22 (6)	17 (16)	17 (28)	<0.001
ACLF triggered by infection	-	37 (97)	24 (63)	<0.001
Concomitant medications				
PPI	223 (64)	74 (68)	43 (70)	0.518
Beta-blockers	151 (43)	47 (43)	26 (43)	0.991
Rifaximin	50 (14)	6 (6)	3 (5)	0.010
Quinolone	7 (2)	1 (1)	1 (2)	0.749
Comorbidities				
CCI	6.0 (5.0-7.4)	6.0 (4.8-7.4)	6.2 (4.4-7.4)	0.915
HCC	76 (22)	33 (30)	17 (29)	0.150
Diabetes (any stage)	122 (35)	39 (36)	19 (31)	0.795

*p<0.05 vs no bacterial infection; §p<0.05 vs all;

MELD: model for end stage liver disease; ACLF: acute-on-chronic liver failure; CLIF-C: chronic liver failure consortium; AD: acute decompensation; CCI: Charlson Comorbidity Index; PPI: proton pump inhibitors; HCC: hepatocellular carcinoma.

Characteristics of infections

Bacterial infections were classified as community acquired in 69 (41%) cases, healthcare-associated in 39 (23%) cases and hospital acquired in 61 (36%) cases. Pneumonia was the leading cause of infection (41, 24%), followed by primary BSIs (30, 17%), UTI (26, 15%) SBP (25, 15%), and SSTI (15, 9%). Primary BSI were more frequent among hospital acquired infection, while UTI and SST were more prevalent among infections diagnosed at admission (Table 17).

Table 17. Source of infection according with epidemiological classification in the 169 patients with bacterial infection at hospital admission or during the hospital stay.

SITE	ALL INFECTIONS	COMMUNITY ACQUIRED	HEALTHCARE ASSOCIATED	HOSPITAL ACQUIRED	*P*
n	169 (100)	69 (39%)	31 (23%)	61 (39%)	-
SBP	26 (15)	11 (16)	7 (18)	8 (13)	0.797
BSI	30 (18)	4 (6)	5 (13)	21 (34)	<0.001
Pneumonia	41 (24)	16 (23)	15 (38)	10 (16)	0.041
UTI	26 (15)	13 (19)	6 (15)	7 (11)	0.509
IAI	18 (11)	5 (7)	3 (8)	10 (16)	0.191
SSTI	15 (9)	11 (16)	3 (8)	1 (2)	0.016
Other[#]	13 (8)	9 (13)	0 (0)	4 (7)	0.046

Other infection included 9 cases of biliary tract infection, 1 case of transjugular intrahepatic porto-systemic shunt infection, 1 case of bone and joint infection, 1 case of Ludwig's angina,1 case of Clostridium difficile infection; *one case of Clostridium difficile; § one case of Legionella pneumophila.

SBP: spontaneous bacterial peritonitis; BSI: bloodstream infection; UTI: Urinary tract infection; IAI: intrabdominal infection; SSTI: skin and soft tissue infection;

ESBL: extended spectrum beta-lactamase; CRE: Carbapenem-resistant Enterobacteriaceae; MDR: multi drug resistant.

Regarding the severity of infection, the median SOFA score was 4 (3-7) and 25 (15%) patients had a qSOFA ≥ 2 points. Furthermore, 65 (38%) patients presented with sepsis and 13 (8%) patients with septic shock.

A microbiological diagnosis was obtained in 93 (55%) patients. The etiology of culture-positive infection is reported in table 18.

Table 18. Etiology of 93 culture-positive bacterial infections collected among the 169 bacterial infections recorded during the study. Percentages refers to the total of culture-positive infection.

	SBP	Primary BSI	Pneumonia	UTI	IAI	SSTI	Other[#]
n	26 (28)	30 (32)	41 (44)	26 (28)	18 (19)	2 (2)	13 (14)
Gram-positive cocci	1 (11)	9 (30)	4 (22)	4 (21)	3 (25)	1 (50)	3 (43)
Staphylococcus aureus	0 (0)	3 (10)	3 (17)	0 (0)	0 (0)	1 (50)	0 (0)
Enterococcus faecalis	0 (0)	1 (3)	1 (6)	3 (16)	3 (25)	0 (0)	0 (0)
Enterococcus faecium	1 (11)	2 (7)	0 (0)	1 (6)	0 (0)	0 (0)	2 (29)
CoNS	0 (0)	4 (13)	0 (0)	0 (0)	0 (0)	0 (0)	1 (14)
Other GP[*]	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (14)
Gram-negative bacilli	7 (78)	19 (63)	11 (68)	18 (78)	7 (11)	1 (50)	4 (50)
Enterobacteriacee	7 (78)	16 (53)	6 (35)	16 (84)	6 (54)	‘0 (0)	4 (50)
ESBL	2 (18)	3 (10)	2 (12)	6 (33)	3 (27)	0 (0)	1 (14)
CRE	0 (0)	3 (10)	0 (0)	2 (10)	4 (36)	0 (0)	0 (0)
Non-fermenting	0 (0)	5 (17)	4 (25)	0 (0)	1 (9)	1 (50)	0 (0)
Other GN[§]	0 (0)	0 (0)	1 (6)	0 (0)	0 (0)	0 (0)	0 (0)
MDR pathogens	4 (36)	19 (58)	6 (33)	10 (53)	4 (36)	1 (50)	3 (37)
Fungi	1 (11)	2 (7)	0 (0)	0 (0)	1 (9)	0 (0)	1 (14)
Polymicrobial	1 (11)	2 (7)	1 (6)	2 (11)	0 (0)	0 (0)	3 (47)
Culture-negative	17 (65)	0 (0)	25 (61)	8 (30)	7 (39)	13 (87)	6 (46)

Other infection included 9 cases of biliary tract infection, 1 case of transjugular intrahepatic porto-systemic shunt infection, 1 case of bone and joint infection, 1 case of Ludwig’s angina,1 case of Clostridium difficile infection; *one case of Clostridium difficile; § one case of Legionella pneumophila.

SBP: spontaneous bacterial peritonitis; BSI: bloodstream infection; UTI: Urinary tract infection; IAI: intrabdominal infection; SSTI: skin and soft tissue infection; ESBL: extended spectrum beta-lactamase; CRE: Carbapenem-resistant enterobacteriaceae; MDR: multi drug resistant.

Briefly, Gram-positive cocci were detected in 25 (27%) of cases, consisting mostly in *Staphylococcus aureus* (7, 7%), *Enteroccocus faecalis* (8, 9%), and *Enterococcus faecium* (6, 6%). Gram-negative bacteria were identified in 63 cases (67%). Of these, Enterobacteriaceae were identified in 54 (58%) cases, including *Escherichia coli* and *Klebsiella pneumoniae* in 33% and 14% of cases, respectively. Non-fermenting bacilli were

isolated in 12 out of 93 (13%) cases of culture-positive infections. Lastly, fungal infections were identified in 4% of cases. In all cases of fungal infection, *Candida albicans* was the causative pathogen. Overall, 40 (43%) pathogens were classified as MDR including extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (19% of all isolates, 33% of Enterobacteriaceae) and carbapenem-resistant Enterobacteriaceae (CRE) (8% of all isolates, 14% of Enterobacteriaceae).

Finally, culture-negative infections were mostly SSTI (13, 68%), pneumonia (27, 58%), and SBP (13, 54%).

Comparison of infected patients complicated or not by ACLF

Patients with bacterial infection were more prone to present ACLF (61/169 [41%] vs 89/347 [26%], p<0.002) patients (Figure 7). Namely, a concomitant diagnosis of ACLF and infection was placed in 39 (64%) patients, while 21 (36%) patients developed ACLF after diagnosis of infection with a median delay of 8 (IQR: 2-17) days. Moreover, 15 patients presented ACLF before the development of a bacterial infection and therefore were excluded from the subsequent analysis.

Table 19 shows the demographic and clinical characteristics of patients with or without ACLF at the time of infection diagnosis. Patients with infection-related ACLF were more likely to have alcohol-related cirrhosis (31% vs 17%, p=0.04), MELD (23 [18-30] vs 13 [10-17], p<0.001) scores at admission.

Regarding the characteristics of bacterial infections (Table 19), patients with ACLF had more frequently hospital-acquired infections (39% vs 25%, p<0.001), healthcare related infections (36% vs 19%, p=0.02), bloodstream infection (BSI) including primary BSI (26% vs 11% p=0.01) or any bacteremic infection (46% vs 14%, p<0.001).Furthermore, the prevalence of MDR bacteria was significantly higher in patients with ACLF compared to patients with uncomplicated bacterial infection (37% vs 13%, p<0.001). Overall, the severity of bacterial infections in patients with ACLF was significantly higher than in patients without ACLF.

Table 19. Demographic, clinical and microbiological characteristics of patients with bacterial infection complicated or not by ACLF.

	UNCOMPLICATED INFECTIONS	INFECTION COMPLICATED BY ACLF	P
N	93	61	
Demographic data			
Age (years)	64 ± 13	62 ± 13	0.319
Sex (male)	60 (65)	39 (64)	0.941
Etiology of cirrhosis[1]			
Viral	47 (50)	24 (39)	0.173
Alcohol	16 (17)	19 (31)	0.043
NASH	7 (7)	5 (8)	1.000
Viral and alcohol	6 (6)	2 (3)	0.480
Viral and NASH	1 (1)	1 (1)	1.000
Alcohol and NASH	0 (0)	3 (5)	0.060
Other	16 (17)	10 (16)	0.895
Clinical data			
MELD score	14 (10-17)	23 (18-30)	<0.001
Comorbidities			
Diabetes	32 (34)	23 (37)	0.67
Chronic renal failure	9 (10)	11 (18)	0.13
HCC	29 (31)	17 (28)	0.66
CCI	6.2 (4.8-7.35)	6.1 (4.7-7.3)	
Admission diagnosis			
Ascites	34 (37)	34 (56)	0.02
Hepatic encephalopathy	10 (11)	19 (31)	0.002
Bleeding	4 (4)	2 (3)	1
Worsening of liver function	6 (6)	9 (14)	0.09
Suspected infection	62 (67)	32 (52)	0.07
Infection classification			
Community acquired	51 (55)	16 (21)	<0.001
Hospital acquired	23 (25)	24 (39)	0.05
Healthcare associated	18 (19)	22 (36)	0.02
Infection severity			
qSOFA	0 (0-0)	1 (0-2)	<0.001
qSOFA≥2	3 (3)	16 (29)	<0.001
SOFA	3 (0-6)	7 (4-9)	<0.001
Septic shock	0 (0)	15 (18)	<0.001
Infection source			

Pneumonia	22 (24)	17 (22)	0.83
Primary bloodstream infection	10 (11)	16 (26)	0.01
SBP	12 (13)	9 (15)	0.74
Urinary tract	20 (21)	6 (10)	0.06
SSTI	11 (12)	4 (6)	0.28
Bacteriemic infection	13 (14)	28 (46)	<0.001
Bacteria			
GNB	28 (30)	29 (47)	0.03
GPC	9 (10)	15 (20)	0.06
Staphylococcus aureus	5 (5)	2 (3)	0.46
Enteroccus faecalis	3 (3)	5 (6)	0.47
Enterococcus faecium	1 (1)	5 (6)	0.09
Fungi			
Candida spp	1 (1)	3 (5)	0.30
Microbiological features			
Polimicrobial	5 (5)	8 (9)	0.05
Negative cultures	51 (51)	23 (29)	0.003
Any MDR bacteria	12 (13)	23 (37)	<0.001

MELD: model for end stage liver disease; ACLF: acute-on-chronic liver failure; CLIF-C: chronic liver failure consortium; AD: acute decompensation; CCI: Charlson Comorbidity Index; PPI: proton pump inhibitors; HCC: hepatocellular carcinoma; SBP: spontaneous bacterial peritonitis; BSI: bloodstream infection; UTI: Urinary tract infection; IAI: intrabdominal infection; SSTI: skin and soft tissue infection; SOFA sequential organ failure assessment; ESBL extended spectrum beta-lactamase; CR carbapenem resistant; MDR multidrug-resistant; GNB Gram-negative bacilli; GPC Gram-positive cocci.

At multivariate analysis, the factors independently associated with ACLF were MELD-Na score [OR 1.17 (95% CI: 1.07-1.27), p=0.004], bacteremic infection [OR 4.59 (95% CI: 1.64-12.28), p=0.004], infection caused by a MDR pathogen [OR 2.88 (95% CI: 1.01- 8.20), p=0.048] and having a quickSOFA score ≥ 2 points [OR 9.39 (95% CI: 2.04-43.28) p=0.004]. (Table 20).

Table 20. Multivariate logistic regression analysis of factors associated to the development of acute-on-chronic liver failure (ACLF) in patients with bacterial infection.

Covariate	Odds ratio	95% CI	*P*
Bacteremic infection	4.59	1.64 – 12.18	0.004
MELD-Na score (1-point increase)	1.17	1.07 – 1.27	0.001
QuickSOFA ≥2 points	9.39	2.04 - 43.28	0.004
Infection caused by a MDR pathogen	2.88	1.01-8.20	0.048

The model calibration was assessed with Hosmer-Lemeshow goodness-of-fit test which showed a P value 0.71. The area under the receiver operating characteristic curve assessing the discriminatory power of the model was 0.88 (95% CI 0.82-0.93). Other variables included in the model were healthcare associated infection, urinary tract infections, alcoholic cirrhosis, ascites at hospital admission.

MELD: model for end stage liver disease; SOFA sequential organ failure assessment; MDR multidrug-resistant.

Finally, in patients with infection (169 cases), we sought differences between those developing delayed ACLF (21 cases) and patients never developing ACLF. Patients with delayed ACLF were more likely to have pneumonia (52% vs 24%, p=0.009), bacteremic infection (53% vs 15%, p=0.003), infection caused by a ESBL-producing Enterobacteriaceae (33% vs 4%, p<0.001) or any MDR (43% vs 13% p=0.001), and did not receive adequate empirical treatment in the first 24 hours (73% vs 0%, p=0.008). Lastly, in this group of patients UTI were less frequent than patients without ACLF (0% vs 20%, p=0.01).

Survival

After 1-year of follow-up of the 516 patients included in the analysis, 14 (3%) patients were lost to follow-up and 53 patients (10%) underwent liver transplantation. Overall 189 (37%) patients died after a median (IQR) time of 90 (32-207) days from the study inclusion. Differences among survivors and non-survivors after 1-year of follow up are depicted in table 21

Table 21. Demographic, biochemical and clinical characteristics of survivors and non-survivors after 1-year follow-up.

	SURVIVORS	NON-SURVIVORS	*P*
N	313	189	
Anthropometric data			
Age (years)	59 (50-69)	66 (57-76)	<0.001
Male sex	202 (64)	111 (59)	0.217
Etiology of cirrhosis[1]			
Viral	119 (38)	90 (48)	0.035
Alcohol	67 (21)	39 (21)	0.830
NASH	23 (7)	9 (5)	0.346
Viral and alcohol	42 (13)	13 (7)	0.023
Viral and NASH	7 (2)	6 (3)	0.568
Alcohol and NASH	6 (2)	4 (2)	1.000
Other	54 (17)	32 (17)	0.926
AD at admission			
Ascites	119 (38)	99 (52)	0.002
HE	73 (23)	62 (33)	0.020
Liver failure	35 (11)	33 (17)	0.046
Renal failure	17 (5)	19 (10)	0.052
GI bleeding	29 (9)	6 (3)	0.011
Bacterial Infection	59 (19)	49 (26)	0.062
Any bacterial infection	88 (28)	81 (43)	0.001
Biochemical and hemodynamic data			
WBC (10^9/L)	5.2 (3.5-8.0)	6.1 (4.1-9.3)	0.003
CRP (mg/dL)	0.94 (0.32-2.35)	1.63 (0.81-5.27)	<0.001
Platelets (10^9/L)	89 (57-143)	87 (55-139)	0.786
Sodium (mmol/L)	137 (134-140)	136 (133-139)	0.004
Bilirubin (mg/dL)	1.92 (0.94-3.66)	2.94 (1.48-6.10)	<0.001
Creatinine (mg/dL)	0.90 (0.72-1.24)	1.11 (0.82-1.53)	<0.001
Albumin (mg/dL)	3.2 (2.9-3.7)	3.0 (2.7-3.4)	<0.001
INR	1.36 (1.22-1.56)	1.46 (1.27-1.70)	0.001
MAP (mmHg)	87 (78-93)	83 (77-92)	0.004
HR (bpm)	75 (66-85)	78 (68-86)	0.194
Clinical data			

Child-Pugh score	8 (6-9)	9 (8-11)	<0.001
Child-Pugh Class			
Class A	84 (24)	25 (23)	0.088
Class B	159 (46)	55 (51)	0.619
Class C	104 (30)	28 (26)	0.186
MELD	14 (10-18)	17 (14-24)	<0.001
MELD-Na	16 (12-21)	20 (15-26)	<0.001
CLIF-C-AD[3]	49 (43-55)	55 (50-62)	<0.001
Any ACLF	71 (23)	92 (49)	<0.001
Grade 1	45 (14)	40 (21)	
Grade 2	22 (7)	38 (20)	<0.001
Grade 3	4 (1)	13 (7)	
Comorbidities			
Charlson Comorbidity Index (CCI)	5.5 (4.4-6.9)	6.8 (5.7-8.6)	<0.001
HCC	58 (18)	55 (29)	0.006
Diabetes (any stage)	104 (33)	74 (39)	0.179

AD: acute decompensation; MELD: model for end stage liver disease; ACLF: acute-on-chronic liver failure; CLIF- C: chronic liver failure consortium; AD: acute decompensation; COPD: chronic obstructive pulmonary disease; PPI: proton pump inhibitors; WBC: white blood cells; INR: international normalized ratio; CRP: C-reactive protein; HCC: hepatocellular carcinoma.

Overall, bacterial or fungal infections were associated to a worse 1-year survival as compared to patients without bacterial infection (50 vs 65%, p=0.001). Interestingly, the Kaplan Meier survival analysis showed that 1-year survival was similar in infected and non-infected patients without ACLF (71% vs 67% p=0.337), while bacterial infections complicated by ACLF were associated to a significantly lower survival rate than ACLF precipitated by other events (23 vs 47%, p=0.010). To further evaluate the impact of ACLF and bacterial or fungal infections complicated or non-complicated by ACLF on 1-year survival we first analyzed the accuracy of 4 cirrhosis-specific scores (MELD, MELD-Na, Child-Pugh and CLIFc-AD score) and Charlson Comorbidity Index in predicting 1-year mortality using ROC curves. Both Charlson Comorbidity Index [AUROC 0.70 (95% CI 0.65-0.74)] and CLIFc-AD [AUROC 0.70 (95% CI 0.65-0.74) best predicted 1-year mortality. Therefore, the final Cox regression model for 1-year mortality included CCI and CLIFc-AD score as continuous variables. Uncomplicated bacterial infection was not associated with an increased risk of mortality after 1 year [AHR 0.84 (95% CI 0.53-1.33) p<0.481]. Conversely, ACLF triggered by infection [AHR 3.14 (95% CI 2.10-4.69) p<0.001 were independent predictors of mortality.

DISCUSSION

In this prospective study, we aimed to identify cirrhotic patients with bacterial infection at higher risk of ACLF and we compared the long-term mortality of patients according with the presence of ACLF and/or bacterial infections. The main findings are that patients with bacteriemic, infections or caused by MDR pathogens are at high risk to develop ACLF. In addition, our results indicate that bacterial infections do not change the natural history of cirrhosis unless they are associated by ACLF.

To date few studies evaluated risk factors for ACLF in patients admitted with acute decompensation and there is a lack of data regarding the main subtype of bacterial infection associated with this syndrome. This aspect seems of pivotal importance as the bacterial infection are the main cause of ACLF.

In the study of Fernandez et al.(84) based on a multicenter enrollment, patients with bacterial infections associated with ACLF were compared with patients with AD. Like in our study, patients with ACLF were characterized by severe infections, nosocomial infections and

isolation of a MDR pathogen. They also found that SBP and pneumonia were more likely to be associated to ACLF. Our results are partially in contradiction with this report. Particularly, even if pneumonia was associated with delayed ALCF we did not find any association between SBP and ACLF. The possible explanations are several. First, the epidemiology of infection may be different in our settings. In fact, the overall rate of SBP were lower than previous reports but similar to that of recent Italian studies(9, 20, 21). Second, management and prevention of cirrhosis complication may have been different, including the rate of patients in antibiotic prophylaxis or in treatment with albumin. Finally, the definitions of infection were slightly different and, in addition, in our study every case of infection was reviewed by a team of infectious disease consultants and hepatologists. In our study bacteremic infection, infection caused by MDR were independent risk factors for ACLF whereas urinary tract infections were found to have a lower propensity to ACLF. This latter aspect may be controversial. A previous study in cirrhotic patients found that UTI, gastrointestinal infection and SBP were characterized by higher incidence of renal failure (85). On the other hand, studies with a population that included also non-cirrhotic patients found that UTI are associated with a lower mortality rate, even if caused by MDR pathogens. (86, 87). Bacteremic infection and infections caused by MDR have already demonstrated to be associated with worse outcome if compared with other source and etiology of infection(9, 31, 58). Thus, it is not surprising that they have an impact also in determining the risk of ACLF.

Another important finding of our study is that bacterial infection itself is not a marker of poor prognosis. The hallmark of bacterial infection as an event that change the history of the disease is due to several previous studies.(8, 19, 88). However, none of the studies stratified patients for the presence of ACLF. According with our finding, bacterial infection accelerates the course of the disease only if complicated by ACLF. However, patients with ACLF precipitated by infection exhibited worse prognosis when compared with patients with non-infectious ACLF.

If our data will be furtherly confirmed, new criteria may be proposed to differentiate cirrhotic patients with complicated bacterial infection including those with bacteremic infection, non-UTI or those presenting with ACLF. This new classification may be useful to prioritize the medical treatment, select patients who may benefit form a broader spectrum antibiotic therapy and prioritize transplantation evaluation. In fact, in patients with ACLF and bacterial infections the 30,90,180 and 365-day mortality was 32%, 40%, 48% and 54% of cases, respectively.

Our study has some limitations. First, most patients were enrolled in a large university teaching hospital with a transplant center. This may have selected patients with advanced liver disease. Therefore, we found a lower rate of SBP than reported by other studies(4, 17). Second, due to Italian laws informed consent for participation in the study cannot be given in case of unconsciousness. Thus, several ICU patients were not included in the study.

Notwithstanding these limitations, we believe that our study can give some novel and robust information on bacterial infection in cirrhotic patients, identifying risk factors for ACLF and consequently for long-term prognosis.

CONCLUSION

In this work several aspects of patients with ESLD were evaluated. The main findings may be summarized as follows:

- Infection remain a worthy cause of morbidity and mortality of patients with ESLD. Among all patients admitted for an acute decompensation 32% develop a bacterial or fungal infection.
- Bloodstream infections (BSI) are an important cause of infection. Among all source of infection, primary BSI occurs in 18% of all patients and in 34% of patients with nosocomial infection. Moreover, bacteremic infections (one third of infections) are independently associated to ACLF.
- In Europe 31% of BSI are caused by MDR pathogens with a wide difference between countries. Isolation of a MDR pathogen is associated to a significative high risk of failure of first-line empiric treatment.
- Different causative pathogens of BSI are found in patient with alcoholic liver disease when compared with other etiology of cirrhosis. In patients with alcoholic cirrhosis a significant high prevalence of Gram-positive cocci was found.
- Despite previous studies our results indicate that bacterial infection changes the course of cirrhosis only when complicated by ACLF. Patients with bacterial infection without ACLF have a similar prognosis of patients with other cause of acute decompensation. However, patients with ACLF caused by infection show a higher mortality after 1 year of follow-up.
- Among patients with BSI, appropriated empirical treatment in the first 24h from diagnosis of infection is associated to improved survival. In addition, continuous or extended infusion of beta-lactams seems more effective than intermittent administration, especially when administered empirically.

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