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“Bacterial and fungal infections in patients with cirrhosis: epidemiology across the world, definition of sepsis and predictors of post discharge outcomes”

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ABSTRACT

Bacterial or fungal infections in patients with cirrhosis: epidemiology across the world, definition of sepsis and predictors of post discharge outcomes

Background and aims: Bacterial infections are frequently observed in patients with cirrhosis and confers a poor survival. In recent years an increase in mortality rate has been observed in patients with cirrhosis. In addition, an increase in the prevalence of multi drug (MDR) bacterial infections has been described, reducing the efficacy of commonly used antibiotics such as third generation cephalosporins. In this thesis we conducted 3 studies aimed to: a) provide data about epidemiology of infections across countries and continents; b) assess the ability of the new criteria for sepsis (Sepsis-3) in predicting poor outcomes in patients with cirrhosis and bacterial infections; c) to assess predictor of early readmission (within 30 days) and mid-term survival (6 months) after discharge for bacterial infections

Methods: For the first study, we conducted a prospective, multicenter, intercontinental, cross sectional study including patients with cirrhosis and bacterial/fungal infections (1,302 patients). For the second study, we conducted a prospective cross sectional study in two cohort of patients (primary cohort [259 patients] and validation cohort [197 patients]). For the third study, patients discharged after a hospitalization for bacterial/fungal infections were followed up for 6 months (199 patients). In all the 3 studies, demographic, clinical, laboratory and microbiological characteristics were collected at inclusion in the study and during the follow up.

Results: In the first study we showed several differences among countries in the prevalence and the type of MDR bacterial infections, that were more prevalent in Indian or other Asian centers as well as in Southern American centers. These findings were associated with a significantly lower probability of microbiological susceptibility to antibiotic regimens suggested by the current guidelines. Remarkably, some infections were poorly treated even in countries with a low prevalence of MDR bacteria. The second study showed that Sepsis-3 criteria and quick SOFA have a significantly greater discrimination for in-hospital mortality (AUROC=0.784 and 0.732, respectively) than SIRS (AUROC=0.606;p<0.01 for both). Sepsis-3 had higher incidence of acute-on-chronic liver failure, septic shock and transfer to ICU than those without Sepsis-3. Similar results were observed in the validation cohort. In the third study, 69 patients (35%) were readmitted within 30 days from discharge. C-reactive protein (CRP) value at discharge (OR=1.91;p=0.022), diagnosis of acute-on-chronic liver failure during the hospital stay (OR=2.48;p=0.008) and the

hospitalization in the last 30 days previous to the admission/inclusion in the study (OR=1.50;p=0.042) were found to be independent predictors of readmission. CRP at discharge was also found to be an independent predictor of 6 month mortality (HR=1.85;p=0.001) as well as age (HR=1.05;p=0.001), MELD score (HR=1.13;p<0.001), refractory ascites (HR=2.22;p=0.007) and diabetes (HR=2.41;p=0.010). Patients with a CRP>10 mg/l at discharge had a significantly higher probability of being readmitted within 30 days (44 vs 24%; p=0.007) and a significantly lower probability of 6-month survival (62 vs 88%;p<0.001) than those with a CRP≤10 mg/L

Conclusions: The first studies allowed to clarify the different epidemiology of bacterial infections in different centers suggesting different approach for the empirical treatment. The second study suggests that Sepsis-3 criteria and qSOFA are reliable tools to define the severity of infections and thus “sepsis” in patients with cirrhosis. Accordingly, an algorithm has been provided for the application of these criteria in patients with cirrhosis. Finally, the third study suggest that CRP levels may be potentially used to guide the antimicrobial stewardship in patients with cirrhosis and bacterial infections.

BACKGROUND

Liver cirrhosis results from different mechanisms of liver injury that lead to necroinflammation and fibrogenesis[1]. Liver cirrhosis is histologically characterized by marked distortion of the liver parenchyma due to large fibrous septa, due to deposition of collagen, that surround regeneration nodules of hepatocytes. The alterations in liver architecture impairs the portal vein circulation through the liver causing an increase in portal pressure. The parenchymal extinction causes an impairment of the liver synthetic function. Prevalence of cirrhosis is of about 1%, however, a recent study from England reported that the incidence of cirrhosis has increased by 51% during a 10-yr period, from 1998 to 2009, for all etiology[2]. Cirrhosis is an increasing cause of morbidity and mortality in more developed countries. It is the 14th most common cause of death in adults worldwide, but the fourth in central Europe; it results in 1.03 million deaths per year worldwide, 170,000 per year in Europe[3]. The natural history of cirrhosis is characterized by a phase characterized by the absence of relevant symptoms of the disease (compensated cirrhosis) and a phase characterized by the appearance of complications of cirrhosis, the main one represented by the appearance of ascites, gastrointestinal bleeding, hepatic encephalopathy and or bacterial infections. When decompensation occurs, the 1 year survival probability rate decreases to 50%[4]. The most common cause of death in these patients is the development of a syndrome characterized by organ failures (kidney, brain, liver, coagulation, circulation and lung) named acute-on-chronic liver failure (ACLF)[5], a condition that is frequently precipitated by a bacterial infection[5–7]

Prevalence of infections in patients with cirrhosis

Patients with cirrhosis of the liver have a high risk of bacterial infections[8]. A large study performed in the U.S. (175 million hospital discharge) showed that patients with cirrhosis had a

risk of sepsis 2.6 fold higher than those without[9]. In addition, the risk of dying due to sepsis was 2 fold higher in patients with cirrhosis than those without cirrhosis. More in detail, the prevalence of bacterial infections is about 25-46% in patients hospitalized for an acute decompensation of cirrhosis[5,10]. In hospitalized patients with cirrhosis, bacterial infections are more common in those with ascites than in those without ascites (45 vs 17 %: $p < 0.001$)[10]. Spontaneous bacterial peritonitis (SBP) and urinary tract infections (UTI) are the most common infections in patients with cirrhosis followed by pneumonia, skin and soft tissue infections and spontaneous bacteremia[10–12]. Risk factors for the development of infection are poor liver function, variceal bleeding, low ascitic fluid protein levels, and prior SBP[13]. Both infections due to gram positive and gram negative bacteria are increased in patients with cirrhosis[9] and cirrhosis of the liver is also considered a risk factor for fungal infections.

Pathogenesis of bacterial infections in cirrhosis

Several factors are involved in the pathogenesis of bacterial infections in patients with cirrhosis. In patients with SBP and spontaneous bacteremia, the *conditio sine qua non* is a pathological bacterial translocation from the intestinal lumen to mesenteric lymph node. The pathological bacterial translocation is related to several factors, including: quantity and quality of intestinal bacteria, increased intestinal permeability and local defects in host immunity[14]. The occurrence of the other infections, as well as SBP is facilitated due to a cirrhosis associated immune dysfunction[15]

Genetic predisposition

Extracellular bacteria are recognized by membrane-bound of pattern recognition receptors (PRRs) such as Toll-like receptors (TLR) and intracellular Nod-like receptors (NLR), including NOD2 and NLRP3, which lead to activation of nuclear factor NF κ B and stimulate the release of antimicrobial peptides[8]. TLR2 recognizes tri-acylated lipoprotein from gram-positive bacteria,

TLR4 detects lipopolysaccharide (LPS), and NOD2 senses muramyl dipeptide, a cell wall component of gram-negative bacteria.

Genetic polymorphisms NOD2 and TLR2, have been associated with the onset of SBP[16,17]. The presence of the nucleotide-binding oligomerization domain containing 2 (NOD2) variants p.R702W, p.G908R, and c.3020insC increased the risk of SBP 3 fold, probably due to an increased intestinal permeability[16].

SBP was significantly more frequent in patients with the TLR2 -16934 TT genotype and in carriers with two long tandem GT repeat alleles[17]. Furthermore, the simultaneous presence of NOD2 and TLR2 polymorphisms indicated a particularly high risk for SBP [17].

Intestinal bacterial overgrowth

The intestinal bacterial overgrowth plays a key role in BT in cirrhosis and is the result, at least partly, of decrease in small-bowel motility and the delayed intestinal transit existing in these patients [18,19]. It has been suggested that autonomic dysfunction, increased NO synthesis and the oxidative stress of the mucosa are the main causes for decreased intestinal motility

Intestinal bacteria quality

The full microbial richness in the human population reaches up to 40,000 species [20]. However, only 30–40 species amount to about 98–99% of the microbiota, and Firmicutes and Bacteroidetes are the predominant intestinal phyla across all vertebrates[21]. The microbiota of cirrhosis has been associated with the depletion of the beneficial phyla Lachnospiraceae (particularly clostridia) and enrichment in the phyla Proteobacteria (mainly Enterobacteriaceae) and enterococcae[22–24]. Gram-negative bacteria (GNB) (specifically *E. coli*, *K. pneumoniae*, *P. aeruginosa* and other Enterobacteriaceae), enterococci and other streptococci, have been found to be the most adept at translocating to MLN[25]. Interestingly, these species and among those particularly *E. coli* are those that most frequently cause spontaneous bacterial infections in

cirrhotic patients[11,26]. Recently Bajaj JS and coll. found that the ratio between “good” vs “bad” species (cirrhosis dysbiosis ratio) was reduced in hospitalized patients with cirrhosis and infection and was negatively correlated with endotoxemia ($r = -0.4$, $p = 0.002$)[24].

Intestinal permeability

Intestinal mucosal barrier represents a physical and biological hurdle to bacterial translocation. The mucins, the secretory mucosal Immunoglobulin A antibodies, alfa-defensins, lysozyme and the tight junctions protect the epithelium against local and systemic bacterial invasion[14].

An increased intestinal permeability (IP) was found in cirrhotic patients compared with healthy controls[27]. Furthermore, a significant correlation between the increased IP and the severity of the liver disease and history of SBP was found. Several factors increase IP in cirrhosis, ultra-structural changing of intestinal mucosa such as widening of intracellular spaces, vascular congestion, edema of the lamina propria, fibromuscular proliferation, a decreased villous/crypt ratio, wall thickening and tight junctions disruption was previously reported[28]. Oxidative stress of intestinal mucosa increases lipid peroxidation and glycosylation of the brush border membranes and mucins[29]. Inflammatory pathway also appears to affect IP. In experimental cirrhosis, the over production of tumor necrosis factor-alpha (TNF-alpha), also described in cirrhotic patients, alters the structure of the intestinal mucosa, decreasing expression of tight junctions (zona occludens 1; ZO-1)[30] and increasing IP. Interestingly, anti-TNF monoclonal antibody as well as pentoxifylline treatment significantly decreases bacterial translocation in experimental cirrhosis[31]. Finally, the hyperactivity of the autonomic nervous system has been associated with alteration in IP. In cirrhotic ascitic rats splanchnic specific sympathectomy has been shown to prevent translocation and spreading of E. coli, being associated with increased chemotaxis and phagocytic capacity of mononuclear cells[32]. Additional proposed beneficial effects of sympathectomy are accelerated intestinal transit time, prevention of gram-negative bacteria overgrowth and improvement in gastrointestinal permeability[14]. Propranolol has

likewise been used and found to lower incidence of SBP in cirrhotic patients[33].

Host immunity in cirrhosis

Cirrhosis is associated with several abnormalities in innate and adaptive components of the immune system's response to microbial challenge, leading to a state of acquired immunodeficiency[15].

The impairment of liver function causes a reduction in the synthesis of innate immunity proteins such as complement, reducing the bactericidal capacity of phagocytic cells[34].

The overall number of leukocytes is frequently reduced in stable patients with cirrhosis, due to hypersplenism. In addition, a reduction of bone marrow stem cells niche has been recently reported and may contribute to the reduced circulating leukocytes[35].

Among immune cells involved in innate immunity, neutrophils are reduced in numbers and shows a reduced phagocytosis of opsonized bacteria[36–38]. The reduction of intracellular killing is due to defective superoxide anion O_2^- production and myeloperoxidase activity[36]. These alterations seem to involve intracellular signaling mechanisms[36–38]. Neutrophils also show impaired chemotaxis to the infection focus. Moving to monocytes, they are markedly increased in number and present an over expression of the TLRs[39] with an over production of TNF-alpha[40] in patients with cirrhosis. However, monocytes shows a in Fc gamma-receptors function[41] that has been linked to a risk of developing bacterial infections. In addition, in patients with acute decompensation of cirrhosis shows an overproduction of the cyclooxygenase-derived eicosanoid prostaglandin E_2 [42]. In vitro administration of plasma from patients with acute decompensation of cirrhosis to healthy monocytes caused a suppression of macrophage proinflammatory cytokine secretion and bacterial killing, that was restored by PGE2 receptor antagonists[42]. Interestingly, the administration of albumin, which reduces PGE2 bioavailability, improved the macrophage proinflammatory response to LPS[42]. Finally, in the most severe patients with cirrhosis, namely those with ACLF, monocytes showed an

overexpression of MERTK receptors that mediates a blunted inflammatory response to LPS stimulation[43]. In addition, in patients with ACLF an increase in mononuclear myeloid-derived suppressor cells was shown, that decreased T cell proliferation, produced less TNF-alpha in response to TLR stimulation, and reduced bacterial uptake of Escherichia coli[44]. Interestingly, it has been shown that patients with ACLF have a very high short-term occurrence of bacterial and fungal infections[7]

Among immune cells mostly involved in acquired immunity, both B and T lymphocytes are reduced in number and show an impairment in immune function. The most striking anomaly observed in the B cell compartment is memory B cell dysfunction. Specifically, cirrhosis leads to a loss of CD27+ memory B cells causing an hypo-responsiveness to CD40/TLR9 activation[45]. T cell depletion is due to an impairment in the production of new naive T cells due to accelerated aging and atrophy of the thymus, reduction of the T cell memory subset, due to spleen sequestration and cell consumption, and impaired compensatory peripheral proliferation[15,46].

Clinical impact of bacterial/fungal infections in cirrhosis

Bacterial infections are a common cause of decompensation in patients with cirrhosis[5]. Specific bacterial components such as LPS from gram negative bacteria or tri-acylated lipoprotein from gram-positive bacteria (also called pathogens associated molecular patterns [PAMPs]) are recognized by PRRs on immune cells, causing the release of proinflammatory mediators. Thus, bacterial infections may cause a status of exaggerated systemic inflammation in patients with cirrhosis[47]. This inflammation can be responsible of tissue damage and organ failure[48]. Interestingly, according to a new hypothesis, systemic inflammation is the main driver of decompensation, organ failures and ACLF in patients with cirrhosis[49]. In fact several lines of evidence suggests that PAMPs may exacerbate splanchnic arterial vasodilation; reduce cardiac contractility; induce renal dysfunction and brain edema[49]. Therefore, bacterial

infections may frequently cause acute kidney injury (AKI) and are the most common precipitants of hepatorenal syndrome (HRS)[10,50,51]. In addition, a cognitive impairment with clinical characteristics of hepatic encephalopathy is frequently caused by bacterial infections[52]. Thus, it is not surprising that bacterial infections are the most common trigger of ACLF[5]. Finally, bacterial infections increase 4 fold the risk of mortality in patients with cirrhosis, reaching 30% at 1 month and 63% at 1-year[53].

Diagnosis of bacterial infections in cirrhosis

Signs and symptoms of infections may be very subtle in patients with cirrhosis, thus in all patients hospitalized for an acute decompensation of cirrhosis the presence of infections should be investigated. Systemic inflammatory response syndrome (SIRS) criteria (at least 2 among body temperature <36°C or >38°C; heart rate >90 bpm, respiratory rate >20/min, white blood cells [WBC] <4.000/μL or >12.000/μL or immature neutrophils >10%) may increase the suspicion of infection[13], however, the application of these criteria is problematic in patients with cirrhosis. Indeed, patients with cirrhosis may have: a) leukopenia due to hypersplenism, b) tachypnea due to hepatic encephalopathy or ascites, c) bradycardia due to the use of beta-blockers[8,13]. Thus, SIRS criteria are neither specific nor sensitive in the diagnosis of infections in patients with cirrhosis although they can still identify patients at higher risk of in-hospital mortality[54].

Acute phase protein, such as C-reactive protein (CRP) and procalcitonin (PCT) are widely used in general population to diagnose bacterial infections[55,56]. CRP is mainly produced by the hepatocytes in response to inflammatory mediators while PCT is produced by several cells. In spite of some concerns about the use of CRP as a biomarker of bacterial infections in patients with advanced cirrhosis, it was proven to be very accurate, with AUC ranging from 0.64 to 0.93[57–59]. Sensitivity and specificity of a threshold of CRP of 10 mg/L was 84% and 91%, respectively[57]. The diagnostic accuracy of PCT seems to be good in the cirrhotic population, similar to those of CRP[57]. The cut-off value proposed for PCT in cirrhosis is identical to that

used in the general population, 0.5 ng/ml[13]. The usefulness of CRP and PCT in the stewardship of antibiotic treatment needs further investigation in cirrhosis.

Diagnostic criteria for bacterial infections in patients with cirrhosis are similar to those used in the general population. Anyway, some specific spontaneous infections occurring in patients with cirrhosis, namely SBP, spontaneous empyema and spontaneous bacteremia deserve a more detailed description. SBP is defined as a bacterial infection of ascitic fluid developed in patient without any intra-abdominal, surgically treatable source of infection[60]. The diagnosis of SBP is based on a polymorphonuclear (PMN) cells in ascitic fluid above 250 cells/ μ l [60]. In patients with haemorrhagic ascites with a fluid red blood cell count > 10 000/ μ l (due to concomitant malignancy or traumatic tap), a correction factor of 1 neutrophil per 250 red blood cells (RBC) has been proposed, since this is the maximum expected ratio of neutrophils to RBCs normally present in peripheral blood[60]. The vast majority of cirrhotic patients with ascites and peritoneal infection have SBP. However, a small group of patients have bacterial peritonitis secondary to perforation or acute inflammation of intra-abdominal organs, abdominal wall infections or previous abdominal surgical procedures[61].

The differentiation is important because secondary peritonitis usually does not resolve unless patients are treated surgically. Secondary peritonitis should be suspected when at least one of the following features is present[62]: a) reduction in ascitic fluid neutrophil count of less than 25% (or even an increase) of the pretreatment value after two days of antibiotic treatment; b) More than one organism isolated from ascites, strongly suggestive of perforated bowel (particularly when the growth of anaerobic bacteria or fungi is observed); c) Runyon's criteria, represented by neutrocytic ascites with at least two of three criteria: ascitic fluid total protein >1 g/dl (in contrast to SBP which selectively occurs in low-protein ascites), glucose <50 mg/dl (due to bacterial glucose utilization), or LDH >225 mU/ml (most likely due to more rapid metabolic rate and disintegration of ascitic PMN)[62].

These criteria are to be very sensitive in the detection of secondary peritonitis, although their

specificity is low. In clinical practice to distinguish 'secondary peritonitis' from SBP, patients should undergo appropriate radiological investigation like chest X ray and abdominal computed tomography scan[61].

Spontaneous empyema is a spontaneous infection of the pleural fluid and is diagnosed when PMN count in pleural fluid is above 250 cells/ μ L. Finally, spontaneous bacteremia is defined by the presence of positive blood cultures with no apparent cause of bacteremia[13].

Antibiotic resistance in cirrhosis

In recent years there has been concern about the increasing isolation of bacteria resistant to common antibiotics used in these patients. Observational studies coming from Europe[11,26,63,64], North America[12,65] and Asia[66] showed a high rate of infections due to multi-drug resistant (MDR) bacteria (bacteria resistant to 3 or more of the main antibiotic families) in patients with cirrhosis.

The most common MDR bacteria isolated in patients with cirrhosis are extended spectrum beta lactamase (ESBL) producing *Enterobacteriaceae*, methicillin resistant *Staphylococcus Aureus* (MRSA) and *Enterococcus Faecium*[11,26,65,67]. In addition, the appearance of extensively resistant (XDR) bacteria (bacteria resistant to at least one antibiotic in all classes excepting 2) such as carbapenemase producing *Klebsiella Pneumoniae* has been recently reported[68,69].

The site of acquisition of infection determines the risk of MR bacterial infection with higher rates of MR bacteria in infections acquired in the healthcare environment: 23–39% in nosocomial infections, 14–41% in healthcare-associated (HCA) episodes and 0–16% in infections acquired in the community[11,13,64]. Other risk factors correlated with MDR bacteria were norfloxacin prophylaxis, use of beta lactams in the previous 3 months and infection due to MDR in the previous 6 months[11,26,65].

According to the above mentioned epidemiological changes, the efficacy of 3rd generation cephalosporins as well as alternative therapies such as amoxicillin-clavulanic acid or quinolones has decreased[11,26,63–67,70–72]. Indeed, MDR infections are more difficult to be treated and frequently the empirical antibiotic treatment is ineffective in these infections [8,11]. As a consequence incidence of septic shock and mortality rate is significantly higher in patients with MDR bacterial infections [11,73].

Thus, there is the urgent need to develop new strategies in the prevention and treatment of bacterial infections in patients with cirrhosis. However, in this field, large epidemiological studies are lacking and/or limited to single center experiences. As a result, a global view of the epidemiology of bacterial infections across the world is currently missing.

Treatment of infections in patient with cirrhosis

General management

Blood urine and ascites cultures should be collected at the diagnosis of infections, before the administration of antibiotics. Vital signs (body temperature, arterial blood pressure, respiratory rate, heart rate and oxygen saturation, cognitive status etc) should be checked. In hypotensive patients, resuscitation fluid should be administered as soon as possible. The assessment of renal and liver function tests should be performed to diagnose AKI or ACLF. Patients with AKI should be managed according to the current International Club of Ascites recommendations[74]. Patients with severe sepsis or septic shock should be treated with a early goal directed therapy[13,75]

Antibiotic treatment

Empirical antibiotic treatment should be initiated as soon as possible after the diagnosis of a bacterial infection[8,13]. This statement is due to the demonstration that any delay in the administration of an effective antibiotic treatment is associated with an increased risk of

mortality in patients with cirrhosis and septic shock[76]. The administration of an effective antibiotic treatment is critical to improve the outcomes of patients with cirrhosis and bacterial infections. Indeed, several studies have shown that the inefficacy of empirical antibiotic treatment is the strongest predictor of short-term mortality in patients with cirrhosis and bacterial infections[26,67,70,72]. The decision of the empirical antibiotic treatment should be guided by the following considerations: a) type of infection; b) risk of a multi drug resistant (MDR) bacterial infection (health care associated [HCA] or nosocomial, recent exposure to systemic antibiotics; norfloxacin prophylaxis etc.) and c) the severity of infection and d) local epidemiology[8]. Ideally, the empirical treatment should cover all potential organisms responsible for infection, but should consider also the potential side effects and the need to spare antibiotics active against MDR bacteria (carbapenems, glycopeptides etc.)[13]. In Table 1 the current recommended empirical antibiotic treatment has been reported[8].

In community acquired infections, third generation cephalosporins are considered safe and effective for treating SBP, spontaneous bacterial empyema and spontaneous bacteremia. Amoxicillin/clavulanic acid is a good alternative to 3rd generation cephalosporins in patients with spontaneous infections. Community acquired UTI should be treated with quinolones or cotrimoxazole in patients without complication and in center with a low prevalence of quinolone resistant Enterobacteriaceae. Patients with complicated UTI should be treated with 3rd generation cephalosporins or amoxicillin/clavulanic acid. The treatment of community acquired pneumonia should include the combination of 3rd generation cephalosporins or amoxicillin/clavulanic acid plus macrolides. An alternative strategy is the administration of monotherapy with respiratory quinolones. The treatment suggested for community acquired skin and soft tissues infections (SSTIs) is the combination of 3rd generation cephalosporins or amoxicillin/clavulanic acid plus oxacillin. The antibiotic regimen suggested for uncomplicated HCA and nosocomial UTI is the use of piperacillin/tazobactam (in center with a low prevalence of MDR bacteria) or carbapenems. The addition of glycopeptides (vancomycin or teicoplanin) may be suggested in

centers with high prevalence of methicillin resistant *Staphylococcus Aureus* (MRSA) or vancomycin sensitive *enterococci* (VSE). Linezolid is suggested in centers with high prevalence of vancomycin resistant *enterococci* (VRE).

Table 1: Recommended empirical antibiotic treatment for community-acquired and nosocomial bacterial infections in cirrhosis (modified from Jalan R et al ref.8)

Type of infection	Community-acquired infections	Nosocomial infections*
SBP, spontaneous bacterial empyema and spontaneous bacteremia	Cefotaxime or ceftriaxone or amoxicillin/clavulanic acid	Piperacillin/tazobactam Δ or meropenem \S \pm glycopeptide $\#$
UTI	Uncomplicated: Ciprofloxacin or cotrimoxazole If sepsis: cefotaxime or ceftriaxone or amoxicillin/clavulanic acid	Uncomplicated: nitrofurantoin or fosfomycin If sepsis: piperacillin/tazobactam $^{\circ}$ or meropenem \pm glycopeptide $\#$
Pneumonia	Amoxicillin/clavulanic acid or ceftriaxone + macrolide or levofloxacin or moxifloxacin	Piperacillin/tazobactam $^{\circ}$ or meropenem/ceftazidime + ciprofloxacin \pm glycopeptide $\#$ should be added in patients with risk factors for MRSA \S
Cellulitis	Amoxicillin/clavulanic acid or ceftriaxone + oxacillin	Meropenem/ceftazidime + oxacillin or glycopeptides $\#$

Legend: SBP, spontaneous bacterial peritonitis; SBE, spontaneous bacterial empyema; MRSA, methicillin-resistant *Staphylococcus Aureus*.

*, Recommended empirical treatment also for health-care associate (HCA) urinary infections and pneumonia. Empirical antibiotic treatment of HCA spontaneous infections and cellulitis will be decided on the basis of the severity of infection (patients with severe sepsis should receive the schedule proposed for nosocomial infections) and on the local prevalence of multiresistant bacteria in HCA infections.

$^{\circ}$, In areas with a low prevalence of multi drug resistant bacteria.

$\#$, IV vancomycin or teicoplanin in areas with a high prevalence MRSA and vancomycin-susceptible *enterococci*. Glycopeptides must be replaced by linezolid in areas with a high prevalence of vancomycin-resistant *enterococci*.

\S , Ventilator-associated pneumonia, previous antibiotic therapy, nasal MRSA carriage

In patients with nosocomial spontaneous infections (SBP, spontaneous bacterial empyema and spontaneous bacteremia), the treatment suggested is piperacillin/tazobactam (in center with a low prevalence of MDR bacteria) or carbapenems are suggested. The addition of glycopeptides should be reserved to centers with a high prevalence of MRSA and VSE. Interestingly, a randomized controlled trial, in a center with a high prevalence of extended spectrum beta lactamases (ESBL) *enterobacteriaceae*, MRSA and *enterococci*, the combination of meropenem plus daptomycin showed to be more effective than ceftazidime in the treatment of nosocomial SBP[67]. In addition, the administration of an effective empirical treatment was found to be an independent predictor of survival. The treatment suggested for HCA infections should be titrated according to the local epidemiology (broad spectrum for centers with high prevalence of MDR bacteria and those provided for community acquired infections in centers with a low prevalence of MDR). In a randomized controlled trial, comparing a broad spectrum antibiotic treatment versus standard treatment, in patients with cirrhosis and HCA infections, the broad spectrum treatment was more effective and associated with a lower mortality than standard treatment[70].

The treatment suggested for HCA or nosocomial pneumonia includes piperacillin/tazobactam (in center with a low prevalence of MDR bacteria) or carbapenems plus a respiratory quinolone. The addition of glycopeptides should be reserved to centers with a high prevalence of MRSA. Linezolid is suggested in centers with high prevalence of vancomycin resistant *enterococci* (VRE). Nosocomial SSTIs should be treated with carbapenems or ceftazidime plus oxacillin, while glycopeptides can be added in centers with a high prevalence of MRSA. The treatment of other infections should follow international guidelines. Aminoglycosides use should be avoided in patients with cirrhosis because it is associated with a high risk of AKI[77]. If microbiological cultures allows the identification of the strain responsible for the infection, the antibiotic regimen should be narrowed according to the antibiotic susceptibility test, to decrease the likelihood of emergence of antibiotic resistance[8,13].

Prevention of acute kidney injury (AKI)

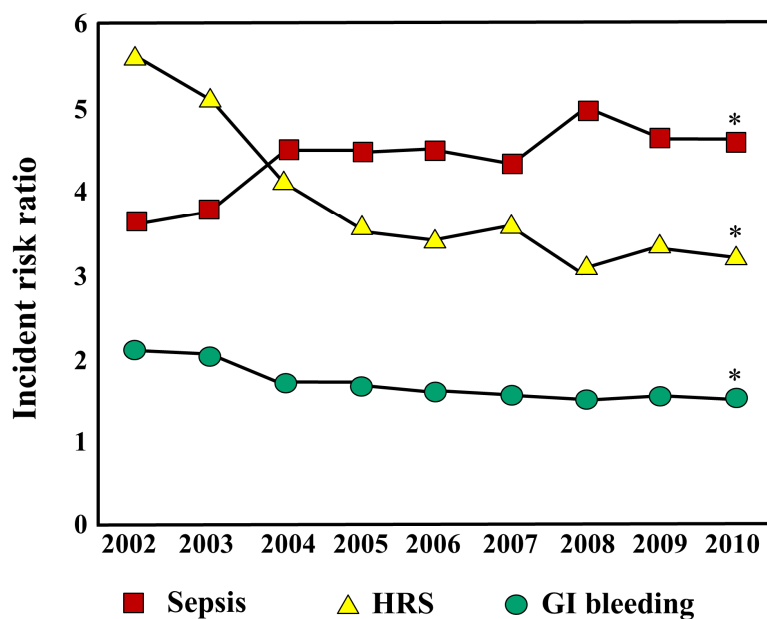
Patients with bacterial infections and in particular SBP, have a high risk to develop AKI. Thus, they represent optimal candidates for the development of strategies aimed to prevent the development of AKI. A randomized controlled clinical trial showed that the administration of antibiotics + albumin (1.5 g per kg of body weight on day 1 and 1 g per kg of body weight on day 3) in patients with SBP is associated with a lower risk to develop HRS than antibiotic alone[78]. Furthermore, mortality rate was significantly lower in patients treated with albumin than in those treated with standard of care[78].

In patients with bacterial infections other than SBP results are controversial. Guevara M et al found a significant improvement in renal function and a trend towards an improvement in survival with the administration of albumin[79]. On the contrary, Thevenot T et al. found albumin able to delay the onset of renal failure, although the three-month renal failure rate and survival rate were not different between the two groups[80]. Importantly, they reported 8 cases of pulmonary edema in patients treated with albumin.

RATIONALE OF THE STUDIES

As mentioned in the background section, bacterial infections in cirrhosis are common, are associated with the development of complications of cirrhosis and confer a high mortality rate. Thus, any effort should be made to improve the management of patients with cirrhosis and bacterial infection. It should be highlighted that in the eighties and nineties, the management of infections in cirrhosis has been significantly improved. In fact, a recent meta-analysis of studies including patients with cirrhosis and bacterial infections reported a reduced 30-day mortality in studies performed between 2000 to 2009 than in those performed between 1978 to 1999 (26 vs 37%; $P= 0.01$)[53]. However, thereafter, mortality for bacterial infections raised up. In fact, a retrospective analysis of 781,515 hospitalizations from 2002 through 2010 in the U.S. showed that the risk of dying for a cirrhosis patient with sepsis was 28% higher in 2010 than in 2002[81]. Remarkably, this finding was in contrast with the observation that the risk of dying for several other complications of cirrhosis such as HRS, GI bleeding or hepatocellular carcinoma significantly decreased overtime[81] (Figure 1).

Figure 1. Incident risk ratios of in-hospital mortality for cirrhosis complications, each year from 2002 to 2010.



Legend: HRS, hepatorenal syndrome; GI, gastrointestinal; *, $p < 0.01$ vs 2002. Modified from ref.81.

This findings are alarming and occurred despite the ongoing “surviving sepsis campaign”[82]. The reasons are not clear, but the increase in the spread of MDR bacteria and the lack of new effective antibiotics may be one. The problem of antimicrobial resistance is so important that on September 2016 the United Nations hold a meeting on this topic that resulted in a declaration of actions to counteract the spread of MDR strains[83]. As reported before, there is the urgent need to develop new strategies in the prevention and treatment of bacterial infections in patients with cirrhosis. In our mind, the first step to be done was to perform large epidemiological studies to understand better the epidemiology of infections across the world. Thus, we performed a multicenter, prospective intercontinental study to evaluate epidemiology and clinical impact of bacterial/fungal infections across the world (Study 1). Another important step is to identify patients with cirrhosis at high risk of short term mortality that deserve a more intensive management and could be the target of trials of intervention. The current definition of sepsis, according to the presence of SIRS criteria has several limitations in cirrhosis. Thus, we evaluated for the first time the new consensus definitions of the Society of Critical Care Medicine and the European Society of Intensive Care Medicine (Sepsis-3 criteria)[84], in patients with cirrhosis and bacterial/fungal infections comparing the new criteria with the SIRS criteria(Study 2). Finally, for patients discharged after a hospitalization for bacterial infections there is a high rate of short term readmission[85,86]. Thus, we conducted a study assessing predictors of early readmissions and mid-term mortality in patients with cirrhosis and bacterial infections after the discharge (Study 3). The results of Study 2 and Study 3 have been recently published [87,88].

STUDY 1:

**EPIDEMIOLOGY AND CLINICAL IMPACT OF BACTERIAL
INFECTIONS IN PATIENTS WITH CIRRHOSIS ACROSS THE WORLD:
THE “GLOBAL STUDY”**

Introduction

In recent years there has been concern about the increasing isolation of bacteria resistant to common antibiotics used in patients with cirrhosis and bacterial infections. Observational studies coming from Europe[11,26,63,64], North America[12,65] and Asia[66] showed a high rate of infections due to MDR bacteria in patients with cirrhosis.

Furthermore, XDR bacteria such as carbapenemase producing *Klebsiella Pneumoniae*, *Acinetobacter Baumannii* etc. are becoming a relevant issue for the management of these patients[68,69].

Norfloxacin prophylaxis for SBP, the use of antibiotics in the 3 months before on set of infection and nosocomial acquisition of infections has been found to be associated with a high risk of infections due to multi drug resistant bacteria[11,13,26,64,65].

These epidemiological studies highlight the urgent need to develop new strategies in the prevention and treatment of bacterial infections in patients with cirrhosis. However, in this field, large epidemiological studies are lacking and/or limited to single center experiences. Thus, they are not fully able to give a global view of the epidemiology of bacterial infections across the world.

A large multi-center international observational study may be helpful to better understand the epidemiology of bacterial infections and the pattern of antibiotic resistance in patients with cirrhosis in the different countries. It may also be helpful in the selection of empirical antibiotic treatment in these patients. This is a critical point, since in cirrhotics with bacterial infections, an

inappropriate empirical antibiotic treatment of SBP has been associated with a poor survival[26,67,70] and with severe sepsis or septic shock[76]. For these reasons a consensus among international experts was held to suggest new guidelines on the antibiotic treatment of bacterial infections in patients with cirrhosis[8]. Nevertheless, the Authors highlight the need to base the antibiotic treatment on the local pattern of antibiotic resistance. For these reasons an international survey including centers coming from all the continents may be useful to better define antibiotic treatment in these patients. Thus, in 2014, the International Club of Ascites, planned a prospective, cross sectional, multicenter, intercontinental study to investigate the epidemiology and pattern of antibiotic resistance, as well as the clinical impact of bacterial infections in patients with cirrhosis. The study had the acronym “Global study”

Materials and methods

Patients

From October 2015 to October 2016 consecutive patients with cirrhosis and bacterial infections were included at 46 centers (15 from Asia, 15 from Europe, 11 from Southern America and 5 from Northern America). Inclusion criteria were the following: a) diagnosis of cirrhosis according to histological, clinical, biochemical, ultrasound and/or endoscopic findings; b) diagnosis of bacterial and/or fungal infection during hospitalization; c) age > 18 years old. Exclusion criteria were: a) hepatocellular carcinoma (HCC) beyond the Milan criteria; b) extrahepatic malignancy; c) severe extrahepatic disease (congestive heart failure stage NYHA ≥ 3 , chronic obstructive pulmonary disease stage GOLD ≥ 3 ; chronic kidney disease requiring renal replacement therapy[RRT]); d) previous transplant; e) HIV infection; f) use of immunosuppressive drugs other than corticosteroids for the treatment of severe acute alcoholic hepatitis; g) inability to provide written informed consent.

The protocol was approved by the local Ethics Committee at each center and all patients provided written informed consent.

Design of the study

Once informed consent was obtained, a physical examination, routine laboratory and microbiological analyses were performed. Demographic, clinical, laboratory and microbiological data, as well as treatment administered were collected. Information about concurrent medications (quinolones prophylaxis, rifaximin and beta-blockers) were collected as well. The following potential risk factors for the development of MDR and XDR infections were collected: i) antibiotic treatment for at least 5 days in the previous 3 months; ii) Isolation of MDR bacteria in the previous 6 months; iii) invasive procedures (surgery, central venous catheterization, bladder catheterization, paracentesis etc.) in the month before the hospitalization.

Bacterial/fungal infections were classified as community acquired (CA), health-care associated (HCA) and nosocomial infections as previously shown[11]. Bacterial/fungal infections were diagnosed according to the following conventional criteria:

- Spontaneous bacterial peritonitis (SBP): polymorphonuclear cell count in ascitic fluid $\geq 250/\text{mm}^3$ [60]
- Urinary tract infections (UTI): Patient had at least one of the following signs or symptoms (fever $\geq 38^\circ \text{C}$, urgency, frequency, dysuria, or suprapubic tenderness) and a positive urine culture or at least two of the following signs or symptoms (fever $\geq 38^\circ \text{C}$, urgency, frequency, dysuria, or suprapubic tenderness) and at least one of the following (more than 10 leukocytes/ μL in urine)[89].
- Pneumonia: radiologic evidence of a new, or progression of a previous, pulmonary infiltrate, consolidation or cavitation plus at least one of the following criteria (fever $\geq 38^\circ\text{C}$, leucocyte count of $>12,000/\text{mm}^3$ or $<4,000/\text{mm}^3$) plus at least one of the following symptoms (new onset of purulent sputum or change in character of sputum, new onset of cough, dyspnea or tachypnea >20 breaths per minute, rales or bronchial breath sounds or worsening of gas exchange) and/or organisms cultured from blood, pleural fluid or a

specimen obtained by transtracheal, aspirate, bronchoalveolar lavage, or biopsy

- Spontaneous bacteremia: at least 1 of the following signs or symptoms: fever ($\geq 38^{\circ}\text{C}$), chills, or hypotension and a positive blood culture (at least two positive blood cultures for common skin contaminant), in the absence of a known source of infection [2]
- Other infections were diagnosed according to the Centers for Disease Control and Prevention Criteria[89].

Microbiological cultures and antibiotic susceptibility tests, were performed according to standard international criteria. Patients were followed-up until death, liver transplantation (LT) and/or discharge. Patients discharged before 28 days were followed up until 28 days since the diagnosis of infection. Data on the development of new bacterial/fungal infections, septic shock, AKI, ACLF, the transfer to the ICU, the use of vasopressors, mechanical ventilation and/or RRT during the hospitalization were collected. Microbiological data about second infections during the hospitalization were collected. Data were collected using an electronic case report form using the Research Electronic Data Capture Software REDCap[90] hosted at the Department of Medicine – DIMED of the University of Padova, Italy

Definitions

MDR bacteria were defined as bacteria resistant at least one antibiotic in 3 or more classes[91].

XDR bacteria were defined as bacteria resistant to at least one antibiotic in all classes excepting 2[91]. For the assessment of MDR and XDR definitions intrinsic resistance were not considered (e.g. *Enterococci* are constitutively resistant to cephalosporins).

ACLF was defined according to the EASL-CLIF consortium definition in patients developing an acute decompensation of cirrhosis[5]: *Grade 1*: Patients with serum creatinine (SCr) ≥ 2 mg/dl; patients with single failure of the liver, coagulation, circulation, or respiration who had a SCr level ranging from 1.5 to 1.9 mg/dL and/or mild to moderate hepatic encephalopathy (grade 1 or 2 according to West Haven criteria); and patients with single cerebral failure who had a SCr level

ranging from 1.5 to 1.9 mg/dL; *Grade 2*: Patients with 2 organ failures; *Grade 3*: Patients with 3 or more organ failures.

AKI was defined as an increase in SCr ≥ 0.3 mg/dL with respect to baseline according to International Club of Ascites criteria [5]. AKI stages were defined as follows: *Stage 1*: increase in SCr ≥ 0.3 mg/dL (26.5 mmol/L) or an increase in 1.5-fold to 2-fold from baseline; *Stage 2*: increase in SCr between 2-fold and 3-fold from baseline; *Stage 3*: increase of SCr >3 -fold from baseline or SCr ≥ 4.0 mg/dL with an acute increase ≥ 0.3 mg/dL or initiation of renal-replacement therapy.

Efficacy of first line antibiotic treatment was defined by each investigator according to clinical improvement and microbiological tests.

For the assessment of microbiological efficacy of currently suggested recommendations for the treatment of infections in patients with cirrhosis (Table 1 - Background) [8] we considered recommendations adequate if the isolated strain was sensitive to at least one of the antibiotic suggested. We differentiated the antibiotic strategy suggested for HCA and nosocomial infections as follows: a) treatment suggested including carbapenems; b) treatment suggested including piperacillin/tazobactam and no carbapenems (for spontaneous infections, UTI and pneumonia); c) treatment suggested including carbapenems and glycopeptides/ oxazolinidones).

Management of infections

The empirical antibiotic treatment was administered according to local protocols and changed according to antibiotic susceptibility test and clinical evolution. Patients with septic shock received resuscitation with plasma expanders and vasopressors. The decision to transfer patient to the intensive care unit and to administer mechanical ventilation and RRT was made by the physician in charge for the patient according to clinical conditions and standard recommendations [75,92].

Statistical analysis

The primary end point of the study was the presence of MDR and XDR infections. Secondary end-points were in-hospital mortality, 28-day mortality, development of ACLF, septic shock, transfer to the ICU, and need for organ support (mechanical ventilation or RRT) during the hospitalization. Continuous variables were reported as mean \pm standard deviation or median with interquartile range (IQR) according to normal or non-normal distribution, respectively. They were compared using Student's T-test or Mann-Whitney U test, respectively. Categorical variables were reported as proportions and compared with chi-square or Fisher's exact test. Variables found to be associated with MDR and XDR bacteria with a p-value <0.1 in the univariate analysis were included in a multivariate step-wise logistic regression analysis, with backward elimination (entry p <0.05 ; drop p >0.1). The odds ratios (OR) and their 95% CI were calculated. A similar approach was used to identify predictors of in-hospital mortality. When scores of liver disease were included in the model, their component were excluded to avoid multicollinearity. Similarly, in case of a correlation >0.5 between variables and or scores, they were not included in the model to avoid multicollinearity. Non-normally distributed continuous variables were log-transformed to be included in the multivariate models. All statistical tests were two-tailed and P-values <0.05 were considered significant. The statistical analysis was performed using SPSS statistical package, and SAS version 9.4.

Results

Study population

During the study period 1,302 patients were enrolled, 565 (43%) from Europe, 416 (32) from Asia and 321 (25%) from South and North America. Demographic and clinical characteristics of patients included in the study have been reported in Table 1.1

Table 1.1. Characteristics of patients included in the study

Variable	N= 1,302
Continent – n (%)	
Asia	416 (32)
America	321 (25)
Europe	565 (43)
Geographic area – n (%)	
India	250 (19)
Other Asian centers	166 (13)
North Europe	137 (11)
South Europe	428 (33)
North America	69 (5)
South America	252 (19)
Age (years) – mean (SD)	57 (13)
Gender (Male) – n (%)	898 (69)
Etiology – n (%) *	
HBV	100 (8)
HCV	259 (20)
Alcohol	697 (54)
NASH	146 (11)
Other	236 (18)
Mean arterial pressure (mmHg) – mean (SD)	82 (13)
Heart rate (bpm) – mean (SD)	88 (17)
Body temperature (°C) – mean (SD)	37 (1)
Respiratory rate (breath/min) – mean (SD)	19 (5)
Beta-blockers use – n (%)	423 (33)
Ascites – n (%)	1,002 (77)
Hepatic encephalopathy – n (%)	496 (38)
ACLF – n (%)	460 (35)
ACLF grade – n (%)	
Grade 1	190 (15)
Grade 2	164 (13)
Grade 3	106 (8)
MELD score – mean (SD)	21 (8)
Child Pugh score – mean (SD)	10 (2)
INR – median (IQR)	1.8 (0.7)
Bilirubin (mg/dL) – median (IQR)	3.7 (1.7 – 8.0)
Albumin (g/dl) – median (IQR)	2.6 (2.2 – 3.0)
Serum creatinine (µmol/L) – median (IQR)	1.1 (0.8 – 1.9)
Serum sodium (mmol/L) – mean (SD)	133 (7)
Leukocytes (x 10⁹/L) – median (IQR)	8.4 (5.2 – 13.0)
C-reactive protein (mg/L) – median (IQR)[°]	35 (15 – 77)

Legend: n, number; SD, standard deviation; HCV, hepatitis C virus; HBV, hepatitis B virus; NASH, non alcoholic fatty liver disease, ACLF, acute-on-chronic liver failure; MELD, model of end stage liver disease; INR, international normalized ratio; *, 129 patients had more than one etiology of cirrhosis; °, available in 1,102 patients

The mean age was 57 ± 13 years old and 69% of patients were male. Alcohol consumption was the most common etiology of cirrhosis followed by HCV infection and NASH. The patients had advanced liver disease as shown by the high prevalence of ascites, hepatic encephalopathy and the mean MELD score and CTP score. Notably, 35% of patients had ACLF at the diagnosis of infection. Among potential risk factor for MDR bacterial infections, 130 patients (10%) were on quinolones prophylaxis, 376 (29%) on treatment with rifaximin, 574 (44%) received an antibiotic treatment for at least 5 days in the previous 3 months and 569 (44%) underwent to invasive procedures the month before the admission. Finally, 69 patients had the isolation of MDR bacteria in the previous 6 months.

Table 1.2. Characteristics of the first infection.

Variable	N= 1,302
Site of infection – n (%)	
Urinary tract infection	289 (22)
Spontaneous bacterial peritonitis	354 (27)
Pneumonia	242 (19)
Spontaneous bacteremia	100 (8)
Skin and soft tissue infections	101 (8)
Other	216 (17)
Type of infection – n (%)	
Community acquired	628 (48)
Health care acquired	336 (26)
Nosocomial	338 (26)
SIRS – n (%)*	405 (36)
qSOFA – n (%)*	255 (23)
Septic shock – n (%)	143 (14)
Positive culture – n (%)	740 (57)
Number of strains isolated	925
Type of strain isolated – n (%)	
Gram –	536 (58)
Gram +	351 (38)
Fungi	38 (4)
Most frequently isolated bacteria – n (%)	
<i>Escherichia Coli</i>	222 (30)
<i>Klebsiella Pneumoniae</i>	104 (14)
<i>Staphylococcus aureus</i>	58 (8)
<i>Enterococcus faecalis</i>	40 (5)
<i>Enterococcus Faecium</i>	39 (5)
MDR bacteria – n (%)°	322 (35)
XDR bacteria – n (%)§	73 (8)

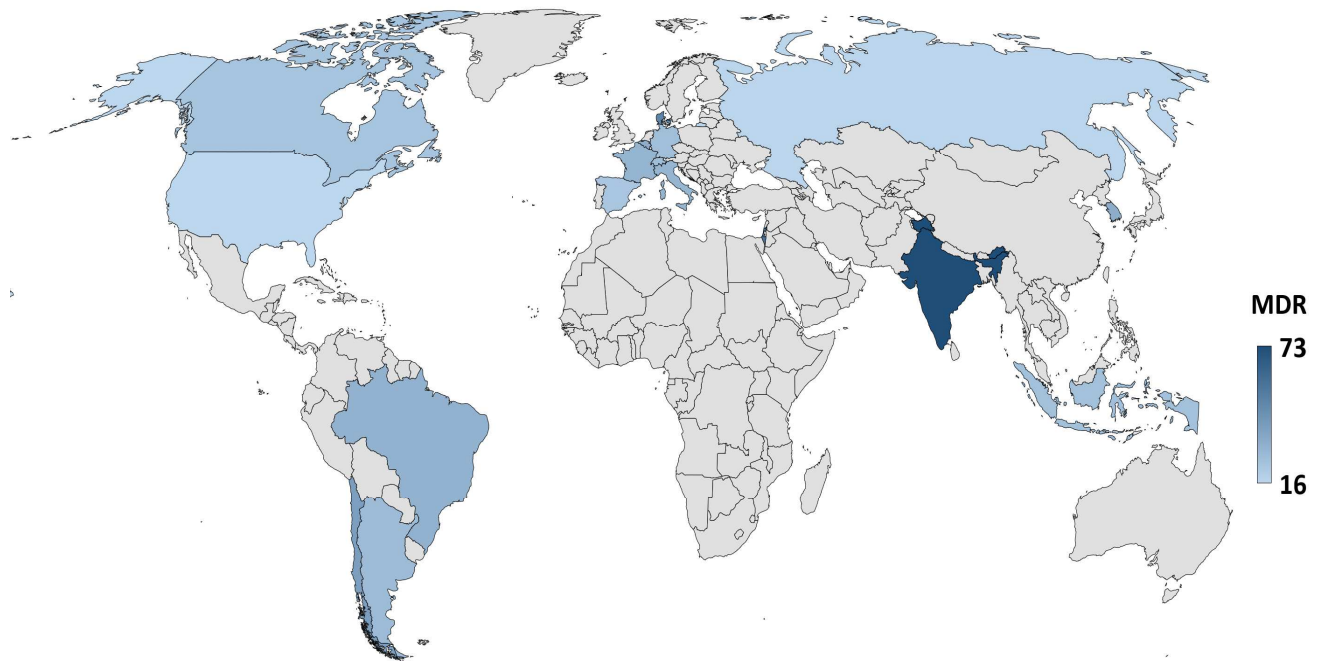
Legend: SIRS, systemic inflammatory response syndrome; qSOFA, quick sequential organ failure assessment; MDR, multi drug resistant; XDR, Extensively drug resistant; *, available in 1,119 patients; °, in 253 patients (34%); §, in 62 patients (8%)

The characteristics of bacterial infections have been reported in table 1.2. The most common infections were SBP (27%), UTI (22%) and pneumonia (19%). About one half of the infections were community acquired (48%), while there was a similar proportion of HCA and nosocomial infections (26%). SIRS and a positivity of qSOFA were found in 36 and 23% of patients, respectively, while 14% had septic shock at the diagnosis of infection. Microbiological cultures were positive in 57% of patients and globally 925 bacteria were isolated. Gram negative bacteria were the most common isolates (57%), while gram negative accounted for 38% of positive cultures and only 4% showed positivity for fungi. In details, most commonly isolated bacteria were *Escherichia Coli* and *Klebsiella Pneumoniae* (among gram negative bacteria) and *Staphylococcus Aureus* and *Enterococci* among gram positive bacteria. Overall, 322 MDR bacteria were isolated in 253 patients (19% of patients; 34% of those with positive cultures). Most commonly isolated MDR bacteria were ESBL producing *Enterobacteriaceae*, methicillin resistant *Staphylococcus Aureus*, vancomycin resistant *Enterococci*, *Pseudomonas Aeruginosa* and *Acinetobacter baumannii*. Overall, 73 XDR bacteria were isolated in 62 patients (5% of patients, 8% of positive cultures). The most common isolated XDR bacteria were carbapenemase producing *Enterobacteriaceae*, *Pseudomonas Aeruginosa* and *Acinetobacter Baumannii*.

Prevalence of MDR and XDR bacteria across the different countries

Relevant differences were observed in the prevalence of MDR and XDR bacterial infections across the different centers. Remarkably, MDR bacterial infections were very common in Indian centers (73% of isolates), while their prevalence was quite low in North American centers (16% in U.S. and 24% in Canada). The prevalence of MDR was quite high also in other Asian centers and in Southern American center (Figure 1.1), while there was a relevant variability across Europe, ranging from 57% of cultures in Israel and 17% in Russia.

Figure 1.1 Prevalence of MDR bacteria across the world



Legend. Light blue identifies countries with a low prevalence, dark blue those with a high prevalence. No data are available for countries reported in grey

Similarly, the prevalence of XDR infections was strikingly high in Indian centers (33% of isolates), while ranging between 0 and 16% of isolates in the other countries. In table 1.3 a detailed description of the prevalence of MDR bacterial infections and the type of MDR strain has been reported. Relevant differences were observed among countries, in particular regarding the prevalence of ESBL producing and carbapenemases producing *Enterobacteriaceae* (CPE) and *Acinetobacter baumannii*. The latter was particularly prevalent in Asian centers. As regards CPE it should be highlighted the high prevalence in centers in the North of Europe (France and Denmark), a finding unexpected according to the data coming from the European Center for Disease Prevention and Control.

Table 1.3. Prevalence of infections due to MDR bacteria across countries

	MDR	XDR	ESBL producing Enterobacteriaceae	MRSA	VRE	Pseudomonas Aeruginosa	Acinetobacter baumannii	CPE
India	73	33	21	2	4	5	13	22
Indonesia	25	0	8	0	0	8	8	0
South Korea	33	5	8	4	2	3	2	1
Belgium	33	0	13	0	0	0	0	0
Denmark	50	17	17	8	0	0	0	17
France	30	10	0	0	0	0	0	10
Germany	26	5	11	0	0	0	0	5
Israel	57	0	43	0	0	0	0	0
Italy	30	7	8	3	3	3	1	4
Russia	17	0	17	0	0	0	0	3
Spain	23	3	6	3	0	6	1	0
Switzerland	26	0	9	0	0	0	0	0
Canada	24	0	18	0	0	0	0	0
U.S.A.	16	3	11	0	3	2	0	0
Argentina	27	3	17	0	3	4	0	0
Brazil	31	16	13	0	3	3	3	6
Chile	39	4	26	0	4	0	0	0
P value	<0.001	<0.001	0.020	0.748	0.982	0.945	<0.001	<0.001

Legend: Results are reported as percentages of patients with positive cultures. MDR, multi drug resistant; XDR, extensively drug resistant; ESBL, extended spectrum beta-lactamases, MRSA, methicillin resistant *Staphylococcus Aureus*; VRE, vancomycin resistant *enterococci*; CPE, carbapenemases producing *Enterobacteriaceae*.

Variables associated with MDR and XDR infections

In order to better understand the differences across countries, we performed a univariate and multivariate analysis of predictors of infections due to MDR bacteria.

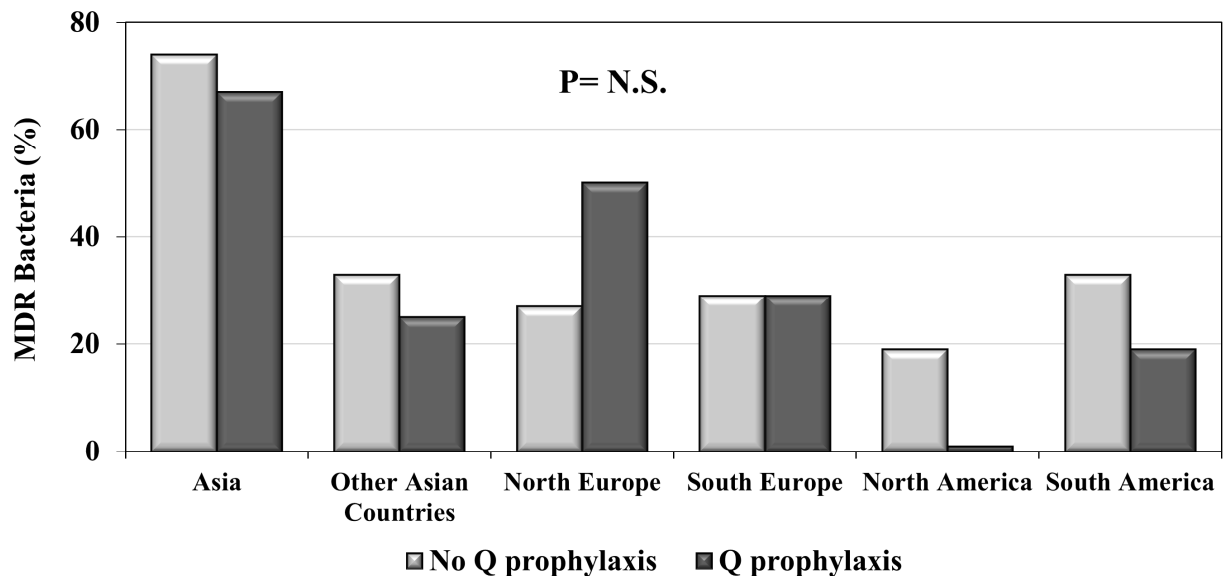
Table 1.4. Comparison of patients' characteristics according to the presence or not of an infection due to a MDR bacteria

Variable	No MDR (N=487)	MDR (N=253)	P
Geographic area – n (%)			
India	23 (5)	63 (25)	<0.001
Other Asian centers	71 (15)	34 (13)	
North Europe	62 (13)	27 (11)	
South Europe	191 (39)	77 (30)	
North America	45 (10)	9 (4)	
South America	95 (20)	42 (17)	
Age (years) – mean (SD)	58 (13)	56 (13)	0.003
Gender (Male) – n (%)	305 (63)	177 (70)	0.057
Mean arterial pressure (mmHg) – mean (SD)	81 (13)	82 (14)	0.560
Norfloxacin prophylaxis – n (%)	45 (9)	21 (8)	0.772
Treatment with rifaximin – n (%)	147 (30)	81 (32)	0.669
Antibiotic treatment in the previous 3 months – n (%)	186 (38)	156 (62)	<0.001
Invasive procedures in the previous month – n (%)	188 (39)	143 (57)	<0.001
Isolation of MDR bacteria in the previous 6 months – n (%)	29 (6)	22 (9)	0.214
Acute-on-chronic liver failure – n (%)	161 (33)	87 (34)	0.779
MELD score – mean (SD)	20 (8)	22 (8)	0.023
Child Pugh score – mean (SD)	9.7 (2.2)	10.2 (2.3)	0.016
Site of infection – n (%)			
Urinary tract infection	157 (32)	90 (36)	<0.001
Spontaneous bacterial peritonitis	94 (19)	28 (11)	
Pneumonia	47 (10)	58 (23)	
Spontaneous bacteremia	72 (15)	22 (9)	
Skin and soft tissue infection	25 (5)	19 (8)	
Other	92 (19)	36 (14)	
Type of infection – n (%)			
Community acquires	242 (50)	75 (30)	<0.001
Health care associated	123 (25)	77 (30)	
Nosocomial	122 (25)	101 (40)	
SIRS – n (%)	158 (40)	75 (34)	0.138
qSOFA – n (%)	89 (23)	53 (24)	0.802
Septic shock – n (%)	64 (13)	41 (16)	0.307
Leukocytes (x 10⁹/L) – median (IQR)	7.7 (4.4 – 12.1)	8.8 (5.6 – 13.4)	0.003
C-reactive protein (mg/L) – median (IQR)	32 (12 – 80)	37 (17 – 74)	0.396

Legend: SIRS, systemic inflammatory response syndrome; qSOFA, quick sequential organ failure assessment; MDR, multi drug resistant; MELD, model of end stage liver disease.

In the univariate analysis MDR infections were more common in young patients, in male and in those with a worse liver function according to MELD and CTP score (Table 1.4). Among known risk factors for MDR, the use of antibiotics in the previous 3 months, invasive procedures in the previous month, health-care associated infections and nosocomial ones were more frequent in those with MDR bacteria than in those without. Surprisingly, norfloxacin prophylaxis was not found to be more frequent in patients with MDR infections. Subgroup analysis did not find any area of the world in which norfloxacin prophylaxis was found to be associated with a higher risk of MDR infections (Figure 1.2)

Figure 1.2. Prevalence of MDR bacteria in patients with or without quinolones prophylaxis across the different geographic areas



Legend: MDR, multi drug resistant; Q, quinolones

MDR infections were more commonly observed in patients with UTI, pneumonia and SSTIs than those with SBP or spontaneous bacteremia. After adjusting for all these confounders, patients from Indian (OR=7.94; $p < 0.001$), Southern American (OR=2.23; $p = 0.053$) and Other Asian centers (OR=2.79; $p = 0.017$) still had an increased risk of infections due to MDR bacteria (Table 1.5).

Table 1.5. Multivariate analysis of predictors of an infection due to a MDR bacteria.

Variable	OR	95% CI	P
Geographic area – n (%)*			
South America	2.23	0.99 – 5.00	0.053
India	7.94	3.30 – 19.11	<0.001
Other Asian centers	2.79	1.20 – 6.46	0.017
North Europe	1.91	0.82 – 4.48	0.136
South Europe	1.64	0.77 – 3.49	0.197
Antibiotic treatment in the previous 3 months	1.92	1.32 – 2.80	0.001
Site of infection °			
UTI	2.48	1.59 – 3.87	<0.001
Pneumonia	3.20	1.83 – 5.59	<0.001
Skin and soft tissue infection	2.92	1.41 – 6.09	0.004
Other infections	1.45	0.85 – 2.49	0.175
Type of infection§			
HCA	1.62	1.04 – 2.52	0.032
Nosocomial	2.65	1.75 – 4.01	<0.001

Legend: OR, odds ratio; CI, confidence interval; UTI, urinary tract infection; HCA, health care acquired. *, Northern American patients were used as a reference group; °, spontaneous infections (SBP and spontaneous bacteremia) were used as reference group; §, community acquired infections were used as reference group.

The analysis of variables associated with XDR infections confirmed that patients from Indian centers had an increased risk of infections due to XDR bacteria (OR= 20.05; p=0.005 – Table 1.6). Interestingly, pneumonia showed to be independently associated with a higher risk of infections due to XDR bacteria (OR= 2.46; P=0.035).

Table 1.6. Multivariate analysis of predictors of an infection due to a XDR bacteria.

Variable	OR	95% CI	P
Geographic area – n (%)*			
South America	2.84	0.33 – 24.13	0.339
India	20.05	2.46 – 163.10	0.005
Other Asian centers	2.95	0.32 – 27.04	0.338
North Europe	2.06	0.22 – 19.60	0.529
South Europe	2.54	0.32 – 20.11	0.376
Isolation of MDR bacteria in the previous 6 month	4.88	1.98 – 12.02	0.001
Site of infection °			
UTI	2.04	0.93 – 4.49	0.077
Pneumonia	2.46	1.07 – 5.65	0.035
Skin and soft tissue infection	0.46	0.06 – 3.81	0.474
Other infections	0.80	0.27 – 2.31	0.673
Type of infection§			
HCA	2.52	1.05 – 6.09	0.039
Nosocomial	5.63	2.47 – 12.87	0.003

Legend: OR, odds ratio; CI, confidence interval; MDR, multi drug resistant; UTI, urinary tract infection; HCA, health care acquired. *, Northern American patients were used as a reference group; °, spontaneous infections (SBP and spontaneous bacteremia) were used as reference group; §, community acquired infections were used as reference group.

Clinical impact of MDR infections

MDR bacterial infections were more difficult to be treated, being associated with a lower efficacy of first line empirical antibiotic treatment, a more frequent need to add a second line antibiotic treatment, a longer duration of antibiotic treatment and a lower rate of resolution of the infection than those with sensitive bacterial infections (Table 1.7). In addition, patients with MDR bacterial infections had a higher incidence of septic shock, need to be transferred to the ICU and to need mechanical ventilation or renal replacement therapy (RRT) than those with sensitive bacterial infections. Length of hospital stay was significantly longer in patients with MDR bacterial infections than in those without. Most important, patients with MDR infections had a significantly higher in-hospital and 28-day mortality rate.

Table 1.7. Clinical impact of infections due to MDR bacteria.

Variable	No MDR (N= 487)	MDR (N= 253)	P
Efficacy of first line antibiotic treatment – n (%)	331 (68)	100 (40)	<0.001
Administration of a second line treatment – n (%)	197 (41)	159 (63)	<0.001
Duration of antibiotic treatment (days)– M (IQR)	10 (7 – 15)	12 (7 – 18)	0.013
Resolution of the infection – n (%)	398 (82)	182 (72)	0.003
Development of new infections – n (%)	101 (21)	53 (21)	1.000
Transfer to ICU – n (%)#	94 (23)	72 (36)	0.001
Development of ACLF during hospitalization – n (%)*	92 (28)	59 (36)	0.118
Development of septic shock during hospitalization – n (%)**	62 (13)	57 (27)	<0.001
Administration of mechanical ventilation – n (%)***	53 (12)	48 (20)	0.003
Administration of hemodialysis – n (%)	34 (7)	42 (17)	<0.001
Length of hospital stay (days) – M (IQR)	15 (9 – 26)	18 (10 – 30)	0.032
In-hospital mortality – n (%)°	97 (21)	75 (31)	0.004
28-day mortality – n (%)§	99 (22)	72 (34)	0.002

Legend: n, %; MDR, multi drug resistant; ICU, intensive care unit; ACLF, acute-on-chronic liver failure; #, patients with indication to ICU admission at inclusion (n=121) were excluded from this analysis; *, Patients with ACLF at inclusion (n=248) have been excluded from this analysis; **, patients with septic shock at inclusion (n= 105) were excluded from this analysis; ***, patients on mechanical ventilation at inclusion were excluded from this analysis (n=47) °, patients transplanted during hospitalization (n=25) have been excluded from this analysis; § patients transplanted or lost to follow up (n=73) have been excluded from this analysis.

Microbiological sensitivity of currently suggested guidelines in patients with cirrhosis and bacterial infections

After having shown significant differences in the prevalence of MDR and XDR bacterial infections across the different countries we explored the microbiological sensitivity to the current suggested empirical antibiotic treatment in patients with cirrhosis and bacterial infections. The suggested antibiotic protocol was adequate to guarantee an antibiotic coverage in more than 80% of isolated strain in Northern and Southern American centers, in Northern European centers while being poorly effective in Indian centers (51%) and just below the 80% threshold in Southern Europe (77%) and Asian centers (79%) (Table 1.8).

Table 1.8. Microbiological efficacy of currently suggested empirical antibiotic treatment* in patients with cirrhosis and bacterial infections across different countries.

	Overall (N= 740)	P value	South Europe (N= 268)	North Europe (N= 89)	South America (N=137)	North America (N=55)	Indian hospitals (N= 86)	Other Asian Hospital (N= 105)	P value
Overall efficacy – n (%)	573 (77)	--	205 (77)	76 (85)	119 (87)	46 (84)	44 (51)	83 (79)	<0.001
Type of infection – n (%)									
Community acquired	246 (78)	0.002	83 (77)	30 (81)	44 (82)	21 (78)	12 (48)	56 (85)	--
HCA*	170 (85)		56 (84)	21 (81)	39 (91)	16 (100)	12 (65)	14 (82)	
Nosocomial	157 (70)		66 (71)	25 (96)	36 (90)	9 (75)	20 (40)	13 (59)	
Site of infection – n (%)									
UTI	205 (83)	<0.001	77 (84)	28 (82)	41 (85)	16 (73)	14 (67)	29 (97)	--
SBP	105 (86)		22 (92)	24 (92)	39 (100)	3 (100)	6 (50)	11 (61)	
Pneumonia	67 (64)		15 (60)	6 (67)	8 (73)	2 (100)	16 (49)	20 (80)	
Spontaneous Bacteremia	75 (80)		34 (79)	3 (100)	16 (80)	9 (100)	2 (25)	11 (100)	
SSTI	28 (64)		8 (53)	3 (60)	3 (75)	4 (100)	4 (50)	6 (75)	
Other	93 (73)		49 (61)	12 (100)	12 (80)	12 (80)	2 (50)	6 (46)	

Legend: HCA, health care associated; UTI, urinary tract infections; SBP, spontaneous bacterial peritonitis; SSTI, skin and soft tissues infections.

*, treatment suggested by Jalan R et al ref 8 including carbapenems for health care associated and nosocomial infections and excluding glycopeptides

The microbiological efficacy of the suggested empirical antibiotic treatment decreased moving from community acquired to nosocomial infections, in particular in Asian centers (Table 1.8). Considering the treatment suggested for specific type of infection, it is remarkable that treatment suggested for pneumonia and SSTI was microbiologically effective only in 64% of isolates involved in those infections, while the treatment suggested for UTI, SBP and spontaneous bacteremia were quite effective (Table 1.8 and Table 1.9). Interestingly, treatment suggested for community acquired pneumonia was microbiologically effective in 83% of isolates and the efficacy of empirical antibiotic treatment decrease moving to HCA and nosocomial infections (63 and 44%, respectively). Conversely, treatment suggested for community acquired and HCA SSTIs had a trend toward less microbiological efficacy than those provided for nosocomial episodes. Interestingly, the addition of clindamycin to beta-lactams in community acquire infections could improve the microbiological coverage (67 vs 52%), although the difference was not significant due to the low number of patients with positive cultures.

Table 1.9. Microbiological efficacy of currently suggested empirical antibiotic treatment* in community acquired, health care associated and nosocomial infections in patients with cirrhosis.

	Community acquired (N=317)	Health care associated (N=200)	Nosocomial (N=223)	P value
Urinary tract infections – n (%)	103 (81)	59 (92)	43 (77)	0.058
Spontaneous bacterial peritonitis – n (%)	43 (83)	41 (91)	21 (84)	0.464
Pneumonia – n (%)	31 (84)	20 (63)	16 (44)	0.002
Spontaneous bacteremia – n (%)	30 (79)	11 (79)	34 (81)	0.968
Skin and soft tissues infections – n (%)	13 (52)	5 (63)	10 (91)	0.082
Other – n (%)	26 (68)	34 (92)	33 (62)	0.006

Legend: *, treatment suggested by Jalan R et al ref 8 including carbapenems for health care associated and nosocomial infections and excluding glycopeptides.

Then, we tried to simulate different approach for health care associated infections considering: a) the feasibility of a strategy including treatment provided for community acquired infections (that may reduce the exposure to broad spectrum antibiotics); b) a broad spectrum antibiotic treatment with the administration of piperacillin/tazobactam (Pip/Tazo) instead than carbapenems (that may reduce the exposure to carbapenems and the risk of XDR infections) c) a broad spectrum treatment including glycopeptides (Table 1.10).

Table 1.10. Microbiological efficacy of different empirical antibiotic strategies for health care associated infections in patients with cirrhosis and bacterial infections across different countries

	Treatment including carbapenems [°]	Treatment with Pip/Tazo ⁺	Treatment suggested for community acquired infections	Treatment including carbapenems and glycopeptides §
Overall efficacy – n (%)	170 (85)	140 (70)***	126 (63)***	175 (88)
Site of infection – n (%)				
UTI	59 (92)	52 (81)	46 (72)*	59 (92)
SBP	41 (91)	34 (76)	31 (69)*	41 (91)
Pneumonia	20 (63)	11 (34)*	10 (31)*	22 (69)
Spontaneous Bacteremia	11 (79)	11 (79)	9 (64)	12 (86)
SSTI	5 (63)	--	4 (50)	7 (88)
Other	34 (92)	27 (73)	26 (70)*	34 (92)
South Europe	56 (84)	46 (69)	40 (60)*	60 (90)
North Europe	25 (96)	23 (89)	19 (73)*	26 (100)
South America	39 (91)	35 (81)	31 (72)*	39 (91)
North America	16 (100)	13 (81)	13 (81)	16 (100)
Indian hospitals	20 (65)	11 (36)*	12 (39)	20 (65)
Other Asian Hospital	14 (82)	12 (71)	11 (65)	14 (82)

Legend: UTI, urinary tract infections; SBP, spontaneous bacterial peritonitis; SSTI, skin and soft tissues infections.

[°], treatment suggested by Jalan R et al ref 8 including carbapenems for health care associated and nosocomial infections and excluding glycopeptides.

⁺, treatment suggested by Jalan R et al ref 8 excluding the use of carbapenems and glycopeptides for health care associated and nosocomial infections.

[§], treatment suggested by Jalan R et al ref 8 including the use of carbapenems and glycopeptides for health care associated and nosocomial infections.

*** p<0.001 vs treatment including carbapenems

* p<0.05 vs treatment including carbapenems

The treatment provided for community acquired infections had a lower efficacy than the standard one for all the infections and for all the centers excepting for North American centers, in which it had an acceptable microbiological efficacy (81%). The use of Pip/Tazo instead of carbapenems had a significantly lower overall microbiological efficacy than the treatment including carbapenems (70 vs 85%; $p < 0.001$) being less effective in the treatment of SBP, pneumonia and other infections, although the level of significance was reached only for pneumonia. Interestingly, the microbiological efficacy of Pip/Tazo reached 81% of UTI and 79% of spontaneous bacteremia. In addition, it was quite effective in Northern European centers and in Southern and Northern American centers (microbiological efficacy $> 80\%$) being a good alternative to carbapenems in these centers. The addition of glycopeptides did not improve the efficacy of an antibiotic regimen based on carbapenems.

Moving to nosocomial infections the treatment with Pip/Tazo showed a significantly lower microbiological efficacy than treatment including carbapenems considering all the infections (55 vs 70%; $p < 0.001$), UTI (55 vs 77%; $p < 0.05$) and SBP (48 vs 84%; $p < 0.05$). Pip/Tazo based regimens were less effective than carbapenem based regimens in all the countries although reached the level of significance only in Southern American centers.

An empirical antibiotic treatment containing both carbapenems and glycopeptides had a significantly higher microbiological efficacy than a carbapenem based antibiotic regimen (80 vs 70%; $p < 0.05$) although the level of significance was reached only for less common infections. The combination of glycopeptides and carbapenems improved the antimicrobial coverage of 12% in pneumonia, although it did not reach the level of significance and the microbial coverage was still very low. When Indian patients were excluded from the analysis (due to the high prevalence of *A. baumannii* and CPE) the combination of carbapenems and glycopeptides (plus quinolones) significantly improved the microbiological efficacy when compared to carbapenems alone (plus quinolones) (71 vs 40%; $p = 0.011$). Regarding the different geographic areas, the combination of carbapenems and glycopeptides was significantly more effective than the use of only

carbapenems in South European centers (85 vs 71%; $p < 0.05$) and had a trend toward the significance for Asian centers other than India (86 vs 59%). Conversely, the combination of carbapenems and glycopeptides had similar antimicrobial coverage than carbapenems alone in American and Northern European centers.

Table 1.11. Microbiological efficacy of different empirical antibiotic strategies for health care associated infections in patients with cirrhosis and bacterial infections across different countries

	Treatment including carbapenems [°]	Treatment with Pip/Tazo+	Treatment including carbapenems and glycopeptides §
Overall efficacy – n (%)	157 (70)	122 (55)***	179 (80)*
Site of infection – n (%)			
UTI	43 (77)	31 (55)*	45 (80)
SBP	21 (84)	12 (48)*	22 (88)
Pneumonia	16 (44)	10 (28)	20 (56)
Spontaneous Bacteremia	34 (81)	30 (71)	34 (81)
SSTI	10 (91)	10 (91)	11 (100)
Other	33 (62)	29 (55)	47 (89)*
South Europe	66 (71)	57 (61)	79 (85)*
North Europe	21 (81)	17 (65)	23 (89)
South America	36 (90)	25 (63)*	36 (90)
North America	9 (75)	7 (58)	9 (75)
Indian hospitals	12 (40)	5 (17)	13 (43)
Other Asian Hospital	13 (59)	11 (50)	19 (86)

Legend: UTI, urinary tract infections; SBP, spontaneous bacterial peritonitis; SSTI, skin and soft tissues infections.

[°], treatment suggested by Jalan R et al ref 8 including carbapenems for health care associated and nosocomial infections and excluding glycopeptides.

+, treatment suggested by Jalan R et al ref 8 excluding the use of carbapenems and glycopeptides for health care associated and nosocomial infections.

§, treatment suggested by Jalan R et al ref 8 including the use of carbapenems and glycopeptides for health care associated and nosocomial infections.

*** $p < 0.001$ vs treatment including carbapenems

* $p < 0.05$ vs treatment including carbapenems

Discussion

The most relevant finding of this study is the wide variability in the prevalence and the type of MDR bacteria in patients with cirrhosis and bacterial infections. The very high prevalence of MDR (3/4 of isolates) and XDR (1/3 of isolates) in Indian center is worrying. This findings were already shown in patients without cirrhosis and are probably related to the antibiotic misuse observed in India[93]. Another explanation may be related to the prevalence of the New Delhi metallo- β -lactamase 1 (NDM-1) which confers resistance to carbapenems and can be easily transferable to other *Enterobacteriaceae*[94]. In addition, a high prevalence of *Acinetobacter baumannii* was found both in India and in other Asian center, which lead to a poor efficacy of currently available antibiotics. Remarkably, even in community acquired infections the prevalence of MDR infections was very high in Indian center.

As previously shown[11], MDR infections were more difficult to be treated being less responsive to the empirical antibiotic treatment and being associated with a lower resolution rate. Furthermore, they were associated with a higher incidence of septic shock, transfer to the ICU and short-term mortality. Considering that the initiation of an effective empirical antibiotic treatment is the strongest predictor of survival in patients with cirrhosis and bacterial infections[13,76] and the relevant regional differences in the epidemiology of these infections, we explored the probability of antimicrobial efficacy of the currently recommended antibiotic regimen in these patients[8]. The results of this analysis have potential relevant implications for clinical practice. The first expected finding is that Indian centers have a very poor antimicrobial coverage with currently suggested recommendations and need to develop local strategies. Considering the lack of effective antibiotics they would probably need to use old antibiotics usually avoided in patients with cirrhosis, such as aminoglycosides and to develop strategies of prevention of development of further resistance. In fact, it will be very important to avoid an irrational use of new effective antibiotics against *Pseudomonas Aeruginosa* and CPE such as

ceftolozane/tazobactam and ceftazidime/avibactam, respectively[95,96]. Interestingly the antimicrobial efficacy of suggested regimens was very good in all the types of SBP, spontaneous bacteremia and UTI. Conversely, the antibiotic regimens suggested for HCA and nosocomial pneumonia are poorly effective, as well as those provided for community acquired SSTIs. The addition of glycopeptides in the former and of clindamycin in the latter may improve the chances of antimicrobial efficacy.

Coming back to the regional differences it should be highlighted that the antimicrobial coverage for community acquired, HCA and nosocomial infections was very good in Northern European and American centers. Interestingly, in Northern American center, HCA infections could be safely treated with the regimen provided for community acquired infections. In addition, HCA infections may be treated avoiding carbapenems, thus with the use of Pip/Tazo based regimens in Northern European and Southern American centers, avoiding the early use of carbapenems for those infections. Conversely, the use of carbapenems could provide better coverage in HCA infections in Southern European centers and Asian centers, confirming the finding of a randomized controlled trial performed in Italy[70].

Moving to nosocomial infections, it should be highlighted that the use of Pip/Tazo instead of carbapenems provided a lower antimicrobial coverage in all the centers and it should be taken into account, particularly in critically ill patients. Conversely, the combination of carbapenems and glycopeptides provided a significantly better coverage, particularly in Southern European centers. As suggested above, the use of glycopeptides in addition to carbapenems could provide a better coverage in pneumonia and in other more rare infections (cholangitis, secondary bacteremia etc.)[97].

Although the early initiation of an effective antibiotic treatment is a main goal of the management of patients with cirrhosis, the prevention of further antibiotic resistance is of equivalent importance. While waiting for the wide distribution of rapid tests for antimicrobial resistance, such as MALDI-TOF, an early de-escalation of antibiotic treatment should be

performed as soon as possible. In our cohort, when we compared patients who de-escalated antibiotic treatment versus those who did not change the treatment (those who received an escalation were excluded), no difference was found in terms of resolution of infections (92 vs 89%; p= N.S.), development of ACLF (18 vs 24%; p= N.S.), septic shock (8 vs 9%; p= N.S.) or in-hospital mortality (12 vs 13%). These findings suggest that de-escalation of antibiotic treatment is feasible and should be implemented. Another important result of our study is that no difference was found in the prevalence of MDR bacteria between patients treated or not with norfloxacin prophylaxis. This results was unexpected and in contrast with previous findings[11]. The reason is not clear and should be further explored, however quinolones prophylaxis should be still prescribed when indicated both in primary[98,99] and in secondary prophylaxis[100].

The study has several strengths, being the largest study performed in patients with cirrhosis and bacterial infections, with a prospective design in several centers across the world. However, it also has potential limitations: the detailed mechanisms of resistance were not studied, although several countries were included, many countries remained excluded from the study due to lack of man power or lack of interest in participating to the study. Another potential limitation is related to the fact that participating centers were tertiary referral hospitals and the epidemiology may be different in other scenarios in the same country. Finally, the microbiological efficacy of antibiotics does not necessarily mean clinical efficacy. However, considering that large multicenter intercontinental interventional studies about the use of antibiotics in patients with cirrhosis are unlikely to be performed in future, the findings of this study may help in clinical decisions-

In conclusion, this study helped to understand better the epidemiology of infections across the world and allowed some suggestions about empirical antibiotic treatment n patients with cirrhosis and bacterial infections

STUDY 2:

ASSESSMENT OF SEPSIS-3 CRITERIA AND QUICK SOFA IN PATIENTS WITH CIRRHOSIS AND BACTERIAL INFECTIONS

Introduction

As reported in the background and methods section, early diagnosis, treatment, and prognostic stratification of patients with cirrhosis and sepsis are crucial[101]. It is well known that the SIRS criteria for the diagnosis of sepsis have poor accuracy in patients with cirrhosis and bacterial infections. In fact, patients with cirrhosis may have: a) leukopenia due to hypersplenism, b) tachypnea due to hepatic encephalopathy or ascites, c) bradycardia due to the use of beta-blockers[8,13]. Recently, a panel of experts proposed new diagnostic criteria for the definition of sepsis in the general population[84]. These criteria define sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Thus, the fulcrum of the definition has been targeted from systemic inflammation to organ dysfunction which is now defined as an acute change in sequential organ failure assessment (SOFA) score ≥ 2 points (Sepsis-3). In addition, a new simplified score, the quick SOFA (qSOFA) score, has been proposed for the screening of sepsis outside of the intensive care unit (ICU). The qSOFA is considered positive when at least 2 among the following criteria are present (alteration of consciousness; respiratory rate ≥ 22 /min; systolic blood pressure ≤ 100 mmHg). This new measure provides simple bedside criteria to identify adult patients with suspected infection who are likely to have poor outcomes. Although qSOFA is less robust than Sepsis-3 in the ICU, it does not require laboratory tests and can be assessed quickly and repeatedly. Thus, the qSOFA should be used to prompt clinicians to further investigate for organ dysfunction and to consider an increase in the frequency of monitoring. Both these criteria have been validated in large series of patients inside and outside the ICU[102–104]. Nevertheless, both of these new scores have never been

tested for prognostic assessment in patients with cirrhosis and bacterial infection so far.

Unlike the qSOFA, the timely application of Sepsis-3 in clinical practice depends on the availability of a SOFA score before the development of the bacterial infection (baseline SOFA score), particularly in patients with a medical history of chronic diseases[84]. However, it should be recognized that a baseline SOFA score was not available in most of the patients who were enrolled in the above mentioned studies. This shortcoming in the application of the Sepsis-3 criteria has been overcome assuming that the baseline SOFA was equal to zero in patients who were admitted to the hospital with a bacterial infection. This assumption could be misleading in patients with cirrhosis, who may have a baseline SOFA higher than zero due to: low platelet count, high values of bilirubin and/or some degree of hepatic encephalopathy.

Thus, the aim of this study was to assess both the applicability and the prognostic accuracy of Sepsis-3 criteria and qSOFA in patients with cirrhosis and bacterial infections.

Materials and methods

Patients

The study was performed in patients from 2 different cohorts. The primary cohort included consecutive patients with cirrhosis and bacterial or fungal infections admitted to the University Hospital of Padua, in Italy, from January 2011 to August 2016. The validation cohort included consecutive patients with cirrhosis and bacterial or fungal infections admitted to the University Hospital of Bologna, in Italy, from March 2014 to February 2016. The patients were prospectively enrolled in the 2 cohorts with the aim to assess the epidemiology and clinical impact of bacterial/fungal infections at each center. The primary end points of the study were: prevalence and risk factors for multi-drug resistant bacterial infections and risk factors for in-hospital, 28-day mortality and 90-day mortality. The studies were subsequently amended including Sepsis-3 and qSOFA assessment. Inclusion criteria were: a) diagnosis of cirrhosis according to histological, clinical, biochemical, ultrasonographic and/or endoscopic findings; b)

diagnosis of bacterial and/or fungal infection during hospitalization; c) age > 18 years old. Exclusion criteria were: a) hepatocellular carcinoma (HCC) beyond the Milan criteria; b) extrahepatic malignancy; c) severe extrahepatic disease (congestive heart failure stage NYHA ≥ 2 , chronic obstructive pulmonary disease stage GOLD ≥ 2 ; chronic kidney disease requiring renal replacement therapy [RRT]); d) previous transplant; e) HIV infection; f) use of immunosuppressive drugs.

The protocol was approved by the local Ethics Committee at each center and all patients provided written informed consent.

Design of the study

Once informed consent was obtained, a physical examination, routine laboratory and microbiological analyses were performed. Demographic, clinical, laboratory and microbiological data, as well as treatment administered were collected. SOFA score was calculated according to these variables [105]. In patients without data on PaO₂, respiratory score was calculated using oxygen saturation/fraction inspired oxygen ratio, has previously suggested and validated both in general population and in patients with cirrhosis (Table 2.1) [5,106]. Since the Sepsis-3 criteria were published in February 2016, the preadmission values of SOFA score were retrospectively collected from outpatient/day hospital visits and/or previous hospitalizations (variables at discharge) as suggested for the application of International Club of Ascites acute kidney injury (ICA-AKI) criteria [74]. In patients without a preadmission SOFA score the baseline was: a) the first SOFA assessment at hospital admission (for those with nosocomial infections); b) considered equal to zero as previously suggested [84]. Scores of liver disease such as Model of End-Stage Liver Disease (MELD), Child-Turcotte Pugh (CTP), chronic liver failure-SOFA (CLIF-SOFA) and CLIF consortium acute decompensation (CLIF-C-AD) scores, were calculated at the diagnosis of infection.

Table 2.1: Sequential Organ Failure Assessment (SOFA) score^o

System	Score				
	0	1	2	3	4
Coagulation Platelet ($\times 10^3/\mu\text{L}$)	≥ 150	<150	<100	<50	<20
Liver Bilirubin (mg/dL)	<1.2	1.2–1.9	2.0–5.9	6.0–11.9	>12.0
Kidney Creatinine (mg/dL)	<1.2	1.2–1.9	2.0–3.4	3.5–4.9	>5.0
Central nervous system GCS	15	13–14	10–12	6–9	<6
Cardiovascular #	MAP ≥ 70 mm Hg	MAP <70 mm Hg	Dopamine <5 or dobutamine or terlipressin	Dopamine 5.1–15 or adrenaline ≤ 0.1 or noradrenaline ≤ 0.1	Dopamine >15 or adrenaline >0.1 or noradrenaline >0.1
Respiration PaO ₂ /FIO ₂ (mmHg) SatO ₂ /FIO ₂	≥ 400 >512	<400 <512	<300 <357	<200* <214*	<100* <89*

Legend: GCS, glasgow coma scale; MAP, mean arterial pressure; PaO₂ partial pressure of oxygen; FIO₂, fraction of inspired oxygen; SatO₂, oxygen saturation; #, Catecholamine doses are given as $\mu\text{g}/\text{kg}/\text{min}$; * with respiratory support. ^oAdapted from references 93-94

Bacterial/fungal infections were classified as community acquired (CA), health-care associated (HCA) and nosocomial infections as previously shown[11]. Bacterial/fungal infections were diagnosed according to standard criteria already reported in the Study 1[60,89]:

Patients were followed-up until death, liver transplantation(LT) and/or discharge. Patients discharged before 90 days were followed up until 90 days from the diagnosis of infection. Data on the development of new bacterial/fungal infections, septic shock, AKI, ACLF, the transfer to the ICU, the use of vasopressors, mechanical ventilation and/or RRT during the hospitalization were collected. If the patients had second infections and/or new admissions for infections during the study period, only the first episode was considered for the purpose of the study.

Definitions

Sepsis, according to Sepsis-3 criteria, was defined by an acute increase of SOFA score ≥ 2 points[84]. Delta SOFA was assessed at admission in patients with CA or HCA infections, while the time frame for the assessment of delta SOFA was 24 hours for patients with nosocomial infections. The qSOFA was considered positive when at least 2 among the following criteria were present (alteration of consciousness; respiratory rate ≥ 22 /min; systolic blood pressure ≤ 100 mmHg)[84]. SIRS was defined by the presence of at least 2 among the following criteria (body temperature $< 36^{\circ}\text{C}$ or $> 38^{\circ}\text{C}$; heart rate > 90 bpm, respiratory rate > 20 /min, white blood cells [WBC] $< 4.000/\mu\text{L}$ or $> 12.000/\mu\text{L}$ or immature neutrophils $> 10\%$). Septic shock was defined as hypotension requiring use of vasopressors to maintain mean arterial pressure (MAP) ≥ 65 mmHg and having a serum lactate level greater than 2 mmol/L despite adequate fluid resuscitation[84].

Bacteria were defined as MDR when resistant to at least one antibiotic in 3 or more classes[91].

ACLF was defined and graded according to the EASL-CLIF consortium definition[5].

AKI was defined and staged according to International Club of Ascites criteria[74].

The efficacy of first line treatment was defined according to the clinical and laboratory improvement. The resolution of infections was defined by the clinical improvement, the

disappearance of signs and symptoms of infection, negative microbiological cultures and the improvement of laboratory and/or radiological examinations.

Management of sepsis

Patients were empirically treated with antibiotics according to the available international recommendation[107,108] and local epidemiology (high prevalence of resistance to quinolones, ESBL-producing *Enterobacteriaceae* and *Enterococci*). From 2014, patients were treated according to EASL recommendations[8]. A group of patients with nosocomial SBP were involved in a randomized controlled trial and treated with meropenem plus daptomycin[67]. In case of positive cultures, antibiotic treatment was adjusted according to an antibiotic susceptibility test. Patients with septic shock received resuscitation with plasma expanders and vasopressors. Patients with type-1 hepatorenal syndrome were treated with vasoconstrictors plus albumin.

Statistical analysis

The primary end point of the study was in-hospital mortality. Secondary end-points were 28-day mortality, development of ACLF, AKI, septic shock, transfer to the ICU, and need for organ support (mechanical ventilation or RRT) during the hospitalization. For estimating sample size, we hypothesized that the primary end-point would have occurred in 20% and 5% in patients with or without Sepsis-3, respectively. We expected two third of patients with cirrhosis and bacterial infections to meet Sepsis-3 criteria and a drop-out rate of 10 % due to LT, thus 257 patients were needed to detect this difference with a 2-sided type-1 error rate of 5% and a power of 90%. Continuous variables were summarized as mean \pm standard deviation or median with interquartile range (IQR) according to normal or non-normal distribution, respectively. They were compared using Student's T-test or Mann-Whitney U test, respectively. Categorical variables were reported as proportions and compared with chi-square or Fisher's exact test. Cumulative incidence of in-

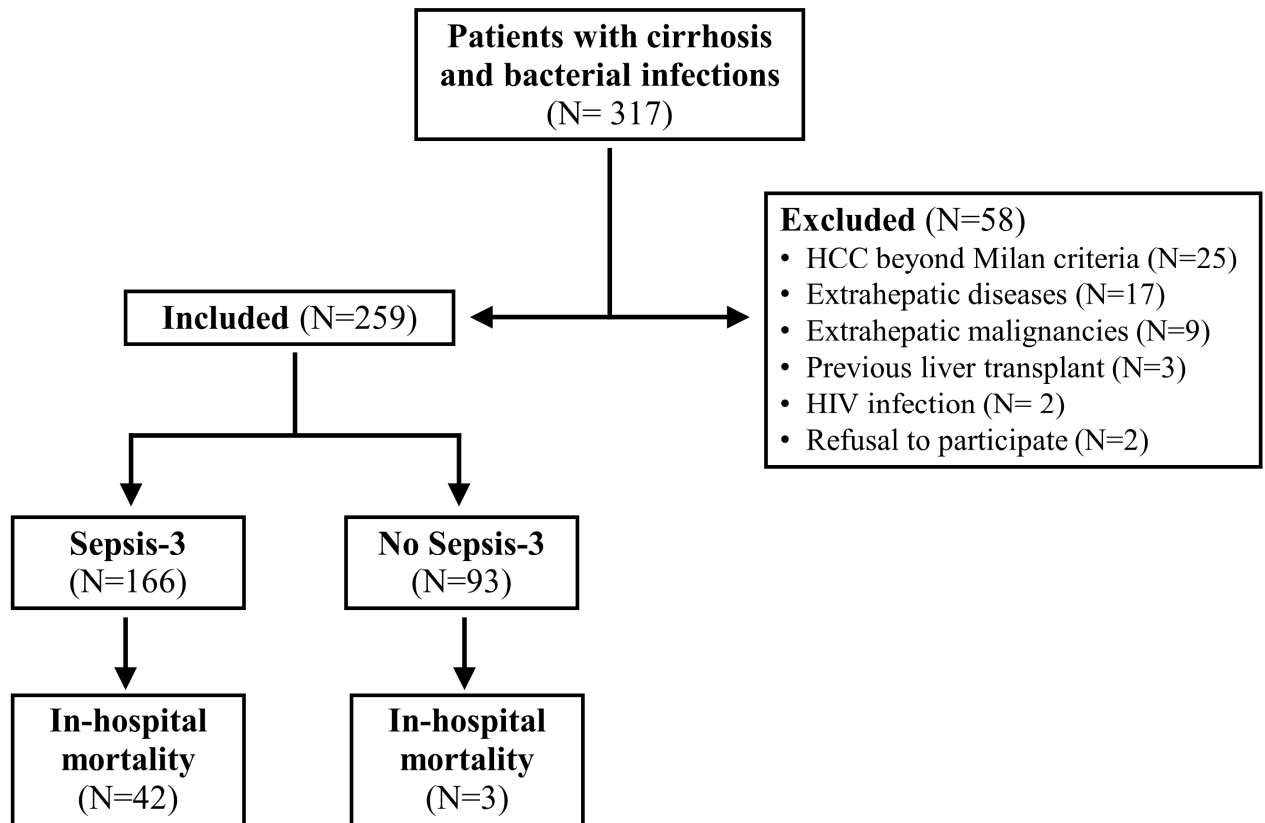
hospital and 28-day mortality was calculated considering LT as a competing event. Cumulative incidence curves were compared using Gray's test. Univariate and multivariate Cox regression analyses of predictors of mortality were performed using a competing risk approach with the Fine and Gray method and results expressed as p-value, subdistribution hazard ratios (sHR) and their 95% confidence intervals (95% CI). Variables found to be associated with in-hospital mortality with a p-value<0.1 in the univariate analysis were included in a multivariate analysis, with stepwise backward elimination (entry p<0.05; drop p>0.1). Variables that by definition could not be available within 24 hours from the diagnosis of infection (i.e. results of cultures, development of new infections and/or new organ failures, response to antibiotic treatment, transfer to the ICU etc.) were not included in the multivariate analysis. Non-normally distributed continuous variables were log-transformed to be included in the multivariate models. When Sepsis-3, SIRS, and qSOFA and/or scores of liver disease were included in the model their components were excluded to avoid multicollinearity. Similarly, in case of a correlation>0.5 between scores of liver disease and above mentioned criteria, the scores were not included in the model to avoid multicollinearity. The discrimination ability of Sepsis-3, SIRS, and qSOFA was assessed with the area under the receiver operating characteristic (AUROC) using the continuous scores. The AUROCs were compared according to the DeLong method. All tests were two-tailed and P-values<0.05 were considered significant. The statistical analysis was performed using SPSS statistical package, version 23.0, MedCalc Software, version 16.2 and SAS version 9.4.

Results

Primary cohort

During the study period, 317 patients hospitalized for an acute decompensation of cirrhosis and with bacterial/fungal infections were evaluated. Among them, 58 were excluded for several reasons and 259 patients were enrolled. In Figure 2.1 a flow chart of patients evaluated in the study has been reported.

Figure 2.1. Flow chart of the study.



Legend: N, number; HCC, hepatocellular carcinoma

The baseline characteristics of patients included are reported in Table 1. The mean age was 61 ± 12 years. The most common etiologies of cirrhosis were alcohol consumption and HCV infection. The majority of patients had an advanced liver disease as shown by the high prevalence of ascites, hepatic encephalopathy and the prognostic scores. Sixty-eight patients (26%) had ACLF and ninety-four (36%) AKI at the diagnosis of infection. Increased levels of C-reactive protein (CRP) were found in most patients (median=22 mg/L; upper normal value=6 mg/L). A baseline SOFA score was available in 207 patients (80%) with a median of 4 (IQR 2-5). The median time elapsed from baseline assessment and the onset of bacterial infection was 21 days (IQR 7-51). All patients were enrolled while in a regular ward. Patients were followed-up for a median of 86 days (IQR 29 - 90).

Table 2.2. Demographic, clinical and laboratory characteristics of patients at the diagnosis of infection in primary cohort.

Variables	N = 259
Age (years) – mean (SD)	61 (12)
Gender (Male) – n (%)	162 (62)
Etiology of cirrhosis – n (%)	
HBV	23 (9)
HCV	78 (30)
Alcohol	104 (40)
HCV + Alcohol	22 (9)
Other	32 (12)
Diabetes – n (%)	83 (32)
MAP (mmHg) – mean (SD)	83 (12)
HR (bpm) – mean (SD)	83 (17)
Body temperature (°C) – mean (SD)	37 (1)
Respiratory rate (breath/min) – mean (SD)	19 (4)
Ascites – n (%)	216 (83)
Hepatic encephalopathy – n (%)	94 (36)
ACLF – n (%)	68 (26)
ACLF grade – n (%)	
Grade 1	45 (66)
Grade 2	20 (30)
Grade 3	3 (4)
MELD score – mean (SD)	20 (7)
Child Pugh score – mean (SD)	9 (2)
CLIF-C AD score – mean (SD)	58 (11)
INR – median (IQR)	1.5 (1.3 – 1.9)
Bilirubin (µmol/L) – median (IQR)	64 (32 – 142)
Albumin (g/dl) – median (IQR)	29 (25 - 34)
SCr (µmol/L) – median (IQR)	100 (73 - 155)
Sodium (mmol/L) – mean (SD)	133 (6)
Platelets (x 10 ⁹ /L) – median (IQR)	91 (56 - 146)
Leukocytes (x 10 ⁹ /L) – median (IQR)	6.6 (4.4 – 10.5)
CRP (mg/L) - median (IQR)	22 (12 - 54)
SOFA score baseline* - median (IQR)	4 (2 – 5)
SOFA score inclusion – median (IQR)	6 (4 – 7)
CLIF-SOFA score inclusion – median (IQR)	6 (4 – 8)

Legend: M, median; m, mean; IQR, interquartile range; SD, standard deviation; n, number; HBV, hepatitis B virus; HCV, hepatitis C virus; MAP, mean arterial pressure; HR, heart rate; MELD, model for end-stage liver disease; CLIF-C AD, chronic liver failure consortium acute decompensation score; ACLF, acute-on-chronic liver failure; INR, international normalized ratio; SCr, serum creatinine; C-reactive protein; SOFA, sequential organ failure assessment; CLIF, chronic liver failure; *, available in 207 patients.

Characteristics of bacterial infections

UTI was the most common infection in our series (33%), followed by SBP (23%), pneumonia (13%) and primary bacteremia(13%) (Table 2.3).

Table 2.3. Clinical and microbiological characteristics of bacterial infections in primary cohort

Variable	N= 259
Site of infection – n (%)	
UTI	85 (33)
SBP	59 (23)
Pneumonia	36 (14)
Primary bacteremia	35 (13)
Skin and soft tissue infection	15 (6)
Others#	29 (11)
Type of infection – n (%)	
CA	82 (32)
HCA	89 (34)
Nosocomial	88 (34)
SIRS – n (%)*	89 (34)
Positive qSOFA score– n (%)	60 (23)
Sepsis according to Sepsis-3 criteria – n (%)	166 (64)
Septic shock – n (%)	6 (2)
Positive microbiological cultures – n (%)	191 (74)
Type of strains isolated – n (%)	
Gram –	87 (46)
Gram +	90 (47)
Fungi	14 (7)
Type of bacteria isolated – n (%)	
<i>Escherichia Coli</i>	43 (22)
<i>Enterococcus Faecium</i>	30 (16)
<i>Klebsiella Pneumoniae</i>	21 (11)
<i>Enterococcus faecalis</i>	20 (10)
<i>Staphylococcus aureus</i>	5 (3)
Other <i>Enterobacteriaceae</i>	12 (6)
Other <i>Staphylococci</i>	24 (13)
Others	36 (17)
MDR Bacteria– n (%)	79 (30)

Legend: n, number; UTI, urinary tract infection; SBP, spontaneous bacterial peritonitis; CA, community acquired; HCA, health-care associated; SIRS, systemic inflammatory response syndrome; qSOFA, quick SOFA score; MDR, multi drug resistant.

#, 6 secondary bacterial peritonitis, 4 clostridium difficile colitis, 4 spontaneous bacterial empyema, 12 infections without identified focus, 2 secondary bacteremia, 1 cholangitis,

*,70 patients (27%) had no SIRS criteria, 100 (39 %) had 1 SIRS criteria, 63 (24) had 2 SIRS criteria, 22 (9) had 3 SIRS criteria and 4 (2%) 4 SIRS criteria. 95 patients (37%) had WBC criteria, 29 (11%) body temperature criteria, 113 (44%) respiratory rate criteria and 71 (27%) heart rate criteria.

There was a balanced proportion between CA (32%), HCA (34%), and nosocomial infections (34%). At least one microbiological culture was positive in 191 patients (74%), with a balance between Gram-positive and Gram-negative strains (47 and 46%, respectively), while fungi were isolated in 7% of patients (mainly *Candida* species). The most common bacterium was *Escherichia coli* (22%), followed by *Enterococcus faecium* (16%) and *Klebsiella pneumoniae* (11%). Seventy-nine patients (30%) showed an infection due to multi-drug resistant (MDR) bacteria. At the diagnosis of infection, 89 patients (34%) had SIRS, 60 (23%) had a positive qSOFA, and 166 (64%) fulfilled Sepsis-3 criteria. Sepsis-3 was significantly more common in patients without an available baseline SOFA than in those with a baseline SOFA score (94 vs 57%; $p<0.001$). In addition, Sepsis-3 was significantly more common in patients with a positive qSOFA than in those with a negative qSOFA (87 vs 57%; $p<0.001$). Conversely, no difference was found in the prevalence of Sepsis-3 among those with or without SIRS ($P=0.10$). Regarding empirical antibiotic treatment, 149 patients (58%) were treated with 2 or more antibiotics. The most used antibiotics were third generation cephalosporins (34%), quinolones (34%), carbapenems (34%), glycopeptides (22%) and piperacillin/tazobactam (18%). The empirical antibiotic treatment was effective in 206 patients (79%) and the final resolution of infection was achieved in 217 patients (84%). Fifty-three patients developed a second infection during the hospitalization.

Survival

During the hospitalization 45 patients died (17%), 19 were transplanted (7%) and 195 survived (75%). In Table 2.4, a comparison of baseline characteristics of survivors versus non-survivors has been reported. Non-survivors had a significantly worse alteration of vital signs, liver and renal function than survivors. Markers of systemic inflammation such as leukocytes and CRP were found to be significantly higher in non-survivors than survivors.

Table 2.4. Demographic, clinical and laboratory characteristics of survivors vs non-survivors* in primary cohort

Variables	Survivors (N=195)	Nonsurvivors (N=45)	sHR (95% CI)	P value
Age (years) – media (DS)	62 (12)	61 (10)	1.02 (0.99-1.04)	0.235
Gender (M) – n (%)	117 (60)	33 (73)	1.65 (0.86-3.14)	0.130
Etiology of cirrhosis – n (%) (Alcohol)	80 (41)	20 (44)	1.18 (0.66-2.10)	0.581
Treatment with β -blockers – n (%)	64 (34)	16 (37)	1.01 (0.55-1.83)	0.985
Diabetes – n (%)	66 (34)	12 (27)	0.84 (0.44-1.59)	0.590
Ascites – n (%)	158 (81)	41 (91)	1.95 (0.77-4.93)	0.159
Hepatic encephalopathy – n (%)	62 (32)	25 (56)	2.48 (1.38-4.44)	0.002
Respiratory rate (breath/min)– mean (SD)	18 (4)	21 (4)	1.13 (1.05-1.21)	0.001
Body temperature (°C) – media (DS)	36.6 (1)	37 (1)	1.35 (1.07-1.70)	0.012
MAP (mmHg) – mean (SD)	85 (11)	81 (13)	0.99 (0.96-1.02)	0.363
Heart rate (bpm) – mean (SD)	81 (14)	87 (18)	1.01 (1.00-1.03)	0.064
INR – median (IQR)	1.5 (1.3 – 1.8)	1.8 (1.6 – 2.1)	3.19 (1.92-5.28)	< 0.001
Bilirubin (μ mol/L) – median (IQR)	53 (27 – 105)	119 (63 – 293)	1.06 (1.03-1.09)	< 0.001
Albumin (g/L)– mean (SD)	31 (6)	27 (6)	0.94 (0.89-0.99)	0.012
SCr (μ mol/L) – median (IQR)	93 (69 – 142)	132 (79 – 197)	1.33 (1.17-1.52)	0.021
Serum Sodium (mmol/L) – media (DS)	134 (6)	131 (6)	0.93 (0.90-0.97)	0.002
CRP (mg/L) – median (IQR)	21 (10 – 52)	35 (15 – 79)	1.01 (1.00-1.01)	0.019
Leukocytes ($\times 10^9/L$) – median (IQR)	5.9 (4.2–9.2)	9.8 (4.7–13.3)	1.03 (1.00-1.07)	0.081
Platelets ($\times 10^9/L$) – median (IQR)	95 (59 – 141)	78 (46 – 150)	1.00 (0.99-1.00)	0.551
MELD score – mean (SD)	18 (7)	26 (7)	1.11 (1.06-1.16)	< 0.001
Child Pugh score – mean (SD)	9 (2)	11 (1)	1.85 (1.47-2.32)	< 0.001
Delta SOFA – median (IQR)	2 (1 – 4)	4 (3 – 7)	1.26 (1.14-1.39)	< 0.001
CLIF – SOFA score – median (IQR)	6 (4 – 7)	8 (7 – 10)	1.50 (1.30-1.72)	< 0.001
CLIF-AD score – mean (SD)	56 (10)	65 (10)	1.07 (1.04-1.10)	< 0.001
AKI at inclusion – n (%)	62 (32)	24 (53)	5.46 (2.40-12.50)	<0.001
ACLF at inclusion – n (%)	41 (21)	18 (40)	1.61 (0.87-2.97)	0.128
SIRS – n (%)	62 (32)	23 (51)	2.24 (1.25-3.98)	0.007
Positive qSOFA score – n (%)	31 (16)	23 (51)	3.18 (1.76-5.71)	< 0.001
Sepsis-3 criteria – n (%)	114 (58)	42 (93)	7.75 (2.38-25.00)	0.001
Nosocomial infections – n (%)	54 (28)	21 (47)	1.09 (0.60-1.99)	0.780
MDR bacteria – n (%)	53 (27)	20 (44)	1.27 (0.71-2.26)	0.791
Efficacy of empirical antibiotic treatment – n (%)	180 (92)	8 (18)	22.92 (10.83-48.52)	< 0.001
Resolution of infection – n (%)	194 (99)	5 (11)	52.4 (21.2-129.6)	< 0.001
Development of septic shock during hospitalization – n (%)	9 (5)	16 (36)	4.50 (2.54-7.94)	< 0.001

Variables	Survivors (N=195)	Nonsurvivors (N=45)	sHR (95% CI)	P value
Trasfer in ICU – n (%)	9 (5)	20 (44)	7.94 (4.50-14.09)	< 0.001
Mechanical ventilation – n (%)	3 (1)	6 (13)	4.07 (2.00-8.20)	0.002
RRT – n (%)	4 (2)	5 (11)	2.37 (1.02-5.53)	0.046

Legend: sHR, subdistribution hazard ratio; CI, confidence interval; M, median; m, mean; IQR, interquartile range; SD, standard deviation; n, number; MAP, mean arterial pressure; MELD, model for end-stage liver disease; CLIF-C AD, chronic liver failure consortium acute decompensation score; AKI, acute kidney injury; ACLF, acute-on-chronic liver failure; INR, international normalized ratio; SCr, serum creatinine; C-reactive protein; SOFA, sequential organ failure assessment; CLIF, chronic liver failure; SIRS, systemic inflammatory response syndrome; qSOFA, quick SOFA score; MDR, multi drug resistant; ICU, intensive care unit; RRT, renal replacement therapy; *, liver transplantation was considered a competing event of death.

The prevalence of SIRS, Sepsis-3, and positive qSOFA was significantly higher in non-survivors than survivors (51 vs 32 %, sHR=2.24; p=0.007; 93 vs 58%, sHR=7.75, p=0.001; and 51 vs 16%, sHR=3.18, p<0.001, respectively). As expected, a high MELD score, hepatic encephalopathy and AKI were all associated with a worse survival rate. An ineffective antibiotic treatment was also found to be strongly associated with a worse survival rate. Interestingly, no difference was found in in-hospital mortality rate according to beta-blockers use. In the multivariate analysis, after excluding MELD and CLIF-SOFA for collinearity with Sepsis-3 criteria, Sepsis-3 (sHR=5.47; p=0.006), positive qSOFA (sHR=1.99; p=0.020), CLIF-C-AD score (sHR=1.05; p=0.001) and CRP (sHR=1.01; p=0.034) were found to be independent predictors of in-hospital mortality (Table 4). The multivariate analysis of 28-day mortality showed similar results. More in detail, Sepsis-3 criteria(sHR=3.57; p=0.016), positive qSOFA (sHR=2.23; p=0.013) and CLIF-C-AD score (sHR=1.05; p=0.001) were found to be independent predictors of 28-day mortality.

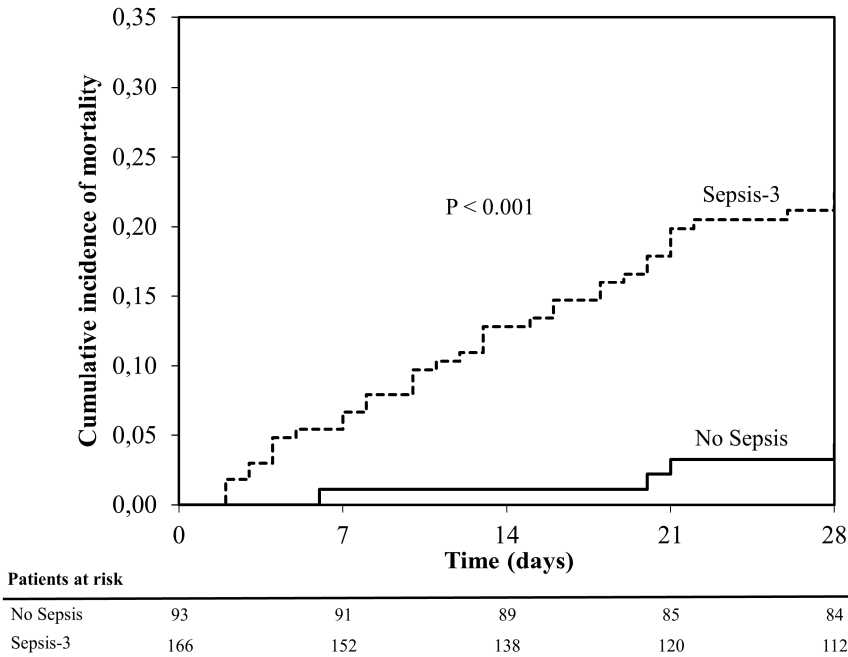
Table 2.5. Multivariate analysis of in-hospital and 28-day mortality in patients with cirrhosis and bacterial/fungal infections in primary cohort

Variable	sHR	95% CI	P
In-hospital mortality			
Sepsis-3 criteria	5.47	1.65 – 18.18	0.006
qSOFA criteria	1.99	1.12 – 3.55	0.020
CRP (g/L)	1.01	1.00 – 1.01	0.034
CLIF-C-AD	1.05	1.01 – 1.08	0.001
28-day mortality			
Sepsis-3 criteria	3.57	1.27 – 9.99	0.016
qSOFA criteria	2.23	1.19 – 4.20	0.013
CLIF-C-AD	1.05	1.02 – 1.08	0.001

Legend: sHR, subdistribution hazard ratio; CI, confidence interval; Sepsis-3, diagnosis of sepsis according to Sepsis-3 criteria; qSOFA, quick sequential organ failure assessment score; CRP, C-reactive protein; CLIF-C-AD; chronic liver failure consortium acute decompensation score.

The cumulative incidence of 28-day mortality was significantly higher in patients with Sepsis-3 than in those without (23 vs 4%; $p=0.001$) (Fig.1 panel-A). In-hospital mortality rate was not significantly different between patients with Sepsis-3 with or without a baseline SOFA score (29vs21%; $p= 0.396$).

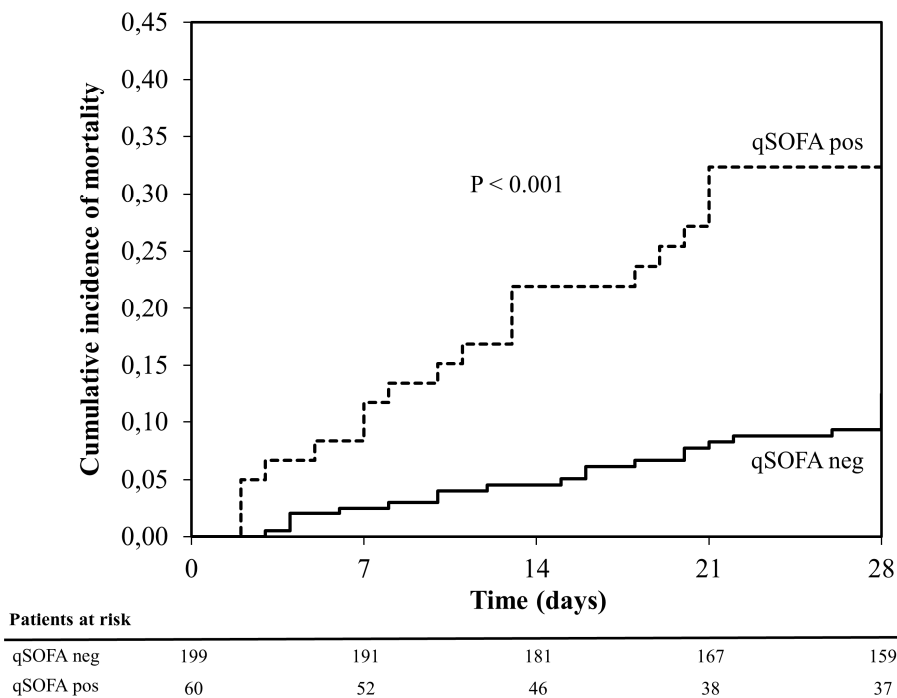
Figure 2.2. Cumulative incidence of 28-day mortality according to fulfillment or not of Sepsis-3 criteria in primary cohort



Legend: Comparison was done using Gray's test

Likewise, patients with a positive qSOFA score had a significantly higher cumulative incidence of 28-day mortality than those with a negative qSOFA score (32 vs 13%; $p < 0.001$) (Figure 2.3).

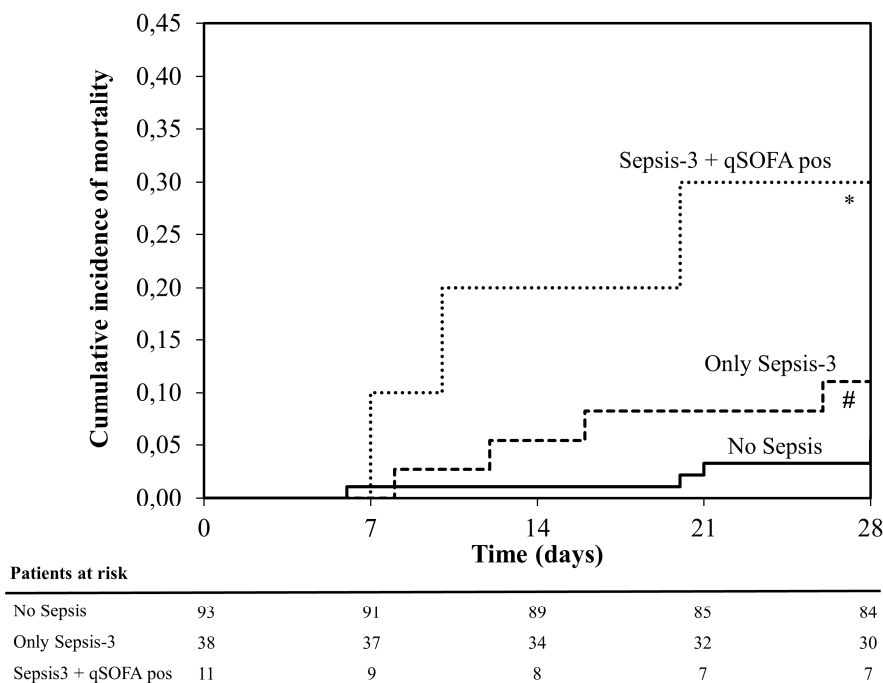
Figure 2.3. Cumulative incidence of 28-day mortality according to the positivity of quick SOFA (qSOFA) score in primary cohort



Legend: pos, positive; neg, negative. Comparison was done using Gray's test

In patients without a baseline SOFA score the combination of both Sepsis-3 and a positive qSOFA helped to identify a group of patients with a worse 28-day mortality that was similar to the one observed in patients with a baseline SOFA and Sepsis-3 (30% and 26%, respectively). Conversely, 28-day mortality was not significantly different between patients without a baseline SOFA and only Sepsis-3 (negative qSOFA) and those without Sepsis-3 (11 vs 6%; $p=0.164$) (Figure 2.4).

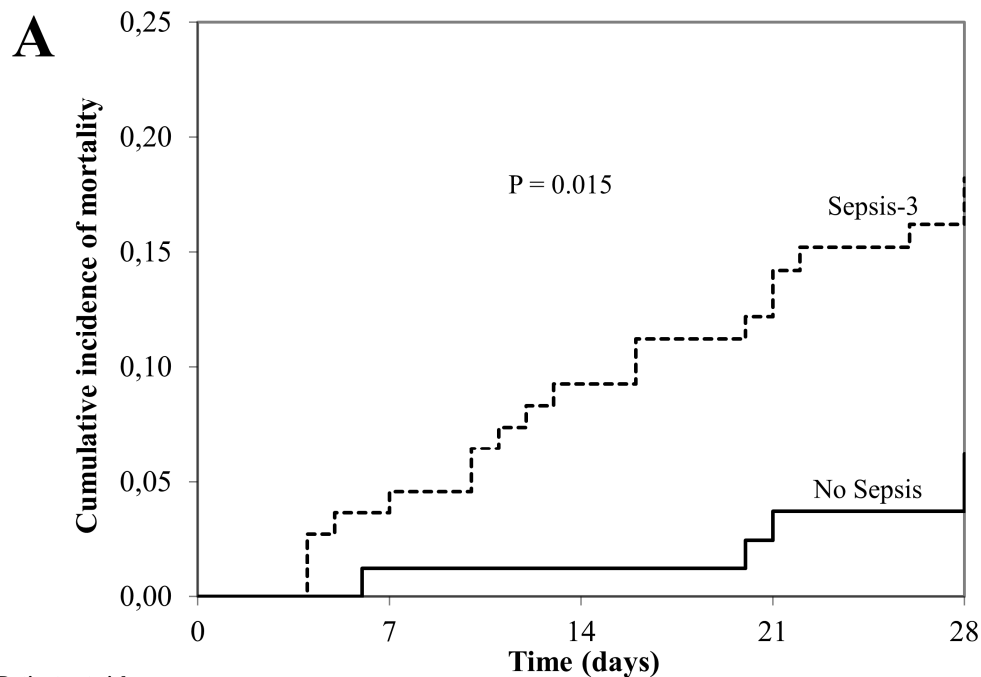
Figure 2.4. Cumulative incidence of 28-day mortality comparing patients without sepsis with those without a baseline SOFA score and only Sepsis-3 criteria or both Sepsis-3 criteria and a positive quick SOFA (qSOFA) in primary cohort



Legend: pos, positive; *, $p= 0.006$ vs No Sepsis; #, $p= 0.164$ vs No sepsis. Comparison was done using Gray's test.

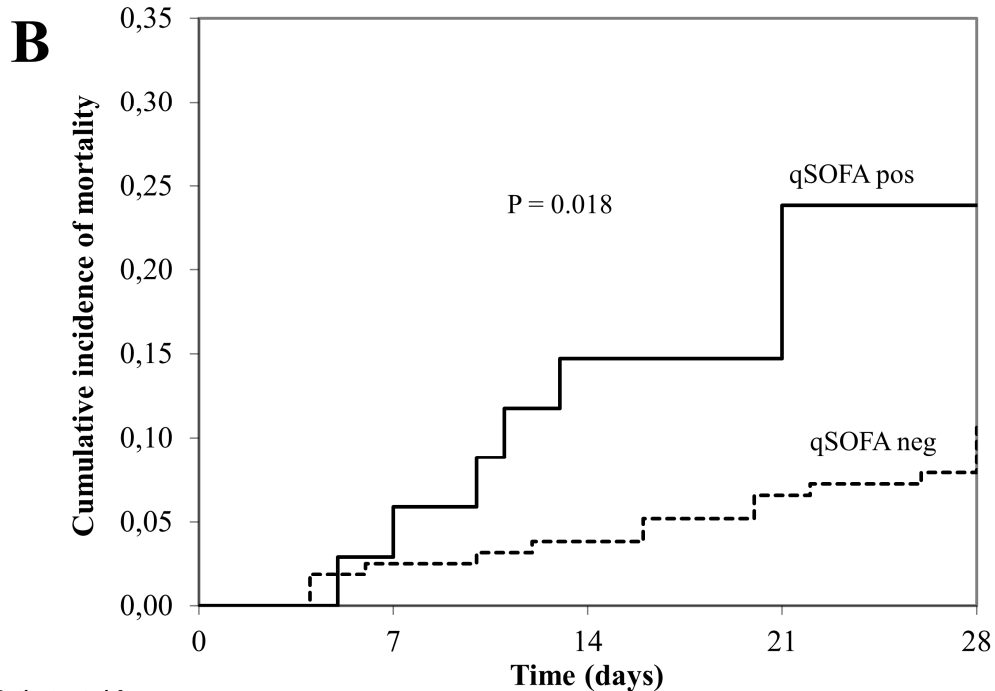
In the subgroup of patients without ACLF or septic shock patients with Sepsis-3 and positive qSOFA had a significantly higher incidence of 28-day mortality than those without (18 vs 6%, $p=0.015$ and 24 vs 11%, $p=0.018$, respectively; Figure 2.5 panel A and panel B, respectively).

Figure 2.5. Cumulative incidence of 28-day mortality according to fulfillment or not of Sepsis-3 criteria (panel A) or the positivity of quick SOFA (qSOFA, panel B) in patients without ACLF/septic shock



Patients at risk

No Sepsis	82	80	79	75	74
Sepsis-3	109	102	94	84	78



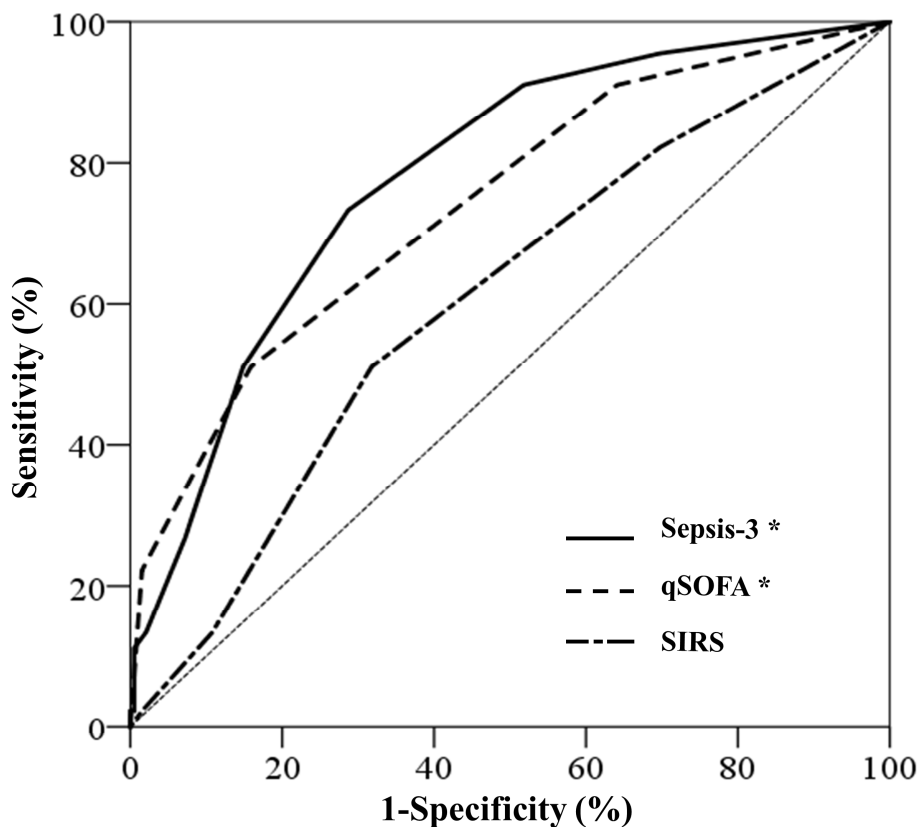
Patients at risk

qSOFA neg	157	150	144	135	129
qSOFA pos	34	32	29	24	22

Legend: pos, positive; neg, negative. Comparison was done using Gray's test.

Interestingly, even in patients with ACLF 28-day incidence of mortality was significantly higher in patients with Sepsis-3 or positive qSOFA than those without (34 vs 0%, $p < 0.001$ and 44 vs 20%, $p = 0.013$). Overall, the AUROC of Sepsis-3 criteria and qSOFA in predicting in-hospital mortality was 0.784 (95% CI=0.729-0.833) and 0.732 (95% CI=0.674-0.785), respectively and it was significantly higher than the one of SIRS (0.606; 95% CI=0.544-0.666; $p < 0.001$ and $p = 0.006$, respectively) (Fig. 2.6).

Figure 2.6: Receiver Operating Characteristic curves for in-hospital mortality of Sepsis-3 criteria, systemic inflammatory response syndrome (SIRS) criteria and quick sequential organ failure assessment (qSOFA) in the primary cohort.



Legend: Sepsis-3 indicates the delta increase in SOFA score; SIRS and qSOFA the final scores given by each SIRS and qSOFA point, respectively. *, $p < 0.01$ vs SIRS.

In addition, the discrimination ability of 28-day mortality of Sepsis-3 (AUROC=0.737; 95% CI=0.676-0.792) and qSOFA (AUROC=0.718; 95% CI=0.656-0.774) was significantly better than SIRS (AUROC=0.609; 95% CI=0.544-0.672; $p = 0.019$ and 0.015, respectively). The use of

a CLIF-SOFA derived “criterion” (delta CLIF-SOFA) to define sepsis did not significantly improve the prognostic accuracy of Sepsis-3 criteria.

Outcomes of infections according to Sepsis-3 and qSOFA

During the hospitalization, 48 patients (25%) developed ACLF, 25 (10%) septic shock and 46 (19%) AKI (Table 2.6). Thus, overall, 116 patients (45%) had ACLF, 31 patients had septic shock (12%), and 140 patients had AKI (54%).

Table 2.6. Outcomes of bacterial infections during follow up.

Variable	N = 259
Response to empirical antibiotic treatment– n (%)	206 (79)
Resolution of infection – n (%)	217 (84)
Development of AKI – n (%)*	46 (28)
Development of septic shock – n (%)**	25 (10)
Development of ACLF – n (%)***	48 (25)
Transfer in ICU – n (%)	29 (11)
Mechanical ventilation – n (%)	9 (3)
RRT – n (%)	10 (4)
In-hospital mortality – n (%)	45 (19)
28-day mortality – n (%)	40 (15)
90-day mortality – n (%)	61 (24)

Legend: AKI, acute kidney injury; ACLF, acute on chronic liver failure; ICU, intensive care unit; renal replacemt therapy.

*, patients with AKI at inclusion (n=94) were excluded from this analysis;

** patients with septic shock at inclusion (n=6) were excluded from this analysis

***patients with ACLF at inclusion (n=68) were excluded from this analysis

Twenty-nine patients (11%) were transferred to the ICU, 9 required mechanical ventilation (3%) and 10 RRT (4%). As expected, patients developing AKI, ACLF and/or septic shock had a significantly higher mortality rate than those who did not (45 vs 3%, 60 vs 1%, 64 vs 13%, respectively; all $p < 0.001$) as well as patients admitted to the ICU (69 vs 12%, $p < 0.001$), or those mechanically ventilated (67 vs 17%, $p = 0.002$) or on RRT (56 vs 17%, $p = 0.013$).

Patients with Sepsis-3 and a positive qSOFA had a significantly lower rate of response to empirical antibiotic treatment (72 vs 93%, $p < 0.001$; 62 vs 85%; $p < 0.001$, respectively) and of final resolution of the infection (77 vs 97%, $p < 0.001$; 67 vs 89%; $p < 0.001$, respectively) than those without (Tables 2.7 and 2.8). Furthermore, patients with Sepsis-3 and positive qSOFA had a significantly higher probability of developing ACLF (36 vs 11%; $p < 0.001$; 44 vs 21%; $p = 0.001$, respectively) and to be transferred to the ICU (16 vs 2%; $p = 0.001$; 23 vs 7%; $p = 0.002$, respectively) and to need mechanical ventilation (5 vs 0%; $p = 0.029$; 10 vs 2%; $p = 0.006$, respectively) than those without (Tables 2.7 and 2.8). Only patients with Sepsis-3 had a higher incidence of septic shock (15 vs 0%; $p < 0.001$) and a need for RRT (6 vs 0%; $p = 0.016$) than those without.

Table 2.7. Outcomes of bacterial infections during follow up in patients with vs without Sepsis-3 criteria.

Variables	No sepsis (N=93)	Sepsis-3 (N=166)	P value
Efficacy of empirical antibiotic treatment – n (%)	86 (92)	120 (72)	< 0.001
Resolution of infection – n (%)	90 (97)	127 (77)	< 0.001
Development of AKI – n (%)*	15 (21)	31 (33)	0.132
Development of ACLF – n (%)**	9 (11)	39 (36)	< 0.001
Development of septic shock during hospitalization – n (%)***	0 (0)	25 (15)	< 0.001
Trasfer in ICU – n (%)	2 (2)	27 (16)	0.001
Mechanical ventilation – n (%)	0 (0)	9 (5)	0.029
RRT – n (%)	0 (0)	10 (6)	0.016
In-hospital mortality – n (%)#	3 (3)	42 (25)	0.001
28-day mortality – n (%)#	4 (4)	36 (22)	0.001
90-day mortality – n (%)#	9 (10)	52 (31)	<0.001

Legend: n, number; AKI, acute kidney injury; ACLF, acute-on-chronic liver failure; ICU, intensive care unit; RRT, renal replacement therapy;

*, patients with AKI at inclusion (n=94) were excluded from this analysis;

**, patients with ACLF at inclusion (n=68) were excluded from this analysis

***, patients with septic shock at inclusion (n=6) were excluded from this analysis

#, comparison made with competing risk analysis (Fine and Gray test)

Table 2.8. Outcomes of bacterial infections during follow up in patients with positive qSOFA vs those with negative qSOFA.

Variables	qSOFA neg (N=199)	qSOFA pos (N=60)	P value
Efficacy of empirical antibiotic treatment – n (%)	169 (85)	37 (62)	< 0.001
Resolution of infection – n (%)	177 (89)	40 (67)	< 0.001
Development of AKI – n (%)*	34 (25)	12 (39)	0.204
Development of ACLF – n (%)**	33 (21)	15 (44)	0.009
Development of septic shock during hospitalization – n (%)***	18 (9)	7 (12)	0.724
Trasfer in ICU – n (%)	15 (7)	14 (23)	0.002
Mechanical ventilation – n (%)	3 (2)	6 (10)	0.006
RRT – n (%)	5 (3)	5 (8)	0.095
In-hospital mortality – n (%)#	22 (11)	23 (38)	<0.001
28-day mortality – n (%)#	21 (11)	19 (32)	<0.001
90-day mortality – n (%)#	36 (18)	25 (42)	<0.001

Legend: n, number; AKI, acute kidney injury; ACLF, acute-on-chronic liver failure; ICU, intensive care unit; RRT, renal replacement therapy;

*, patients with AKI at inclusion (n=94) were excluded from this analysis;

**, patients with ACLF at inclusion (n=68) were excluded from this analysis

***, patients with septic shock at inclusion (n=6) were excluded from this analysis

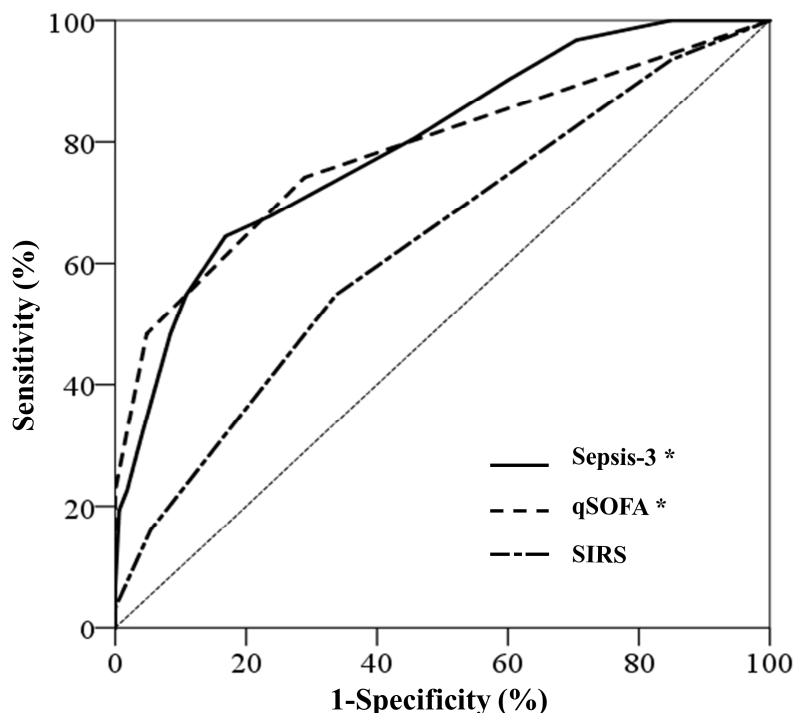
#, comparison made with competing risk analysis (Fine and Gray test)

Validation cohort

The external validation cohort included 197 patients with cirrhosis and bacterial and/or fungal infections followed for a median of 90 days (IQR=86-90). A comparison between validation cohort and primary cohort has been reported in Supplementary Table 8. Demographic characteristics were similar between the 2 groups. Patients in the validation cohort were less frequently alcoholics and had a less advanced liver disease as shown by the lower rate of ascites and the lower values of MELD score, CTP score and SOFA score. The rate of patients fulfilling

Sepsis-3 was significantly lower in the validation cohort than in the primary cohort (50 vs 64%; $p=0.004$) as well as the rate of patients with a positive qSOFA (12 vs 24%; $p=0.004$). Conversely, no difference was found in the prevalence of SIRS criteria. In-hospital mortality was not significantly different between the 2 cohorts, while LT rate was higher in the primary cohort than in the validation cohort. Even in the validation cohort Sepsis-3 was significantly more common in patients without an available baseline SOFA than in those with a baseline SOFA score (95 vs 46%; $p<0.001$) and in patients with a positive qSOFA than in those with a negative qSOFA (92 vs 45%; $p<0.001$). Similarly to the primary cohort, no difference was found in the prevalence of Sepsis-3 among those with or without SIRS ($P=0.810$). In the validation cohort both Sepsis-3 criteria (AUROC=0.797; 95% CI=0.734-0.851) and qSOFA (AUROC=0.784; 95% CI=0.720-0.839) confirmed a significantly better discrimination ability for in-hospital mortality than SIRS criteria (AUROC=0.631; 95% CI=0.560-0.699 [Sepsis-3 vs SIRS, $p=0.009$; qSOFA vs SIRS; $p=0.009$]; Fig. 2.8). Moreover, the discrimination ability for 28-day mortality of Sepsis-3 (AUROC=0.813; 95% CI=0.751-0.865) and qSOFA (AUROC=0.779; 95% CI=0.714-0.835) were significantly better than SIRS (AUROC=0.632; 95% CI=0.561-0.700; $p=0.006$ and 0.017 , respectively).

Figure 2.7. Receiver Operating Characteristic curves for in-hospital mortality of Sepsis-3 criteria, systemic inflammatory response syndrome (SIRS) criteria and quick sequential organ failure assessment (qSOFA)



Legend: Sepsis-3 indicates the delta increase in SOFA score; SIRS and qSOFA the final scores given by each SIRS and qSOFA point, respectively. *, $p < 0.01$ vs SIRS.

Discussion

An accurate prognostic assessment in patients with cirrhosis and bacterial infection is crucial for their management in clinical practice. Until now, it has been based on the characterization of the bacterial infection as sepsis, severe sepsis, and septic shock, on the development of organ failure/s, and/or on CTP and MELD scores. Moving from the definition of sepsis, which has been based on SIRS criteria, it should be recognized that this approach has some relevant drawbacks. It is well known that SIRS criteria have significant shortcomings when applied to patients with cirrhosis. Beyond this, it should be emphasized that recently, according to the Third International Consensus Definition for sepsis and septic shock, they have now been abandoned also in the general population in favor of new evidence-based criteria. Specifically, a new criterion has been validated, namely, the Sepsis-3 which is defined as an acute change in SOFA score ≥ 2 points. In

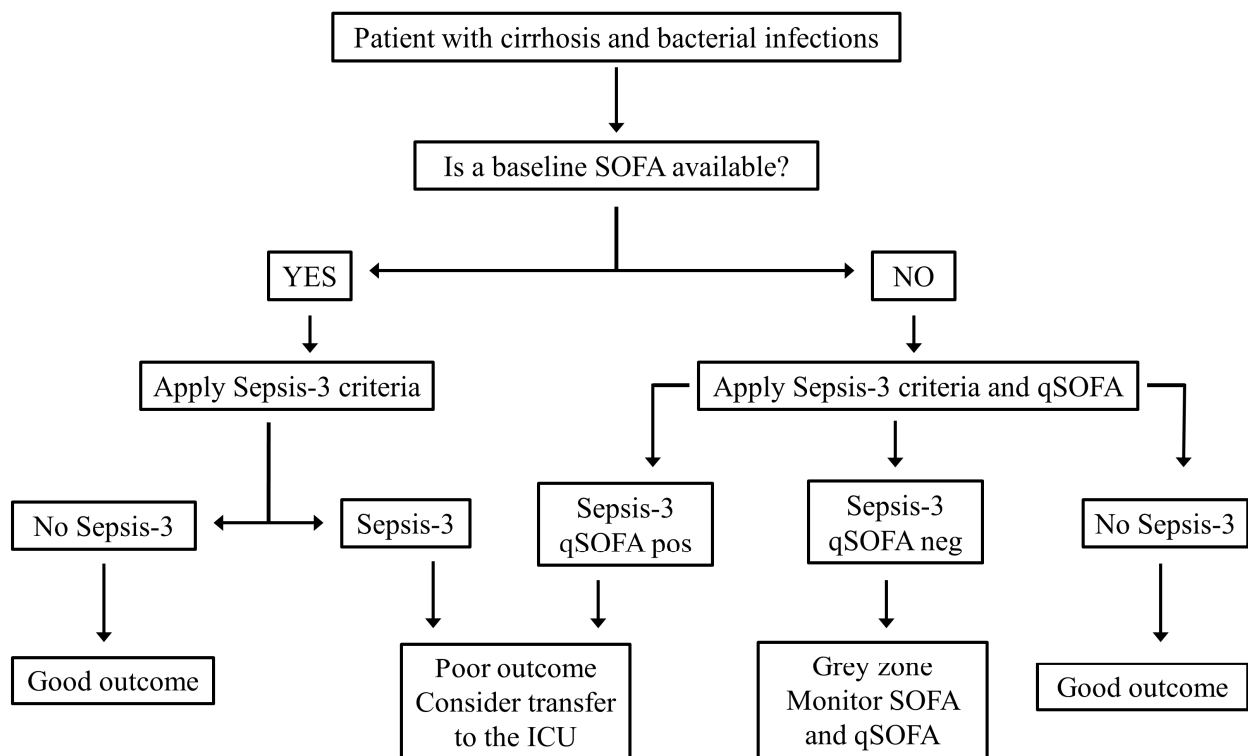
addition, a new simplified tool, namely qSOFA, has been validated for the screening in patients with suspected bacterial infection in order to provide simple bedside criteria to identify patients with suspected infection who are likely to have poor outcomes.

Neither Sepsis-3 criteria nor qSOFA have been tested and validated in patients with cirrhosis and bacterial infection so far. Thus, the main result of the study was to show that the definition of sepsis based on the Sepsis-3 criteria has a better prognostic accuracy than the one based on the SIRS criteria. The relevance of this finding has been further strengthened by the external validation. This finding is probably related to two factors: a) the shortcoming of SIRS criteria, b) the deep prognostic impact of organ dysfunction, detected by Sepsis-3 criteria, in patients with cirrhosis. The latter is not surprising. In fact, the Canonic study has been clearly shown that organ dysfunction is a strong predictor of mortality in patients with an acute decompensation of cirrhosis[5]. Although the discrimination ability of both sepsis-3 criteria and qSOFA was far from being perfect (a finding already shown in general population)[102–104], they were able to identify patients at risk of short term mortality even in a potential low risk group (no ACLF, no septic shock). Interestingly, even in high risk group (patients with ACLF), both Sepsis-3 and qSOFA criteria identified a group with a very high short-term mortality rate. These data suggest that when ACLF and organ failures are acutely precipitated by a bacterial infection the prognosis is the worst.

One potential criticism of our study is that the diagnosis of sepsis according to Sepsis-3 criteria was very common in both the primary and the validation cohorts. This is due to the critical role of having or not having a baseline SOFA score. After all, it is sufficient that a patient has a platelet count <150,000 due to hypersplenism and a total bilirubin ≥ 1.2 mg/dl due to a mild liver dysfunction for a delta SOFA score ≥ 2 if the baseline is not available and thus, is assumed to be equal to 0. Such confirming, both in the primary cohort and the validation cohort the prevalence of Sepsis-3 was significantly lower in patients with a baseline SOFA score than in those without. Despite this limitation, no significant difference was found in terms of in-hospital mortality

between patients diagnosed with sepsis with or without a baseline SOFA (29 vs 21%; $p=0.396$). It means that organ dysfunctions are so important that are able to identify patients with bacterial infections with a poor prognosis regardless of the presence or not of a baseline SOFA. But the question arises: to what extent was organ dysfunction related to bacterial infections in patients with cirrhosis in whom the baseline SOFA was not available? Therefore, Sepsis-3 criteria should be interpreted and applied with caution in these patients. Probably, the only way to exceed this shortcoming is the sequential application of the algorithm proposed for patients outside the ICU, which provides in the first instance the use of qSOFA in the assessment of patients with bacterial infections[84]. In fact, the qSOFA does not include parameters of liver as well as coagulation function that are so frequently impaired in patients with cirrhosis even without infections. In addition, about 90% of patients with a positive qSOFA meet Sepsis-3 criteria being highly suggestive of the presence of sepsis induced organ dysfunction. Such confirming the positivity of both qSOFA and Sepsis-3 in patients without a baseline SOFA score was associated with a dramatic reduction of the probability of 28-day survival. According to these concepts, a new algorithm has been proposed that is specifically devoted to the application of Sepsis-3 criteria and qSOFA in the management of hospitalized patients with cirrhosis and bacterial infection (Fig. 2.8). According to this algorithm, if a baseline SOFA score is available when the diagnosis of bacterial infection is made or suspected, the Sepsis-3 criteria can be applied. On the contrary, when a baseline SOFA score is not available both qSOFA and Sepsis-3 criteria should be applied. If both the criteria are positive, the patient should be intensively cared for, since they predict a high probability of a worse outcome, while the absence of both the criteria identify a group of patients with a very good outcome. Moreover, the only positivity of the Sepsis-3 in these patients should be interpreted with caution since it doesn't necessarily reflect the consequence of the bacterial infection on organ functions and, probably, a close monitoring of the SOFA score should be advised in the management.

Figure 2.8.



Legend: SOFA, sequential organ failure assessment; qSOFA, quick sequential organ failure assessment; ICU, intensive care unit

Moving from the clinical ground to pathophysiology, it should be highlighted that the degree of inflammation still plays a crucial role on the outcome of patients with cirrhosis and bacterial infections. This is proven by the finding that CRP was among the independent predictors of in hospital survival. Finally, it should be highlighted that the ability of CLIF-C-AD score in predicting mortality is well explained by the fact that it intercepts both the degree of inflammation, through the WBC count, and the clinical consequences of a bacterial infection, through its other variables (creatinine, serum sodium concentration and INR) in patients with cirrhosis[109].

In conclusion, our study shows for the first time that the qSOFA and the Sepsis-3 criteria are more accurate than the SIRS criteria to identify patients with cirrhosis and bacterial or fungal infections with a worse outcome. In more detail, the presence of Sepsis-3 when a baseline value of the SOFA score is available or with a simultaneous positivity of qSOFA, can be considered as

a reliable basis to define sepsis in these patients. The only positivity of Sepsis-3 criteria in patients without a baseline value of SOFA score and without a simultaneous positivity of qSOFA, should be interpreted with caution, because they do not necessarily reflect a bacterial-related organ dysfunction.

STUDY 3:

PREDICTORS OF EARLY READMISSION IN PATIENTS WITH CIRRHOSIS AFTER THE RESOLUTION OF BACTERIAL INFECTIONS

Introduction

Thirty-day and 3-month hospital readmissions occur in about 20-35% and 35-50% of patients with cirrhosis, respectively[85,86,110–116]. These rates are higher than those reported in general population in the US (20% and 34%, respectively)[117]. Hospital readmissions represent a very limiting factor for the quality of life, and, thus, a key parameter in the evaluation of the quality of care in patients with cirrhosis. The identification of the causes of early readmission is critical in reducing readmission rates and, thus, improving the quality of care. In a large, multicenter prospective study conducted in the US, 3-month readmission was found to be associated with MELD score, diabetes, and nosocomial infections[86]. Taking into account all of the other studies on the same matter, the final conclusion was quite similar, leading to a general recommendation such as “close monitoring of patients at high risk of early readmission is mandatory”. The close monitoring outpatients with cirrhosis, in a setting of a new model of specialist care, has already been proven to be successful in reducing the burden of hospital readmissions[85]. Nevertheless, the question remains: “can we take further preventive measures before discharging the patient?” Looking at the results of the previously cited US multicenter prospective study[86], nosocomial infections featured prominently among the discharge variables when readmissions due to infections, HE or renal/metabolic causes were considered. The relevance of infection as a predictor of early readmission led us to plan a prospective study to identify predictors of 30-day readmission in a specific cohort of cirrhotic patients, such as that of patients who were discharged after the resolution of a bacterial and/or fungal infection.

Methods

Patients

The study was conducted evaluating all consecutive patients with cirrhosis and ascites discharged from the General Hospital of Padova from January 2010 until June 2016 after a hospitalization for a bacterial or fungal infection. Inclusion criteria were the following: a) diagnosis of liver cirrhosis confirmed by liver biopsy or by combination of clinical, radiological and biochemical markers; b) age >18 years; c) diagnosis of bacterial infection and/or fungal infection at first hospital admission or during first hospitalization. Exclusion criteria were the following: a) acute or subacute liver failure without underlying cirrhosis; b) presence of active malignancy (except HCC within the Milan criteria) or previous malignancies with less than five years of negative follow-up; c) moderate to severe chronic heart failure (NYHA \geq 2); d) severe chronic obstructive pulmonary disease (GOLD stage \geq 2); e) severe psychiatric disorders; f) ongoing or recent immunosuppressant therapy; g) previous organ transplant; h) HIV infection; i) refusal to provide written informed consent. The protocol was approved by the local Ethics Committee and all patients provided written informed consent.

Study protocol

After identification of the bacterial infection and the signature of informed consent, the patients entered in a screening period during the hospitalization. During this period, data regarding demographic, clinical, laboratory, and microbiological data at the time of diagnosis of infection were collected, in addition to information regarding antibiotic treatment administered. Events that occurred during the hospitalization such as the development of acute kidney injury (AKI), acute-on-chronic liver failure (ACLF) or second infections were also collected. Patients were treated with antibiotics according to the available international recommendations[8,107] and local epidemiology. The decision to discharge the patient was made by the attending physicians according to clinical and laboratory data. Those patients who survived through hospitalization

entered in the study protocol and clinical and laboratory data, as well as treatment administered, were reassessed on the day of discharge. Patients were followed up regularly after being discharged for a period of at least 6 months, or until liver transplantation, or death. During the follow up, data on new hospital admissions and/or bacterial infections were collected in the study charts. In cases where patients did not attend the visits, a telephone contact was performed with the patients, family members, or the primary physicians. For the purpose of the study, all unprogrammed urgent hospital readmissions were defined as "readmission". Patients hospitalized for planned procedures (execution of radiological, endoscopic, hemodynamic procedures or programmed paracentesis) were not considered "readmissions". Early readmissions were defined as all unprogrammed, urgent hospital readmissions which occurred within 30 days from discharge. Data regarding the cause of readmission, infections during the readmission as well as microbiological and clinical data at the time of readmission were collected. For each patient, more than 200 variables among demographic and clinical data, vital signs, biochemical, microbiological and radiological tests, hepatic and/or extrahepatic organ failures and treatment administered, were evaluated at the time of diagnosis of infection, during hospitalization, at discharge, and during the follow-up period.

Diagnostic criteria and definitions

Infections were diagnosed according to the Centers for Disease Control and Prevention Criteria[89]. These criteria have been reported in detail in Study 1. Infections were classified as community acquired infections (CA), health care associated infections (HCA), and nosocomial infections as previously shown. Patients who had second infections during the hospitalization were considered as having had a nosocomial infection. Bacteria were defined as multi-drug resistant (MDR) when resistant to at least one antibiotic in 3 or more classes[91].

ACLF was defined according to the EASL-CLIF consortium criteria[5]. AKI was defined according to the International Club of Ascites criteria[74].

Statistical analysis

The primary end point of the study was the occurrence of an early hospital readmission. Patients lost to follow up immediately after discharge were excluded from the analysis. Continuous variables were reported as mean and standard deviation for those with normal distribution, and as median and IQR for those not normally distributed. Categorical variables were reported as frequency and percentage. Comparison between groups was performed using Wilcoxon-Mann-Whitney and Students' T test for normally and not normally distributed continuous variables, respectively and chi-square test or Fisher's exact test for categorical variables. Variables with $p < 0.1$ at univariate analysis between patients readmitted or not within 30 days after discharge, were included in multivariate logistic regression analysis with a stepwise backward elimination (entry $p < 0.05$; exit $p > 0.10$). Results are reported as OR with a 95% confidence interval. The survival curves were developed using the Kaplan Meier method and compared with the Log Rank test. Variables with $p < 0.1$ at univariate analysis between survivors and deaths within 6 months after discharge, were included in a multivariate Cox proportional hazards model with stepwise backward elimination (0.05 entry, exit 0.10). Results were presented as the HR with a 95% confidence interval. All tests were bilateral, and statistical significance was considered for $p < 0.05$. Data were processed using the SPSS Statistics version 23.0.

Results

Study population

Two-hundred-ten consecutive patients with cirrhosis discharged from the hospital after an episode of bacterial or fungal infection were enrolled. Eleven patients were excluded from the analysis because they were lost to follow up immediately after discharge, thus 199 patients were included in the final analysis.

Baseline characteristics of patients have been reported in Table 3.1.

Table 3.1. Characteristics of the study population

Variables	Whole population (N= 199)
Age (years) – m (SD)	61 (13)
Gender (male) – n (%)	117 (59)
Etiology – n (%)	
Alcohol	82 (41)
HCV	72 (36)
HBV	16 (8)
Other	29 (15)
Diabetes – n (%)	65 (33)
Charlson comorbidity score – m (SD)	4.4 (1.9)
Admitted in the previous 90 days – n (%)	73 (37)
Nosocomial infections – n (%)	77 (39)
Main type of infection – n (%)	
Urinary tract infections	84 (42)
Spontaneous bacterial peritonitis	36 (18)
Pneumonia	28 (14)
Skin and soft tissue infections	24 (12)
Spontaneous bacteremia	15 (8)
Other	12 (6)
SIRS during admission – n (%)	89 (45)
AKI during admission – n (%)	71 (36)
ACLF during admission – n (%)	52 (26)
Heart rate (bpm) – m (SD)*	81 (16)
Mean arterial pressure (mmHg) – m (SD)*	84 (12)
Ascites - n (%)*	182 (92)
Refractory ascites – n (%)	63 (32)
Hepatic encephalopathy – n (%)*	31 (16)
White blood cell count ($\times 10^9/L$) – M (IQR)*	5.2 (3.4 – 7.1)
C- reactive protein (mg/L) – M (IQR)*	12.6 (6.4 – 22.7)
Bilirubin ($\mu\text{mol/L}$) – M (IQR)*	41 (23 – 71)
INR – M (IQR)*	1.4 (1.2 – 1.6)
Serum creatinine ($\mu\text{mol/L}$) – M (IQR)*	79 (61 – 110)
Albumin (g/dl) – M (IQR)*	2.9 (2.5 – 3.3)
MELD score – m (SD)*	15 (5)
Child Pugh score – m (SD)	8 (7 – 10)
CLIF-c AD score – m (SD)	50 (8)
Length of hospital stay (days) – M (IQR)	15 (9 – 27)
Duration of antibiotic treatment (days) – M (IQR)	10 (7-19)
Antibiotic treatment at discharge – n (%)	67 (34)
Norfloxacin prophylaxis – n (%)*	40 (20)

Legend: n, number; m, mean; SD, standard deviation; M, median; IQR, interquartile range; SIRS, systemic inflammatory responses syndrome; AKI, acute kidney injury; ACLF, acute-on-chronic liver failure; MELD, model of end stage liver disease; CLIF-c AD, Chronic Liver Failure consortium acute decompensation score. *, variables collected at discharge

The mean age was 61±13 years and the majority were male (59%). Urinary tract infection (UTI) was the most common infection followed by SBP and pneumonia (42%, 18% and 14%, respectively). Those infections were community acquired in 75 patients (38%), health care associated in 47 (24%) and nosocomial in 77 (39%). Microbiological cultures were positive in 128 patients (64%), and 54 patients (27%) had an infection due to a MDR bacteria, mainly ESBL producing *Enterobacteriaceae* (n=14), *Enterococcus faecium* (n=14) and MRSA (4) (Table 3.2).

Table 3.2. Microbiological cultures during the hospitalization

Positive cultures*	N= 167
Blood cultures – n (%)	43 (26)
Urine cultures – n (%)	83 (49)
Ascitic fluid cultures – n (%)	16 (10)
Other – n (%)	25 (15)
Isolated strains	N= 192
Enterobacteriaceae – n (%)	80 (44)
<i>Escherichia Coli</i>	54 (29)
<i>Klebsiella pneumoniae</i>	12 (7)
<i>Proteus mirabilis</i>	4 (2)
<i>Morganella spp.</i>	3 (2)
<i>Enterobacter spp.</i>	3 (2)
Enterococci – n (%)	45 (25)
<i>Enterococcus faecalis</i>	21 (12)
<i>Enterococcus faecium</i>	23 (13)
Staphylococci – n (%)	24 (13)
<i>Staphylococcus aureus</i>	10 (5)
Streptococci – n (%)	4 (2)
Pseudomonas Aeruginosa – n (%)	3 (2)
Acinetobacter baumannii – n (%)	2 (1)
Stenotrophomonas maltophilia – n (%)	2 (1)
Candida spp. – n (%)	14 (8)
Other – n (%)	8 (4)
MDR bacteria – n %	54 (28)

Legend: n, number; MDR, multi drug resistant; * cultures were positive in 128 patients

Fungal infections were diagnosed in 14 patients. During admission, 26% of patients fulfilled ACLF criteria, 36% AKI criteria and 45% SIRS criteria. The most commonly used antibiotics were quinolones, third generation cephalosporins and other betalactams in association with betalactamases inhibitors; 61 patients (31%) were treated with 2 or more antibiotics (Table 3.3).

Table 3.3. Characteristics of antibiotic treatment during the hospitalization

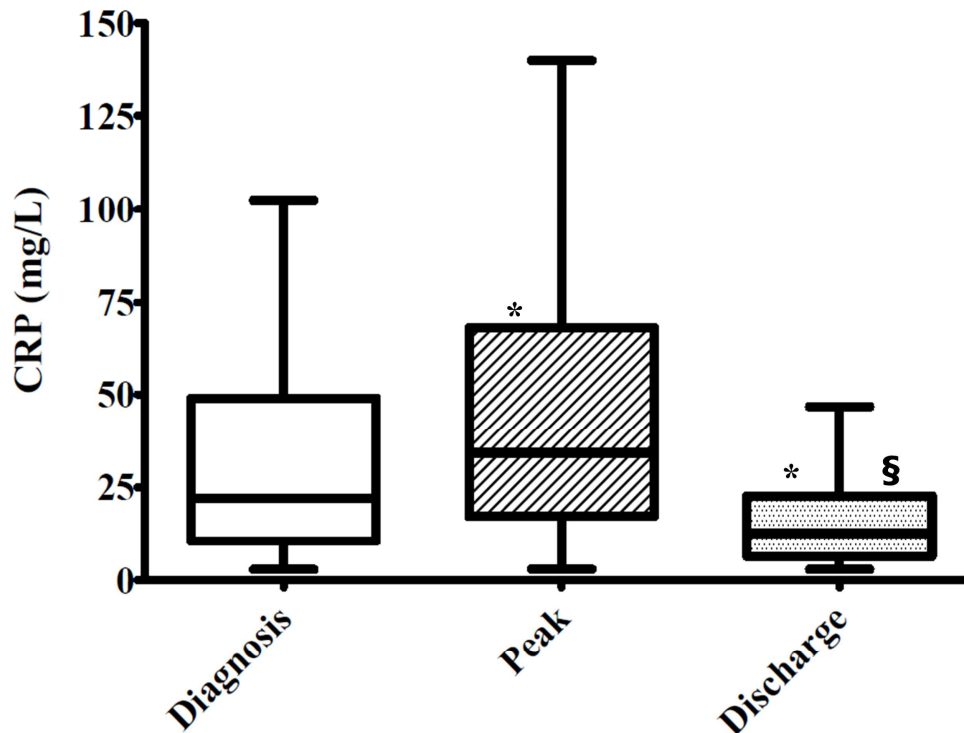
Variables	N= 199	Duration of treatment
Treatment with 2 or more antibiotics – n (%)	61 (31)	--
Time from admission/symptoms of infection to antibiotic treatment (hours) – M (IQR)	1.0 (0.5-2.0)	--
Duration of treatment – M (IQR)	10 (7-19)	--
Antibiotic class used – n (%)		
Beta-lactams plus β -lactamases inhibitors	72 (36)	7 (6 – 10)
3 rd generation cephalosporins	73 (37)	8 (6 – 10)
Quinolones	88 (44)	7 (5 – 9)
Glycopeptides	42 (21)	8 (6 – 10)
Carbapenems	66 (33)	8 (6 – 10)
Lipopeptides	22 (11)	8 (7 – 14)
Tigecycline	10 (5)	10 (9 – 14)
Linezolid	7 (4)	8 (5 – 10)
Aminoglycosides	5 (3)	8 (5 – 12)
Tetracyclines	1 (1)	7 (- -)
Antifungal treatment – n (%)*	24 (12)	9 (6 – 13)
Antibiotic treatment at discharge – n (%)	67 (34)	--

Legend: n, number; IQR, interquartile range; * antifungal agents used were fluconazole and caspofungin.

The median hospital stay was 15 days (IQR= 9-27 days), the patients were treated with antibiotics for a median of 10 days (IQR 7-19) and one third of them continued antibiotic treatment at home. Furthermore, 40 patients (20%) were discharged on quinolones prophylaxis. Almost all patients had ascites (92%) of which 32% had refractory ascites, and the mean MELD score at discharge was 15±5. Among other potential risk factors for frailty and early readmissions, 73 patients (37%) had experienced another hospitalization within 30 days before the index admission; two thirds of them had a comorbidity other than liver cirrhosis, mainly diabetes and chronic kidney disease (CKD), and 8% were discharged in a nursing home facility.

CRP levels significantly increased during the acute phase of bacterial infection (22 vs 34 mg/l; $p<0.001$) and significantly decreased at discharge (12.6 mg/L; $p<0.001$ vs both CRP levels at diagnosis and peak; Figure 3.1).

Figure 3.1. C-reactive protein levels at the diagnosis, during the acute phase of bacterial infection and at discharge.



Legend: CRP, C-reactive protein; * $p<0.001$ vs CRP at diagnosis; §, $p<0.001$ vs CRP at peak

Patients with severe sepsis and/or septic shock ($n=52$) had significantly higher values of CRP both at the diagnosis of infection and at peak than those without (43 vs 18 mg/dl and 65 vs 28 mg/dl, respectively; both $p<0.001$). However, levels of CRP at discharge were not significantly different between the 2 groups (13 vs 12 mg/dl; $p=0.119$). We did not find any correlation between the CRP levels at discharge and scores of liver disease.

Early readmissions

Sixty-nine patients (35%) were readmitted to the hospital within 30 days from discharge. The probability of readmission at 1, 3, and 12-months was 35%, 67%, and 74%, respectively.

Table 3.4 shows a comparison between patients readmitted or not within 30 days from discharge.

Table 3.4. Characteristics of patients readmitted or not within 30 days from discharge.

Variables	Readmitted (N=69)	Non-readmitted (N=130)	P
Age (years) – m (SD)	62 (14)	61 (13)	0.727
Gender (male) – n (%)	39 (57)	78 (60)	0.747
Etiology – n (%)			
Alcohol	24 (35)	58 (45)	0.517
HCV	26 (38)	46 (35)	
HBV	7 (10)	9 (7)	
Other	12 (17)	17 (13)	
Diabetes – n (%)	25 (36)	40 (31)	0.533
Charlson comorbidity score – m (SD)	4.4 (1.6)	4.5 (2.1)	0.775
Admitted in the previous 90 days – n (%)	33 (48)	40 (31)	0.026
Nosocomial infections – n (%)	34 (49)	43 (33)	0.037
Main type of infection – n (%)			
Urinary tract infections	27 (39)	57 (44)	0.179
Spontaneous bacterial peritonitis	17 (25)	19 (15)	
Pneumonia	7 (10)	21 (16)	
Skin and soft tissue infections	7 (10)	17 (13)	
Spontaneous bacteremia	4 (6)	11 (9)	
Other	7 (10)	5 (4)	
SIRS during admission – n (%)	31 (45)	58 (45)	
AKI during admission – n (%)	31 (45)	38 (29)	0.040
ACLF during admission – n (%)	27 (39)	25 (19)	0.004
Mean arterial pressure (mmHg) – m (SD)*	85 (11)	84 (12)	0.472
Ascites - n (%)*	68 (99)	114 (88)	0.014
Refractory ascites – n (%)	24 (35)	39 (30)	0.596
Hepatic encephalopathy – n (%)*	13 (19)	18 (14)	0.471
White blood cell count ($\times 10^9/L$) – M (IQR)*	5.9 (3.5 – 7.3)	4.8 (3.4 – 6.9)	0.172
C- reactive protein (mg/L) – M (IQR)*	16 (9 – 31)	10 (5 – 21)	0.009
Bilirubin ($\mu\text{mol/L}$) – M (IQR)*	45 (28 – 71)	39 (21 – 71)	0.144
INR – M (IQR)*	1.4 (1.2 – 1.6)	1.4 (1.2 – 1.6)	0.954
Serum creatinine ($\mu\text{mol/L}$) – M (IQR)*	87 (67 – 114)	75 (60 – 105)	0.021
Albumin (g/dl) – M (IQR)*	3.0 (2.7-3.5)	2.9 (2.5-3.2)	0.420
MELD score – m (SD)*	16 (5)	15 (6)	0.114
Child Pugh score – m (SD)	9 (8 – 10)	8 (7 – 10)	0.043
CLIF-c AD score – m (SD)	52 (8)	49 (8)	0.049
Length of hospital stay (days) – M (IQR)	16 (9 – 27)	15 (9 – 27)	0.490
Duration of antibiotic treatment (days) – M (IQR)	10 (7 – 20)	11 (7 – 18)	0.984
Antibiotic treatment at discharge – n (%)	20 (29)	47 (36)	0.406
Norfloxacin prophylaxis – n (%)*	15 (22)	25 (19)	0.815

Legend: n, number; m, mean; SD, standard deviation; M, median; IQR, interquartile range; SIRS, systemic inflammatory responses syndrome; AKI, acute kidney injury; ACLF, acute-on-chronic liver failure; CLIF-c AD, Chronic Liver Failure consortium acute decompensation score. *, variables collected at discharge

Patients that were readmitted early had a higher rate of ACLF (39 vs 19%; $p= 0.004$), AKI (45 vs 29%; $p= 0.040$), and nosocomial infections (49 vs 33%; $p=0.037$) during the first hospitalization than those who were not readmitted. In addition, patients readmitted early had more frequently experienced other hospitalizations in the 30 days before the index hospitalization (48 vs 31%; $p=0.026$). Patients readmitted within 30 days more frequently had ascites (99 vs 88%; $p= 0.014$) and higher levels of serum creatinine (87 vs 75 $\mu\text{mol/L}$; $p=0.021$) and CRP (16 vs 10 mg/L ; $p=0.009$) at discharge than those not readmitted. No difference was found between patients readmitted versus those not readmitted in terms of age, sex, comorbidity, site of infection, duration of antibiotic treatment, and/or of hospital stay. The CLIF-c AD score and CTP score were significantly higher in those readmitted than in those not readmitted. In a multivariate analysis, CRP (OR= 1.91; $p=0.022$), ACLF during admission (OR=2.48; $p=0.008$) and a previous hospitalization in the 30 days before the index admission (OR=1.50; $p=0.042$) were found to be independent predictors of early readmission (Table 3.5).

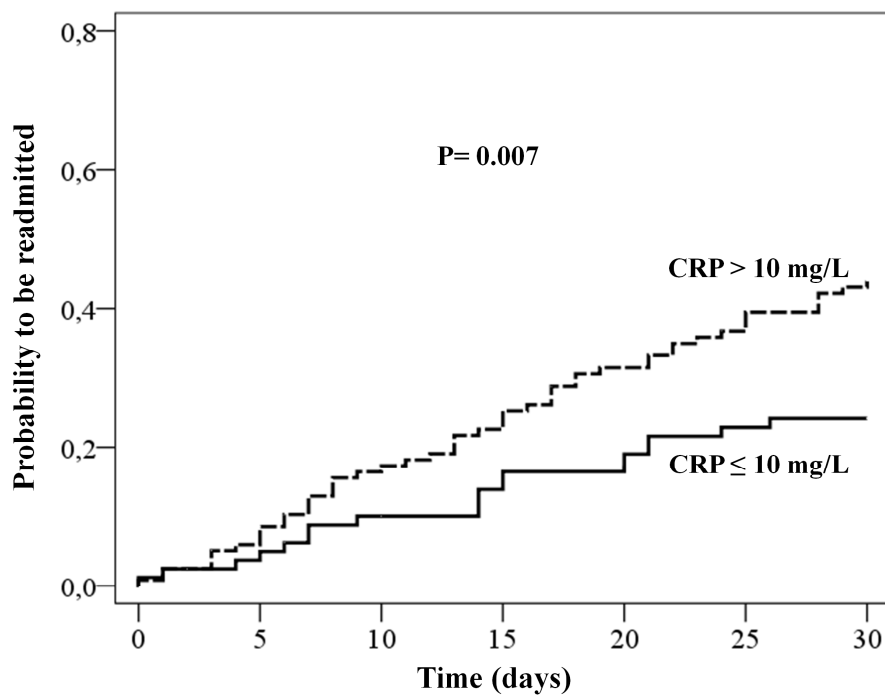
Table 3.5. Independent predictors of readmission within 30 days.

Variables	OR	95% CI	P
ACLF	2.48	1.27 – 4.85	0.008
Admission in the previous 30 days	1.50	1.06 – 2.12	0.042
CRP at discharge (mg/L)	1.91	1.03 – 3.57	0.022

Legend: OR, odds ratio; CI, confidence interval; ACLF, acute-on-chronic liver failure; CRP, C-reactive protein.

Patients with a $\text{CRP} > 10 \text{ mg/l}$ had a significantly higher probability of being readmitted within 30 days than those with a $\text{CRP} \leq 10 \text{ mg/l}$ (44 vs 24%; $p=0.007$; Figure 3.2).

Figure 3.2. Probability to be readmitted within 30 days from discharge according to plasma level of C-reactive protein at discharge.



Patients at risk

CRP > 10 mg/L	118	105	94	85	77	67	62
CRP ≤ 10 mg/L	81	76	70	65	63	60	59

Legend: CRP, C-reactive protein

Forty-seven out of 69 patients (68%) were readmitted early due to an infection. CRP was the only independent predictor of early readmission for an infection (OR=1.76; p=0.004). Among patients readmitted early for an infection, 25 (52%) were readmitted within 14 days, of which 13 had the same infection that had occurred during the first hospitalization. Of the 11 patients readmitted within 14 days with positive cultures in both the admissions, the same strain isolated during the first admission was found in 8 patients(72%). CRP at discharge was higher in patients readmitted within 14 days with the same infection than in those readmitted for a different infection although the difference was not significant, probably due to the sample size (28 vs 17 mg/L; p= N.S.).

Analysis of survival

During the first six months of follow up, 47 patients (23%) died, 19 (10%) were transplanted, 2 (1%) were lost to follow up and 131 (66%) were alive.

A comparison of patients who died versus those who survived at 6 months has been reported in Table 3.6.

Table 3.6. Comparison of characteristics of patients that died versus patients that survived at 6 months.

Variables	Survivors (N=131)	Non-survivors (N=47)	P
Age (years) – m (SD)	61 (12)	67 (13)	0.009
Gender (male) – n (%)	79 (60)	24 (51)	0.353
Etiology – n (%)			
Alcohol	58 (35)	18 (38)	0.489
HCV	46 (44)	16 (34)	
HBV	11 (8)	3 (6)	
Other	16 (12)	10 (21)	
Diabetes – n (%)	37 (28)	23 (49)	0.017
Charlson comorbidity score – m (SD)	4.2 (1.9)	5.3 (2.0)	0.001
Admitted in the previous 90 days – n (%)	44 (34)	18 (38)	0.687
Nosocomial infections – n (%)	34 (49)	43 (33)	0.410
Spontaneous bacterial peritonitis – n (%)	17 (13)	10 (21)	0.261
SIRS during admission – n (%)	56 (43)	25 (53)	0.288
AKI during admission – n (%)	37 (28)	22 (47)	0.032
ACLF during admission – n (%)	28 (21)	17 (36)	0.071
Mean arterial pressure (mmHg) – m (SD)*	85 (12)	83 (9)	0.146
Ascites - n (%)*	116 (88)	46 (98)	0.073
Refractory ascites – n (%)	31 (24)	23 (49)	0.002
Hepatic encephalopathy – n (%)*	21 (16)	7 (15)	1.00
White blood cell count ($\times 10^9/L$) – M (IQR)*	5.0 (3.3 – 6.9)	5.7 (3.8 – 7.8)	0.127
C-reactive protein (mg/L) – M (IQR)*	10 (5 – 19)	18 (13 – 32)	<0.001
Bilirubin ($\mu\text{mol/L}$) – M (IQR)*	34 (21 – 61)	49 (30 – 79)	0.020
INR – M (IQR)*	1.4 (1.2 – 1.5)	1.5 (1.3 – 1.7)	0.006
Serum creatinine ($\mu\text{mol/L}$) – M (IQR)*	76 (60 – 104)	106 (68 – 129)	0.002
Albumin (g/dl) – M (IQR)*	2.9 (2.5-3.3)	3.0 (2.5-3.5)	0.297
MELD score – m (SD)*	14 (4)	18 (6)	<0.001
Child Pugh score – m (SD)	8 (7 – 9)	9 (8 – 10)	0.001
CLIF-c AD score – m (SD)	49 (8)	55 (8)	<0.001
Length of hospital stay (days) – M (IQR)	14 (9 – 26)	16 (8 – 26)	0.971
Duration of antibiotic treatment (days) – M (IQR)	10 (7 – 18)	10 (7 – 19)	0.614
Antibiotic treatment at discharge – n (%)	37 (29)	22 (47)	0.038
Norfloxacin prophylaxis – n (%)*	27 (21)	6 (13)	0.333
Readmission within 30 days – n (%)*	36 (28)	23 (49)	0.012

Legend: n, number; m, mean; SD, standard deviation; M, median; IQR, interquartile range; SIRS, systemic inflammatory responses syndrome; AKI, acute kidney injury; ALCF, acute-on-chronic liver failure; MELD, model of end stage liver disease; CLIF-c AD, Chronic Liver Failure consortium acute decompensation score. *, variables collected at discharge; #, Patients transplanted (19) or lost to follow up (2) were excluded from this analysis

Non-survivors were older, had a higher rate of comorbidity such as diabetes and more advanced liver and renal dysfunction than survivors at 6 months. Refractory ascites was significantly more common among patients who died than in those who survived at 6 months (49 vs 24%; $p=0.002$). As expected, the CTP, MELD, and CLIF-c AD scores were significantly higher in patients that died than in those that survived. CRP levels at discharge were significantly higher in patients that died than in those that survived at 6 months (18 vs 10 mg/L; $p<0.001$). Finally, a readmission within 30 days from discharge was significantly more common in non-survivors than in survivors (49 vs 28 %; $p=0.012$).

In multivariate analysis, age (HR=1.05; $p=0.001$), MELD score at discharge (HR=1.13; $p<0.001$), CRP (HR=1.85; $p=0.001$), refractory ascites (HR=2.22; $p=0.007$), and diabetes (HR=2.41; $p=0.010$) were found to be independent predictors of mortality at 6 months (Table 3.7).

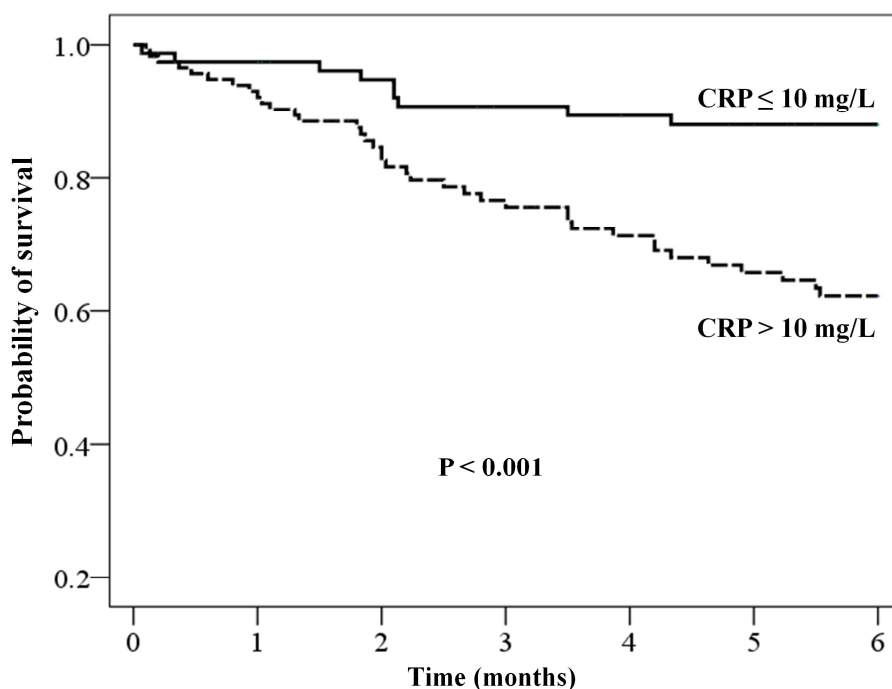
Table 3.7. Independent predictors of 6-month mortality

Variables	HR	95% CI	P
Age	1.05	1.03 – 1.07	0.001
MELD score at discharge	1.13	1.07 – 1.18	<0.001
CRP at discharge (mg/L)	1.85	1.29 – 2.65	0.001
Refractory ascites	2.22	1.24 – 3.97	0.007
Diabetes	2.14	1.20 – 3.82	0.010

Legend: HR, hazard ratio; CI, confidence interval; MELD, model of end stage liver disease; CRP, C-reactive protein.

Patients with a CRP>10 mg/L had a significantly lower probability of survival at 6 months than those with a CRP≤10 mg/L (62 vs 88%; $p<0.001$; Figure 3.3).

Figure 3. Six-months probability of survival according to plasma level of C-reactive protein at discharge.



Patients at risk								
	0	1	2	3	4	5	6	
CRP > 10 mg/L	118	101	84	73	65	58	53	
CRP ≤ 10 mg/L	81	75	71	66	64	61	58	

Legend: CRP, C-reactive protein

The classification of patients according to the presence of diabetes and CRP with a cut-off of 10 mg/dl allowed for the identification of groups of patients with a stepwise decrease in survival rate. In detail, patients with both DM and CRP>10 mg/dl had the lowest survival rate at 6 months (53%) followed by those without DM and a CRP>10 mg/dl (68%), those with DM with a CRP≤10 mg/l (75%), while patients without DM and a CRP≤10 mg/l had the best survival rate (93%; $p<0.001$).

Discussion

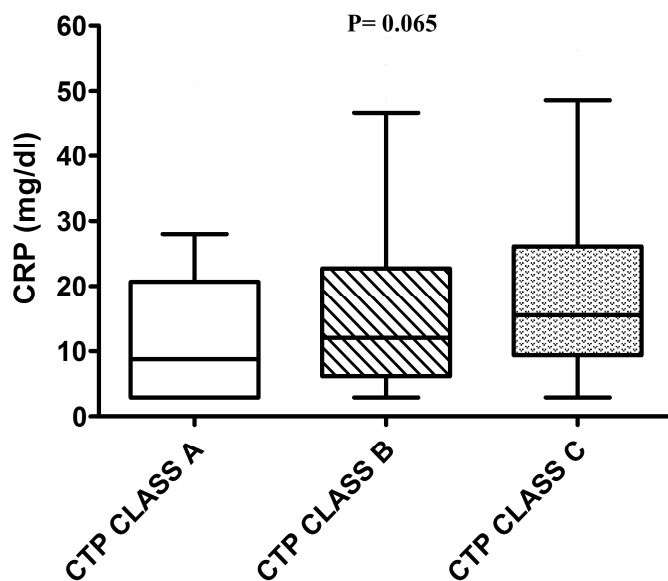
The main and most original finding of the study is the strong power of CRP in predicting both the 30-day hospital readmission and the 6-month mortality in patients with cirrhosis discharged after a bacterial or fungal infection. The finding that high baseline CRP levels could remain

elevated over time despite the resolution of a bacterial infection in many patients with cirrhosis is not completely new, since it has been already described by Cervoni JP et al.[118]. The new finding is the potency of a persistently increased CRP as a predictor of 30-day readmission. Remarkably, it was stronger than all the other demographic or clinical parameters when infection was considered the only cause of readmission. These findings lead to different potential interpretations. From a pathophysiological ground it is well known that CRP is a marker of inflammation and an inflammatory state is the main cause of organ failures and thus of ACLF in patients with cirrhosis. However, we need to address why a group of cirrhotic patients discharged after the resolution of a bacterial or fungal infection maintains high CRP levels.

The first hypothesis is that patients who had higher levels of CRP at discharge probably had higher levels of pathological bacterial translocation. This interpretation is indirectly supported by the evidence that patients who were readmitted early to the hospital had a more severe cirrhosis as proven by the higher values of CTP and CLIF-c-AD scores at discharge from the index hospitalization. However, we did not find a correlation between the levels of CRP and the severity of liver disease (MELD score and CTP score). There was a nonsignificant trend toward higher levels of CRP in patients with CTP class C (Figure 3.4), a finding already highlighted by Papp M et al in noninfected cirrhotic patients[57].

The second hypothesis is that patients readmitted had a more severe inflammatory response to the infection. However, the higher levels of CRP in patients who were readmitted within 30 days cannot be accounted for a more severe degree of inflammation at the time of infection, since the peak value of CRP during the infection was similar to that observed in patients who were not readmitted.

Figure 3.4. C-reactive protein levels according to Child-Turcotte-Pugh class.



Legend: CRP, C-reactive protein; CTP, Child-Turcotte-Pugh

Thus, considering all the data, the most likely explanation is that the higher levels of CRP at discharge in patients who were then re-admitted could be due to a non-complete resolution of the infection. Accordingly, 52% of 25 patients who were readmitted for a bacterial or fungal infection within 14 days had the same site of infection, and in 8 patients among 11 with positive cultures in both admissions, the same organism has been isolated (72%). The threshold of 14 days, although arbitrary, was not chosen randomly, since it represents the verge between a relapse of infection and a re-infection[119]. Other supporting data are the following: a) the WBC count was higher at the time of discharge from the index hospitalization, b) the infection was more frequently “difficult to treat” since it was more frequently both nosocomial and/or complicated by AKI and ACLF. This interpretation makes it possible to introduce the concept that CRP or other biomarkers could be used to guide the antibiotic stewardship strategies, to improve the outcomes in patients with cirrhosis. This is a new concept for hepatologists, while in other fields of medicine, such as in patients with acute respiratory infections, there is evidence that it may be beneficial[120]. Clinical signs such as skin temperature, as well as routine laboratory tests, such as WBC count, are probably unreliable in defining the resolution of a

bacterial or fungal infection in patients with cirrhosis. In fact, a normal or quite close to normal body temperature as well as WBC count within the normal range, cannot exclude the persistence of infection. Thus, a CRP value of 10 mg/dl could be used as a target to drive antibiotic treatment in patients with cirrhosis and bacterial/fungal infections. Although the duration of antibiotic treatment was not standardized in our study, some data may support the concept of a CRP driven stewardship of antibiotic treatment. In fact, we found a significant positive correlation between the duration of antibiotic treatment and the delta reduction of CRP (peak value – discharge value; $r=0.310$; $p<0.001$). However, the comparison between CRP-based algorithms with non-standardized routine care in cirrhotic patients with “difficult to treat” bacterial or fungal infections should be performed in a controlled clinical trial, with major end-points such as 28-day and 3-month mortality rates and 30-day and 3-month hospital readmission rates.

CRP was also proven to be a strong predictor of a 6-month mortality rate in patients with cirrhosis who recovered after a bacterial or fungal infection. The relationship between the persistence of an increased level of CRP and the probability of death in patients with cirrhosis has already been shown by Cervoni JP et al.[118]. However, they considered a very high threshold of CRP, quite close to the median peak value which was observed in the present study, and three times higher than the threshold of 10 mg/l at the resolution of the infection that we have identified. Nevertheless, looking at the liver related causes of death in their study, one may realize that the most common cause was sepsis (44.4%) as it was found in our series (64%).

In our cohort, diabetes was found to be an independent predictor of 6-month mortality. This finding is not a new concept. In fact, diabetes has been shown to be a risk factor of complications and mortality in patients with cirrhosis[121]. Interestingly, even in the group of patients with diabetes, most of the causes of death were liver-related rather than non-liver-related. The reasons of the association of diabetes with complications of cirrhosis is not completely understood, however it probably involves changes in microcirculation (microangiopathy), oxidative stress and immune dysfunction[122]. In conclusion, CRP or other biomarkers of systemic inflammation

could be applied to antibiotic stewardship strategies in order to improve the outcomes in patients with cirrhosis who develop a bacterial or fungal infection particularly if it's difficult to be treated because of it is either nosocomial or associated with ACLF. The efficacy of this strategy when combined with close monitoring of the frailest patients with cirrhosis in lowering the rate of early hospital readmissions should be tested in clinical trials.

CONCLUSIONS

In conclusion, the studies performed in this thesis allowed us to improve the knowledge about bacterial infections in patients with cirrhosis. The epidemiology and the spectrum of resistance in different countries were partially clarified. The results also showed that the antibiotic treatment suggested by the current recommendation has limited efficacy in certain countries and that different strategies should be developed in patients with pneumonia and skin and soft tissues infections. MDR and particularly XDR bacteria are a big concern and although new molecules are currently available for certain strains, their use should be limited to avoid the development of further resistance. The results of Study 2 also showed that the new criteria for sepsis, namely Sepsis-3 criteria are accurate in defining the severity of infection and thus “sepsis”. These criteria may be used to make decisions about the management of patients with cirrhosis and bacterial infections both in terms of intensity of treatment and in planning strategies of intervention. The drawback of not having a baseline SOFA score has been overcome by the combination of Sepsis-3 and qSOFA and an algorithm for the use of Sepsis-3 criteria has been proposed. Finally, C-reactive protein, appear a useful asset to guide the duration of treatment and to predict worse outcomes in patients with cirrhosis.

REFERENCES

- [1] Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014;383:1749–61.
- [2] Ratib S, West J, Crooks CJ, Fleming KM. Diagnosis of Liver Cirrhosis in England, a Cohort Study, 1998-2009: A Comparison With Cancer. *Am J Gastroenterol* 2014;109:190–8.
- [3] Blachier M, Leleu H, Peck-Radosavljevic M, Valla D-C, Roudot-Thoraval F. The burden of liver disease in Europe: A review of available epidemiological data. *J Hepatol* 2013;58:593–608.
- [4] D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: A systematic review of 118 studies. *J Hepatol* 2006;44:217–31.
- [5] Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013;144:1426–1437.e9.
- [6] Piano S, Tonon M, Vettore E, Stanco M, Pilutti C, Romano A, et al. Incidence, predictors and outcomes of acute-on-chronic liver failure in outpatients with cirrhosis. *J Hepatol* 2017. doi:10.1016/j.jhep.2017.07.00.
- [7] Fernández J, Acevedo J, Weist R, Gustot T, Amoros A, Deulofeu C, et al. Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. *Gut* 2017. doi:10.1136/gutjnl-2017-314240.
- [8] Jalan R, Fernandez J, Wiest R, Schnabl B, Moreau R, Angeli P, et al. Bacterial infections in cirrhosis: A position statement based on the EASL Special Conference 2013. *J Hepatol* 2014;60:1310–24.
- [9] Foreman MG, Mannino DM, Moss M. Cirrhosis as a risk factor for sepsis and death: Analysis of the National Hospital Discharge Survey. *Chest* 2003;124:1016–20.
- [10] Fasolato S, Angeli P, Dallagnese L, Maresio G, Zola E, Mazza E, et al. Renal failure and

bacterial infections in patients with cirrhosis: Epidemiology and clinical features. *Hepatology* 2007;45:223–9.

- [11] Fernández J, Acevedo J, Castro M, Garcia O, Rodríguez de Lope C, Roca D, et al. Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: A prospective study. *Hepatology* 2012;55:1551–61.
- [12] Bajaj JS, O’Leary JG, Reddy KR, Wong F, Olson JC, Subramanian RM, et al. Second infections independently increase mortality in hospitalized patients With cirrhosis: The north American Consortium for the study of end-stage liver disease (NACSELD) experience. *Hepatology* 2012;56:2328–35.
- [13] Fernández J, Gustot T. Management of bacterial infections in cirrhosis. *J Hepatol* 2012;56:1–12.
- [14] Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014;60:197–209.
- [15] Albillos A, Lario M, Álvarez-Mon M. Cirrhosis-associated immune dysfunction: Distinctive features and clinical relevance. *J Hepatol* 2014;61:1385–96.
- [16] Appenrodt B, Grünhage F, Gentemann MG, Thyssen L, Sauerbruch T, Lammert F. Nucleotide-binding oligomerization domain containing 2 (NOD2) variants are genetic risk factors for death and spontaneous bacterial peritonitis in liver cirrhosis. *Hepatology* 2010;51:1327–33.
- [17] Nischalke HD, Berger C, Aldenhoff K, Thyssen L, Gentemann M, Grünhage F, et al. Toll-like receptor (TLR) 2 promoter and intron 2 polymorphisms are associated with increased risk for spontaneous bacterial peritonitis in liver cirrhosis. *J Hepatol* 2011;55:1010–6.
- [18] Bauer TM, Steinbruckner B, Brinkmann FE, Ditzen AK, Schwacha H, Aponte JJ, et al. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence and relation with spontaneous bacterial peritonitis. *Am J Gastroenterol* 2001;96:2962–7.
- [19] Chang C-S, Chen G-H, Lien H-C, Yeh H-Z. Small intestine dysmotility and bacterial

- overgrowth in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 1998;28:1187–90.
- [20] Lloyd-Price J, Mahurkar A, Rahnavard G, Crabtree J, Orvis J, Hall AB, et al. Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature* 2017;550:61–6.
- [21] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the Human Intestinal Microbial Flora. *Science* (80-) 2005;308:1635–8.
- [22] Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011;54:562–72.
- [23] Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014;513:59–64.
- [24] Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014;60:940–7.
- [25] Steffen E, Berg R, Deitch E. Comparison of translocation rates of various indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node. *J Infect Dis* 1988;157:1032–8.
- [26] Ariza X, Castellote J, Lora-Tamayo J, Girbau A, Salord S, Rota R, et al. Risk factors for resistance to ceftriaxone and its impact on mortality in community, healthcare and nosocomial spontaneous bacterial peritonitis. *J Hepatol* 2012;56:825–32.
- [27] Scarpellini E, Valenza V, Gabrielli M, Lauritano EC, Perotti G, Merra G, et al. Intestinal Permeability in Cirrhotic Patients With and Without Spontaneous Bacterial Peritonitis: Is the Ring Closed[quest]. *Am J Gastroenterol* 2009;105:323–7.
- [28] Norman DA, Atkins JM, Seelig LLJ, Gomez-Sanchez C, Krejs GJ. Water and electrolyte movement and mucosal morphology in the jejunum of patients with portal hypertension. *Gastroenterology* 1980;79:707–15.

- [29] Ramachandran A, Prabhu R, Thomas S, Reddy JB, Pulimood A, Balasubramanian KA. Intestinal mucosal alterations in experimental cirrhosis in the rat: Role of oxygen free radicals. *Hepatology* 2002;35:622–9.
- [30] Song H-L, Lv S, Liu P. The roles of tumor necrosis factor-alpha in colon tight junction protein expression and intestinal mucosa structure in a mouse model of acute liver failure. *BMC Gastroenterol* 2009;9:70.
- [31] Francés R, Chiva M, Sánchez E, González-Navajas JM, Llovet T, Zapater P, et al. Bacterial translocation is downregulated by anti-TNF-alpha; monoclonal antibody administration in rats with cirrhosis and ascites. *J Hepatol* 2007;46:797–803.
- [32] Worliceck M, Knebel K, Linde HJ, Moleda L, Schölmerich J, Straub RH, et al. Splanchnic sympathectomy prevents translocation and spreading of *E coli* but not *S aureus* in liver cirrhosis. *Gut* 2010;59:1127–34.
- [33] Senzolo M, Cholongitas E, Burra P, Leandro G, Thalheimer U, Patch D, et al. β -Blockers protect against spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. *Liver Int* 2009;29:1189–93.
- [34] Such J, Guarner C, Enriquez J, Rodriguez JL, Seres I, Vilardell F. Low C3 in cirrhotic ascites predisposes to spontaneous bacterial peritonitis. *J Hepatol* 1988;6:80–4.
- [35] Bihari C, Anand L, Rooge S, Kumar D, Saxena P, Shubham S, et al. Bone marrow stem cells and their niche components are adversely affected in advanced cirrhosis of the liver. *Hepatology* 2016;64:1273–88.
- [36] Rajkovic IA, Williams R. Abnormalities of neutrophil phagocytosis, intracellular killing and metabolic activity in alcoholic cirrhosis and hepatitis. *Hepatology* 1986;6:252–62.
- [37] Tritto G, Bechlis Z, Stadlbauer V, Davies N, Francés R, Shah N, et al. Evidence of neutrophil functional defect despite inflammation in stable cirrhosis. *J Hepatol* 2017;55:574–81.
- [38] Rolas L, Makhezer N, Hadjoudj S, El-Benna J, Djerdjouri B, Elkrief L, et al. Inhibition of

- mammalian target of rapamycin aggravates the respiratory burst defect of neutrophils from decompensated patients with cirrhosis. *Hepatology* 2013;57:1163–71.
- [39] Riordan SM, Skinner N, Nagree A, McCallum H, McIver CJ, Kurtovic J, et al. Peripheral blood mononuclear cell expression of toll-like receptors and relation to cytokine levels in cirrhosis. *Hepatology* 2003;37:1154–64.
- [40] Genescà J, Martí R, Rojo F, Campos F, Peribáñez V, González A, et al. Increased tumour necrosis factor α production in mesenteric lymph nodes of cirrhotic patients with ascites. *Gut* 2003;52:1054–9.
- [41] Gomez F, Ruiz P, Schreiber AD. Impaired Function of Macrophage Fc γ Receptors and Bacterial Infection in Alcoholic Cirrhosis. *N Engl J Med* 1994;331:1122–8.
- [42] O'Brien AJ, Fullerton JN, Massey KA, Auld G, Sewell G, James S, et al. Immunosuppression in acutely decompensated cirrhosis is mediated by prostaglandin E2. *Nat Med* 2014;20:518–23.
- [43] Bernsmeier C, Pop OT, Singanayagam A, Triantafyllou E, Patel VC, Weston CJ, et al. Patients with acute-on-chronic liver failure have increased numbers of regulatory immune cells expressing the receptor tyrosine kinase MERTK. *Gastroenterology* 2015;148:603–615.e14.
- [44] Bernsmeier C, Triantafyllou E, Brenig R, Lebosse FJ, Singanayagam A, Patel VC, et al. CD14+CD15-HLA-DR- myeloid-derived suppressor cells impair antimicrobial responses in patients with acute-on-chronic liver failure. *Gut* 2017.
- [45] Doi H, Iyer TK, Carpenter E, Li H, Chang K-M, Vonderheide RH, et al. Dysfunctional B-cell activation in cirrhosis resulting from hepatitis C infection associated with disappearance of CD27-Positive B-cell population. *Hepatology* 2012;55:709–19.
- [46] Lario M, Muñoz L, Ubeda M, Borrero M-J, Martínez J, Monserrat J, et al. Defective thymopoiesis and poor peripheral homeostatic replenishment of T-helper cells cause T-cell lymphopenia in cirrhosis. *J Hepatol* 2013;59:723–30.

- [47] Clària J, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, et al. Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. *Hepatology* 2016;64:1249–64.
- [48] Medzhitov R, Schneider DS, Soares MP. Disease Tolerance as a Defense Strategy. *Science* (80-) 2012;335:936–41.
- [49] Bernardi M, Moreau R, Angeli P, Schnabl B, Arroyo V. Mechanisms of decompensation and organ failure in cirrhosis: From peripheral arterial vasodilation to systemic inflammation hypothesis. *J Hepatol* 2015;63:1272–84.
- [50] Terra C, Guevara M, Torre A, Gilabert R, Fernández J, Martín-Llahí M, et al. Renal failure in patients with cirrhosis and sepsis unrelated to spontaneous bacterial peritonitis: Value of MELD score. *Gastroenterology* 2005;129:1944–53.
- [51] Huelin P, Piano S, Solà E, Stanco M, Solé C, Moreira R, et al. Validation of a Staging System for Acute Kidney Injury in Patients With Cirrhosis and Association With Acute on Chronic Liver Failure. *Clin Gastroenterol Hepatol* 2017;15:438–45.
- [52] Merli M, Lucidi C, Pentassuglio I, Giannelli V, Giusto M, Di Gregorio V, et al. Increased risk of cognitive impairment in cirrhotic patients with bacterial infections. *J Hepatol* 2013;59:243–50.
- [53] Arvaniti V, D’Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010;139:1246–1256.e5.
- [54] Cazzaniga M, Dionigi E, Gobbo G, Fioretti A, Monti V, Salerno F. The systemic inflammatory response syndrome in cirrhotic patients: Relationship with their in-hospital outcome. *J Hepatol* 2009;51:475–82.
- [55] Gabay C, Kushner I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. *N Engl J Med* 1999;340:448–54.
- [56] Schuetz P, Albrich W, Mueller B. Procalcitonin for diagnosis of infection and guide to

- antibiotic decisions: past, present and future. *BMC Med* 2011;9:107.
- [57] Papp M, Vitalis Z, Altorjay I, Tornai I, Udvardy M, Harsfalvi J, et al. Acute phase proteins in the diagnosis and prediction of cirrhosis associated bacterial infections. *Liver Int* 2012;32:603–11.
- [58] Bota DP, Van Nuffelen M, Zakariah AN, Vincent J-L. Serum levels of C-reactive protein and procalcitonin in critically ill patients with cirrhosis of the liver. *J Lab Clin Med* 2005;146:347–51.
- [59] Tsiakalos A, Karatzaferis A, Ziakas P, Hatzis G. Acute-phase proteins as indicators of bacterial infection in patients with cirrhosis. *Liver Int* 2009;29:1538–42.
- [60] Rimola A, García-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, et al. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. *J Hepatol* 2000;32.
- [61] Soriano G, Castellote J, Álvarez C, Girbau A, Gordillo J, Baliellas C, et al. Secondary bacterial peritonitis in cirrhosis: A retrospective study of clinical and analytical characteristics, diagnosis and management. *J Hepatol* 2010;52:39–44.
- [62] Akriviadis E, Runyon B. Utility of an algorithm in differentiating spontaneous from secondary bacterial peritonitis. *Gastroenterology* 1990;98:127–33.
- [63] Fernández J, Navasa M, Gómez J, Colmenero J, Vila J, Arroyo V, et al. Bacterial infections in cirrhosis: Epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002;35:140–8.
- [64] Merli M, Lucidi C, Giannelli V, Giusto M, Riggio O, Falcone M, et al. Cirrhotic patients are at risk for health care-associated bacterial infections. *Clin Gastroenterol Hepatol* 2010;8:979–985.e1.
- [65] Tandon P, DeLisle A, Topal JE, Garcia-Tsao G. High Prevalence of Antibiotic-Resistant Bacterial Infections Among Patients With Cirrhosis at a US Liver Center. *Clin Gastroenterol Hepatol* 2012;10:1291–8.

- [66] Cheong HS, Kang C, Lee JA, Moon SY, Joung MK, Chung DR, et al. Clinical Significance and Outcome of Nosocomial Acquisition of Spontaneous Bacterial Peritonitis in Patients with Liver Cirrhosis. *Clin Infect Dis* 2009;48:1230–6.
- [67] Piano S, Fasolato S, Salinas F, Romano A, Tonon M, Morando F, et al. The empirical antibiotic treatment of nosocomial spontaneous bacterial peritonitis: Results of a randomized, controlled clinical trial. *Hepatology* 2016;63:1299–309.
- [68] Piano S, Romano A, Rosi S, Gatta A, Angeli P. Spontaneous bacterial peritonitis due to carbapenemase-producing *Klebsiella pneumoniae*. *Eur J Gastroenterol Hepatol* 2012;24:1234–7.
- [69] Alexopoulou A, Papadopoulos N, Eliopoulos DG, Alexaki A, Tsiriga A, Toutouza M, et al. Increasing frequency of gram-positive cocci and gram-negative multidrug-resistant bacteria in spontaneous bacterial peritonitis. *Liver Int* 2013;33:975–81.
- [70] Merli M, Lucidi C, Di Gregorio V, Lattanzi B, Giannelli V, Giusto M, et al. An empirical broad spectrum antibiotic therapy in health-care-associated infections improves survival in patients with cirrhosis: A randomized trial. *Hepatology* 2016;63:1632–9.
- [71] Campillo B, Richardet J-P, Kheo T, Dupeyron C. Nosocomial spontaneous bacterial peritonitis and bacteremia in cirrhotic patients: impact of isolate type on prognosis and characteristics of infection. *Clin Infect Dis* 2002;35:1–10.
- [72] Umgelter A, Reindl W, Miedaner M, Schmid RM, Huber W. Failure of current antibiotic first-line regimens and mortality in hospitalized patients with spontaneous bacterial peritonitis. *Infection* 2009;37:2–8.
- [73] Fernández J, Acevedo J, Prado V, Mercado M, Castro M, Pavesi M, et al. Clinical course and short-term mortality of cirrhotic patients with infections other than spontaneous bacterial peritonitis. *Liver Int* 2017;37:385–95.
- [74] Angeli P, Gines P, Wong F, Bernardi M, Boyer TD, Gerbes A, et al. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus

- recommendations of the International Club of Ascites. *Gut* 2015;64:531–7.
- [75] Nadim MK, Durand F, Kellum JA, Levitsky J, O’Leary JG, Karvellas CJ, et al. Management of the critically ill patient with cirrhosis: A multidisciplinary perspective. *J Hepatol* 2016;64:717–35.
- [76] Arabi YM, Dara SI, Memish Z, Al Abdulkareem A, Tamim HM, Al-Shirawi N, et al. Antimicrobial therapeutic determinants of outcomes from septic shock among patients with cirrhosis. *Hepatology* 2012;56:2305–15.
- [77] Felisart J, Rimola A, Arroyo V, Perez-Ayuso R, Quintero E, Gines P, et al. Cefotaxime is more effective than is ampicillin-tobramycin in cirrhotics with severe infections. *Hepatology* 1985;5:457–62.
- [78] Sort P, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, et al. Effect of Intravenous Albumin on Renal Impairment and Mortality in Patients with Cirrhosis and Spontaneous Bacterial Peritonitis. *N Engl J Med* 1999;341:403–9.
- [79] Guevara M, Terra C, Nazar A, Solà E, Fernández J, Pavesi M, et al. Albumin for bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. A randomized, controlled study. *J Hepatol* 2012;57:759–65.
- [80] Thévenot T, Bureau C, Oberti F, Anty R, Louvet A, Plessier A, et al. Effect of albumin in cirrhotic patients with infection other than spontaneous bacterial peritonitis. A randomized trial. *J Hepatol* 2015;62:822–30.
- [81] Schmidt ML, Barritt AS, Orman ES, Hayashi PH. Decreasing mortality among patients hospitalized with cirrhosis in the United States from 2002 through 2010. *Gastroenterology* 2015;148:967–977.e2.
- [82] Dellinger R, Carlet J, Masur H, Gerlach H, Calandra T, Cohen J, et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med* 2004;32:858–73.
- [83] Nations U. Draft Political Declaration of the High-level Meeting of the General Assembly

on Antimicrobial Resistance 2016;16108:0–3.

- [84] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama* 2016;315:801–10.
- [85] Morando F, Maresio G, Piano S, Fasolato S, Cavallin M, Romano A, et al. How to improve care in outpatients with cirrhosis and ascites: A new model of care coordination by consultant hepatologists. *J Hepatol* 2013;59:257–64.
- [86] Bajaj JS, Reddy KR, Tandon P, Wong F, Kamath PS, Garcia-Tsao G, et al. The 3-month readmission rate remains unacceptably high in a large North American cohort of patients with cirrhosis. *Hepatology* 2016;64:200–8.
- [87] Piano S, Bartoletti M, Tonon M, Baldassarre M, Chies G, Romano A, et al. Assessment of Sepsis-3 criteria and quick SOFA in patients with cirrhosis and bacterial infections. *Gut* 2017.
- [88] Piano S, Morando F, Carretta G, Tonon M, Vettore E, Rosi S, et al. Predictors of Early Readmission in Patients With Cirrhosis After the Resolution of Bacterial Infections. *Am J Gastroenterol* 2017;112:1575–83.
- [89] Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;36:309–32.
- [90] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–81.
- [91] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268–81.

- [92] Ginès P, Fernández J, Durand F, Saliba F. Management of critically-ill cirrhotic patients. *J Hepatol* 2012;56.
- [93] Ganguly N, Arora N, Chandy S, Fairoze M, Gill JP, Gupta U, et al. Rationalizing antibiotic use to limit antibiotic resistance in India. *Indian J Med Res* 2011;134:281–94.
- [94] Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010;10:597–602.
- [95] Haidar G, Philips NJ, Shields RK, Snyder D, Cheng S, Potoski BA, et al. Ceftolozane-Tazobactam for the Treatment of Multidrug-Resistant *Pseudomonas aeruginosa* Infections: Clinical Effectiveness and Evolution of Resistance. *Clin Infect Dis* 2017;65:110–20.
- [96] Sader HS, Castanheira M, Flamm RK, Farrell DJ, Jones RN. Antimicrobial Activity of Ceftazidime-Avibactam against Gram- Negative Organisms Collected from U . S . Medical Centers in 2012. *Antimicrob Agents Chemother* 2014;58:1684–92.
- [97] Reuken PA, Torres D, Baier M, Loffler B, Lubbert C, Lippmann N, et al. Risk Factors for Multi-Drug Resistant Pathogens and Failure of Empiric First-Line Therapy in Acute Cholangitis. *PLoS One* 2017;12:e0169900.
- [98] Fernández J, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, et al. Primary Prophylaxis of Spontaneous Bacterial Peritonitis Delays Hepatorenal Syndrome and Improves Survival in Cirrhosis. *Gastroenterology* 2007;133:818–24.
- [99] Fernández J, del Arbol LR, Gómez C, Durandez R, Serradilla R, Guarner C, et al. Norfloxacin vs Ceftriaxone in the Prophylaxis of Infections in Patients With Advanced Cirrhosis and Hemorrhage. *Gastroenterology* 2006;131:1049–56.
- [100] Ginès P, Rimola A, Planas R, Vargas V, Marco F, Almela M, et al. Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double-blind, placebo-controlled trial. *Hepatology* 1990;12:716–24.
- [101] Gustot T, Durand F, Lebrec D, Vincent J-L, Moreau R. Severe sepsis in cirrhosis.

Hepatology 2009;50:2022–33.

- [102] Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of Clinical Criteria for Sepsis. *Jama* 2016;315:762–74.
- [103] Freund Y, Lemachatti N, Krastinova E, Van Laer M, Claessens Y-E, Avondo A, et al. Prognostic Accuracy of Sepsis-3 Criteria for In-Hospital Mortality Among Patients With Suspected Infection Presenting to the Emergency Department. *Jama* 2017;317:301–8.
- [104] Raith EP, Udy AA, Bailey M, McGloughlin S, MacIsaac C, Bellomo R, et al. Prognostic Accuracy of the SOFA Score, SIRS Criteria, and qSOFA Score for In-Hospital Mortality Among Adults With Suspected Infection Admitted to the Intensive Care Unit. *Jama* 2017;317:290–300.
- [105] Vincent J, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;22:707–10.
- [106] Pandharipande PP, Shintani AK, Hagerman HE, St Jacques PJ, Rice TW, Sanders NW, et al. Derivation and validation of Spo₂/Fio₂ ratio to impute for Pao₂/Fio₂ ratio in the respiratory component of the Sequential Organ Failure Assessment score. *Crit Care Med* 2009;37:1317–21.
- [107] Gines P, Angeli P, Lenz K, Moller S, Moore K, Moreau R, et al. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010;53:397–417.
- [108] Gilbert D, Moellering RJ, Eliopoulos G, Chambers H, Saag M. *The Sanford Guide to Antimicrobial Therapy* 2010. 40th ed. Sperryville, VA: Antimicrobial Therapy, Inc; 2010.
- [109] Jalan R, Pavesi M, Saliba F, Amor??s A, Fernandez J, Holland-Fischer P, et al. The CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure. *J Hepatol* 2015;62:831–40.

- [110] Berman K, Tandra S, Forssell K, Vuppalanchi R, Burton Jr JR, Nguyen J, et al. Incidence and Predictors of 30-Day Readmission Among Patients Hospitalized for Advanced Liver Disease. *Clin Gastroenterol Hepatol* 2011;9:254–9.
- [111] Volk ML, Tocco RS, Bazick J, Rakoski MO, Lok AS. Hospital Readmissions Among Patients With Decompensated Cirrhosis. *Am J Gastroenterol* 2012;107:247–52.
- [112] Graupera I, Solà E, Fabrellas N, Moreira R, Solé C, Huelin P, et al. Urine Monocyte Chemoattractant Protein-1 Is an Independent Predictive Factor of Hospital Readmission and Survival in Cirrhosis. *PLoS One* 2016;11:e0157371.
- [113] Tapper EB, Finkelstein D, Mittleman MA, Piatkowski G, Lai M. Standard assessments of frailty are validated predictors of mortality in hospitalized patients with cirrhosis. *Hepatology* 2015;62:584–90.
- [114] Tapper EB, Finkelstein D, Mittleman MA, Piatkowski G, Chang M, Lai M. A Quality Improvement Initiative Reduces 30-Day Rate of Readmission for Patients With Cirrhosis. *Clin Gastroenterol Hepatol* 2016;14:753–9.
- [115] Kanwal F, Asch SM, Kramer JR, Cao Y, Asrani S, El-Serag HB. Early outpatient follow-up and 30-day outcomes in patients hospitalized with cirrhosis. *Hepatology* 2016;64:569–81.
- [116] Singal AG, Rahimi RS, Clark C, Ma Y, Cuthbert JA, Rockey DC, et al. An Automated Model Using Electronic Medical Record Data Identifies Patients With Cirrhosis at High Risk for Readmission. *Clin Gastroenterol Hepatol* 2013;11:1335–41.
- [117] Jencks SF, Williams M V, Coleman EA. Rehospitalizations among Patients in the Medicare Fee-for-Service Program. *N Engl J Med* 2009;360:1418–28.
- [118] Cervoni J-P, Thévenot T, Weil D, Muel E, Barbot O, Sheppard F, et al. C-Reactive protein predicts short-term mortality in patients with cirrhosis. *J Hepatol* 2012;56:1299–304.
- [119] PH C, Cnaan A, Zaoutis T, BV H, RW G, Keren R. Recurrent urinary tract infections in children: Risk factors and association with prophylactic antimicrobials. *JAMA*

2007;298:179–86.

- [120] Schuetz P, Wirz Y, Sager R, Christ-Crain M, Stolz D, Tamm M, et al. Effect of procalcitonin-guided antibiotic treatment on mortality in acute respiratory infections: a patient level meta-analysis. *Lancet Infect Dis* 2017. doi:10.1016/S1473-3099(17)30592-3.
- [121] Elkrief L, Chouinard P, Bendersky N, Hajage D, Larroque B, Babany G, et al. Diabetes mellitus is an independent prognostic factor for major liver-related outcomes in patients with cirrhosis and chronic hepatitis C. *Hepatology* 2014;60:823–31.
- [122] Elkrief L, Rautou P-E, Sarin S, Valla D, Paradis V, Moreau R. Diabetes mellitus in patients with cirrhosis: clinical implications and management. *Liver Int* 2016;36:936–48.