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TITLE OF THE DOCTORAL THESIS

The Impact of Infections and Other Factors on the Progression of Liver Decompensation, with a Focus on Acute Decompensation and Acute-on-Chronic Liver Failure

S.S.D. MED/09

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Summary

Introduction: Liver cirrhosis requires a better prognostic definition due to the identification of different phases characterized by significant differences in mortality rates, primarily with the transition from compensated to non-acute (NAD) and acute decompensation (AD), followed by acute-on-chronic liver failure (ACLF), characterized by the highest mortality. This transition appears to be influenced by various factors involving in particular the progressive worsening of portal hypertension and systemic inflammation, alongside the emergence of infections and renal impairment.

Aim: Our primary objective was to explore the interconnection and impact of bacterial infections on the natural progression of liver cirrhosis. In the second phase, we aimed to identify factors linked to the maintenance of liver compensation or the onset of NAD, AD, and ACLF conditions, considering infections, liver events and current therapy.

Methods: This was a single-center cohort study, with the enrolment of consecutive patients affected by liver cirrhosis who were followed in our Liver Unit between January 2017 and December 2022, either as inpatients or outpatients.

Results: A total of 456 patients were enrolled, with a median follow-up period of 43.31 months (IQR 24-72). Follow-up was discontinued in the event of liver transplant, mortality, or an episode of ACLF as defined by the EASL criteria. 70.6% were male, with a mean age of 64 ± 11 years. Based on recorded liver events and infectious episodes during follow-up, we categorized the cohort into four subgroups: NAD subgroup (70 patients), AD subgroup (151 patients), ACLF subgroup (81 patients) and compensated subgroup (154 patients).

During the study period, 142 patients (31.1%) experienced at least one infectious episode, with 12.7% testing positive for MDROs colonization. Sepsis was the most prevalent type of bacterial infection (30.3%), followed by pneumonia (26.7%), urinary tract infections (20.4%) and spontaneous bacterial peritonitis (18.3%), while MDROs infections accounted for over 20% of cases. Urinary tract infections and MDROs were statistically more prevalent in the ACLF subgroup.

We observed an association between MDROs colonization and hepatic decompensation, with an odds ratio (OR) of 0.33 (95% CI 0.14-0.72) for compensated patients, while colonized patients faced a threefold higher risk of developing AD (OR 3.20; 95% CI 1.65-6.48) and ACLF (OR 2.55; 95% CI 1.19-

5.32). Another factor not significantly associated with liver compensation but strongly linked to the development of both AD (OR 5.69; 95% CI 2.92-11.84) and ACLF (OR 3.58; 95% CI 1.80-7.03) was portal vein thrombosis (PVT).

In terms of current therapy, β-blockers (OR 2.14, 95% CI 1.33-3.49), direct oral anticoagulants (DOACs) (OR 3.28, 95% CI 1.76-6.23), and angiotensin-converting enzyme inhibitors/calcium channel blockers (ACE-i/CCBs) (OR 2.10, 95% CI 1.27-3.47) were strongly associated with liver compensation, while no protective role of statins and rifaximin was confirmed.

Conclusion: Infections were confirmed to be a crucial complication for cirrhotic patients. To our knowledge, this is the first study to assess the impact of MDROs colonization and PVT on the development of liver decompensation, suggesting that a history of PVT and colonization with MDROs may predispose individuals to AD and ACLF.

DOACs, β-blockers and ACE-i/CCBs could potentially influence the natural progression of liver cirrhosis, particularly in preventing AD and ACLF. Future prospective and randomized controlled studies may offer further insights into the pharmacological effects and the impact of infections on the progression of cirrhosis.

Introduction

1) Natural history of liver cirrhosis

The natural history of liver cirrhosis has traditionally involved the progression from a preliminary asymptomatic compensated stage, known as compensated advanced chronic liver disease (cACLD), to the symptomatic phase called decompensated liver cirrhosis. [1] This transition is characterized by the development of clinical signs related to portal hypertension and impaired liver function, such as jaundice, ascites, gastrointestinal bleeding, hepatic encephalopathy, and infections, at a rate of 5-7% per year. The survival rate at 1 year significantly decreases (61% vs 95% in compensated patients), as well as the median survival (2 years vs 12 years in compensated patients). [2, 3]

This simplified view encompasses various prognostic subgroups. Firstly, the outcome of decompensating liver cirrhosis is influenced by the number and type of decompensating events. Ascites, the main and most frequent decompensating event, accounts for nearly 70% of cases transitioning to decompensated cirrhosis. [4] Furthermore, ascites has a worse prognosis compared to variceal bleeding, while the combination of both complications results in the worst outcome. [5] In a cohort of nearly 500 patients, the comparison between different decompensating events showed a 20% 5-year mortality risk for bleeding alone versus 30% for ascites, while the combination of both increases the mortality risk to 88%. [6]

Two primary factors closely linked to the deterioration of liver decompensation are the development of infections and renal impairment. A meta-analysis of nearly 12,000 patients showed that infections increased mortality 4-fold, with a mortality rate of 30% at 1 month and over 60% at 1 year. [7] In terms of renal failure, Tesi et al. reported a 7-fold increased mortality, with 58% of patients dying within one month and 63% at 1 year. [8] Both manifestations appear to result from the progressive worsening of portal hypertension and systemic inflammation, which markedly increases in the advanced disease stages, as demonstrated by the rise in the inflammation biomarker IL-6. [9] Portal hypertension is implicated in the development of recurrent variceal bleeding, encephalopathy, jaundice, hepatorenal syndrome, hyponatremia, and refractory ascites. [10, 11]

To better define the different prognostic subgroups of liver cirrhosis based on the onset of the first decompensating event, whether acute or progressive, experts have described two entities: acute

decompensation (AD), which includes acute-on-chronic liver failure (ACLF), and non-acute decompensation (NAD). [4]

a. AD and ACLF

Following the results of the CANONIC study, a large multicentre observational study conducted on over 1300 hospitalized cirrhotic patients with acute decompensation, a clearer definition of AD was established. [12] AD is characterized by the sudden onset of multiple major complications, including acute gastrointestinal bleeding, first or recurrent grade 2 or 3 ascites in less than 2 weeks, first or recurrent acute hepatic encephalopathy, and any bacterial infection. It is not just the occurrence of a single decompensation event, but the rapidity of onset that distinguishes AD from NAD.

In this context, a further subgroup includes ACLF, which is defined by the development of multiple organ failure, involving six main systems: liver, kidney, brain, coagulation, circulation, and lung. The number of organ failures defines the grade of ACLF, with ACLF grade 3 when more than 3 organ failures are present. Moreover, this condition is characterized by an extremely increased mortality compared to AD: a 5% 28-day mortality rate in AD without ACLF vs 22-77% in ACLF patients. [13-15]

Notably, prior decompensation has no effect on the development of ACLF; in fact, patients with no previous decompensation were more likely to present more severe grades of ACLF and a higher 28-day mortality rate. [14] The mortality rate in patients who develop ACLF is dramatically high, with a stepwise increase according to ACLF stage and the number of failing organ systems: 6%-18% for no-ACLF/ACLF grade 1 to 42%-92% with ACLF grade 2-3. [12, 15] In particular, the systems most strongly associated with mortality were renal and brain impairment. This explains why ACLF grade 1a is identified by the presence of kidney failure, and grade 1b by a single organ failure plus renal dysfunction (defined by a serum creatinine of 1.5-1.9 mg/dl) and/or grade 1-2 encephalopathy (according to West-Haven criteria). [12]

The incidence of ACLF is 14% in outpatient cirrhotic patients, with an incidence that increases in line with the Child Pugh (CP) score: only 2% in CP class A vs. 29% in CP class ≥B at 12 months. [15] The prevalence of ACLF in the CANONIC study patients was 30% (20% at admission and 10% during hospitalization), and the overall 28-day and 90-day mortality rates were 33% and 51%, respectively. Mortality rates in patients without ACLF were low (28-day: 1.9%; 90-day: 10%). The prevalence, 28-

day, and 90-day mortality rates associated with the different grades of ACLF were 15.8%, 22%, and 41% respectively in ACLF-1, 10.9%, 32%, and 55% in ACLF-2, and 4.4%, 73%, and 78% in ACLF-3. [12]

Independent predictors of disease severity were CLIF Consortium ACLF score (CLIF-C ACLFs) and presence of liver failure (total bilirubin ≥12 mg/dL) at ACLF diagnosis. ACLF was observed to resolve or improve in 49.2% of cases, to fluctuate in 30.4%, while in 20.4% of cases it worsened. [16] CLIF-C ACLFs was found to be superior to Model for End-Stage Liver Disease (MELD), MELD-NA score, and CP score in predicting and stratifying mortality in ACLF patients. [17] In particular, the assessment of ACLF with the CLIF-C ACLFs at 3-7 days accurately resulted in the prediction of 28-day and 90-day mortality, as 81% of cases presented their final ACLF grade at 1 week. Evaluating the severity of the syndrome is fundamental for decision-making, as early liver transplant has demonstrated good performance (75% at 1 year), while the presence of more than 4 organ failures or a CLIF-C ACLFs >64 at days 3-7 may be considered an indication for palliative care due to 100% 28-day mortality. [16]

From a pathophysiological perspective, AD and ACLF differ mainly in terms of systemic inflammation, which appears to be the primary driver for developing ACLF, explaining the multi-organ involvement outside the liver rather than a simple hemodynamic disturbance. The systemic inflammation hypothesis posits that the spread of bacterial products, favoured by the presence of portal hypertension, is the primary event, leading to the activation of the innate immune response, which triggers the release of endothelial mediators responsible for arterial vasodilatation, pro-inflammatory cytokines, and reactive oxygen species. The subsequent alteration of tissue homeostasis forms the basis for multi-organ involvement and failure. [18-20] Pro-inflammatory cytokines, markers of oxidative stress, macrophage activation, as well as white blood cell count, neutrophils, monocytes, and plasma C-reactive protein, are increased in patients with ACLF compared with AD. Moreover, the degree of inflammation reflects the severity of circulatory, liver, and kidney failure due to reduced organ perfusion and metabolic cellular impairment. [18]

In most cases, a precipitating factor may be identified, and the number of precipitating factors is linked to the severity of the condition. The PREDICT study revealed that in Western countries, the most frequent precipitating factors are extra-hepatic, such as bacterial infections (41.3%), severe alcohol hepatitis (20.4%), and gastrointestinal bleeding (27.1%), while in Eastern countries, the most frequent precipitating factor is viral reactivation. [21-27] Although an extensive work-up is indicated at admission in all patients with ACLF, in 35% of cases, the precipitating factors remain unknown. [21]

While in ACLF patients, CLIF-C ACLFs was found to better predict 28-day and 90-day mortality compared to MELD, MELD-Na score, and CP scores; in acutely decompensated cirrhosis without ACLF, CLIF-C ADs provides more accurate predictions of 90-day, 180-day, and 365-day mortality compared to the other scores with similar ability to predict the occurrence of ACLF. [17, 28] *Figure 1*

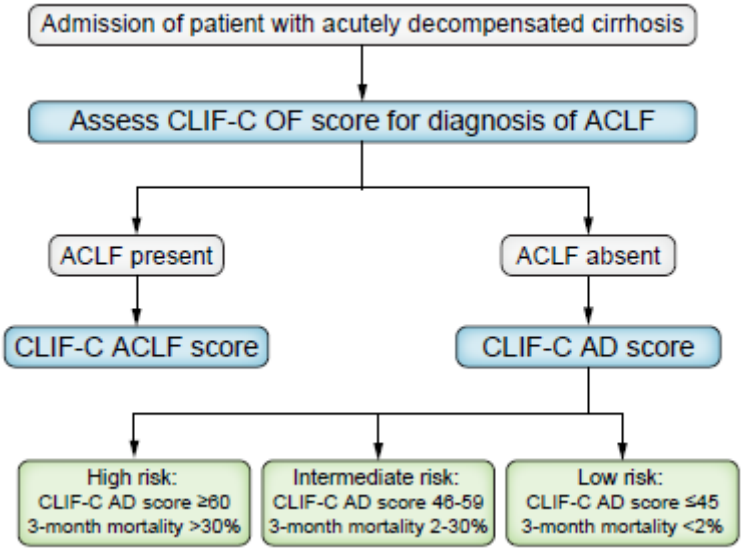

Figure 1 Algorithm for the sequential use of the EASL-CLIF scores in patients with cirrhosis admitted to hospital with acute decompensation (from Journal of Hepatology, August 2023. vol. 79 461–491)

From a pathophysiological and prognostic perspective, AD appears to lie between compensated or stable decompensated cirrhosis and ACLF. The PREDICT study examined hospitalized AD patients, assessing clinical events over the following three months and 3-12 months mortality risk. The study identified three distinct clinical entities with varying 3- and 12-month mortality rates:

- Pre-ACLF: characterized by the development of ACLF in the follow-up period, with a 3-month mortality of 53.7% and 12-month mortality of 67.4%
- Unstable decompensated cirrhosis: involving re-hospitalization without ACLF, with a 3-month mortality of 0% and 12-month mortality of 9.5%
- Stable decompensated cirrhosis: neither progressing to ACLF nor requiring re-admission, with a 3-month mortality of 21% and 12-month mortality of 35.6%.

Markers of systemic inflammation, such as c-reactive protein and white blood cell levels, progressively increase from stable decompensated cirrhosis to the pre-ACLF subgroup, suggesting the pivotal role of systemic inflammation in ACLF progression and decompensation. [21]

b. NAD

While AD is characterized by the development of first or recurrent grade 2-3 ascites within two weeks, acute gastrointestinal bleeding, first or recurrent encephalopathy, and any type of acute bacterial infection, NAD encompasses slow ascites development, mild encephalopathy (grade 1-2), and progressive jaundice in the non-cholestatic form of cirrhosis. NAD usually represents the first decompensation with a single decompensating event in more than 60% of cases without requiring hospitalization, in comparison with AD/ACLF where patients are more critical, usually presenting with two or more decompensating events, and hospitalization is needed. [4, 12]

Proven strategies for preventing or delaying decompensation have been evaluated with the main focus on their impact on the main trigger for decompensation: portal hypertension and systemic inflammation. [29] Patients with a hepatic venous pressure gradient (HVPG) <10 mmHg had a 90% probability of not experiencing decompensation over a 4-year period, while with the presence of clinically significant portal hypertension (CSPH), when HVPG rises above 10 mmHg, the decompensation risk rises consequently. [11, 30]

Currently, the first decompensation event is no longer considered a point of no return, as data suggest the possibility of re-compensation, particularly when the underlying etiological causes are controlled. [11] The cessation of liver injury through the therapeutic control of causal factors is deemed a prerequisite for fibrosis regression. A sustained virological response in hepatitis C virus or viral suppression in hepatitis B virus reduces the incidence of decompensation, as does the suspension of alcohol and the control of metabolic factors such as diabetes, obesity, and dyslipidaemia. [29]

Furthermore, targeting alterations in the gut-liver axis and circulatory dysfunction/portal hypertension with rifaximin, norfloxacin, long-term administration of albumin, β-blockers, and statins is suggested. In fact, reducing portal hypertension with the use of non-selective β-blockers, especially carvedilol, has been shown to lower the incidence of decompensating events and improve the survival rate, as per the PREDESCI study. [31] In recent years, the beneficial effects of statins in

decreasing portal pressure and improving hepatic microcirculation have been proven to decrease decompensation and mortality. [32]

2) Bacterial infections and liver cirrhosis

After the CANONIC study, the criteria to define the presence of AD were expanded to include bacterial infections due to their high prevalence and negative impact on the prognosis of cirrhotic patients. [7, 33] Experts now emphasize the role of bacterial infections as the main precipitating factor for the development of AD and ACLF. [14, 27, 29]

The prevalence of bacterial infections in decompensated cirrhotic patients is estimated to range from 30% to 46%, with various categories such as community-acquired (32-50%), healthcare-associated (25-41%), and nosocomial infections 25-37%). [34-37] Additionally, nearly 25% of cirrhotic patients with a bacterial infection are at risk of developing a second infection, which can significantly worsen their clinical condition and increase the risk of mortality, especially in the case of infections caused by multi-drug resistant organisms (MDROs). [38] The most frequent bacterial infections in cirrhotic patients include spontaneous bacterial peritonitis (SBP, 20-30%), urinary tract infections (UTI, 20-25%), pneumonia (8-20%), sepsis (8-20%), and skin and soft tissue infections (SSTI, 5-10%), with specific bacteria being responsible for each type of infection. [37, 38] For instance, SBP and UTI are mainly sustained by Gram-negative bacteria (Enterobacterales as E. coli and K. pneumoniae), while Gram-positive bacteria are more often responsible for pneumonia and skin and SSTI. [36]

Cirrhotic patients are at a higher risk of bacterial infections due to several factors, including portosystemic shunting, increased intestinal permeability, cirrhosis-associated immune dysfunction, gut dysbiosis, and genetic factors. [39-41] Moreover, the risk of MDROs infections is increasingly concerning, with the prevalence of MDROs infections rising from 8% in the late '90s to 38% nowadays. [35, 36] This increase may be attributed to healthcare exposure of cirrhotic patients, invasive procedures, antibiotic prophylaxis, and repeated hospitalizations with exposure to antimicrobial therapy. The mortality rate for sepsis is rising, while mortality for other major complications (as gastrointestinal bleeding or hepatorenal syndrome) has decreased. Risk factors independently associated with the development of MDROs infections include recent exposure to β-

lactams, nosocomial infection, previous MDROs infection, whereas the role of long-term norfloxacin prophylaxis is still debated. [42-45] The strategies for the prevention and response to novel and targeted MDROs include various measures such as maintaining spatial separation between beds, using privacy curtains, cleaning and disinfecting shared reusable equipment and environmental surfaces, changing personal protective equipment, and performing hand hygiene. [46] Efforts to prevent MDROs transmission are still needed, as these organisms can spread long before being detected. Various guidelines are available for the control of multidrug-resistant Gram-negative bacteria, and they contain broad areas of agreement. MDROs are common in healthcare-associated infections, increasing the severity of infectious complications, negatively affecting morbidity, mortality, and care costs. In addition, MDROs carriage can have a significant impact on the daily lives of carriers and their families, leading to uncertainties and lingering questions about the MDROs.

To mitigate the impact of MDROs carriage on carriers' daily lives, providing clear information to carriers, improving the general knowledge of staff dealing with MDROs, and providing follow-up care for patients beyond the hospital are essential. Further research and efforts are needed to address the challenges posed by MDROs and their impact on patients and healthcare systems. Common isolated MDROs in cirrhotic patients include Extended-spectrum-β-lactamase (ESBL) producing Enterobacteriaceae, Methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE), and carbapenem-resistant Enterobacteriaceae (CRE). [36]

To prevent the spread of MDROs, the application of antibiotic stewardship programs to better evaluate the microbiological local background of the single area is strongly suggested. [46, 47] Infection control practices such as hand hygiene, surveillance swabs, barrier precautions, and rapid microbiological tests are also necessary. [48] Moreover, narrowing the subgroups of cirrhotic patients that could benefit from antibiotic prophylaxis and exploring future strategies such as faecal microbiota transplant, the immunomodulatory effects of statins, therapies to reverse gut dysbiosis, and the use of prokinetics to reduce bacterial overgrowth are important. [49]

Antimicrobial Stewardship Verona

The "SAVE" project at the Verona University hospital, which commenced in May 2018, was designed to be a non-restrictive intervention aimed at reducing overall antimicrobial consumption and MDRO infections. It involved a multidisciplinary antimicrobial stewardship team comprising specialists from

Infectious Diseases, Microbiology, Pharmacy, Infection Prevention and Control, Hospital Epidemiology, and Psychology. The team collaborated with ward physicians to develop and evaluate guidelines for empirical antibiotic therapy and prescriptive appropriateness. In addition, infection control measures such as screening for MDROs in all admitted patients (as rectal MDROs swabs), isolation of positive patients, hand hygiene education, and culture collection before starting antibiotics were implemented. [46, 50]

Aim

Our primary objective was to thoroughly investigate the interconnection and impact of bacterial infections on the natural history of liver cirrhosis. The first phase of our study assessed the microbiological profile of our specific cohort of cirrhotic patients. In the second phase, we aimed to identify factors linked to the maintenance of liver compensation or the development of NAD, AD, and ACLF conditions, considering infections, comorbidities, liver etiology, and current therapy.

Methods

Our study was a single-center cohort study, approved by the local Institutional Ethics Committee (2730CESC-VR), in accordance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the 2013 Declaration of Helsinki. We enrolled consecutive patients affected by liver cirrhosis who were followed in our Liver Unit between January 2017 and December 2022 as inpatients or outpatients.

The inclusion criteria were a diagnosis of liver cirrhosis assessed by abdominal ultrasonography, computed tomography scan, or magnetic resonance imaging and/or histologically, age of 18 years or older, and the signing of an informed consent form. We excluded patients with clinical conditions predisposing to immunosuppression (e.g. HIV infection or AIDS, primary or acquired immunodeficiency, solid organ recipients, or ongoing immunosuppressive therapies), as well as patients affected by cancer except hepatocarcinoma (HCC) and cholangiocarcinoma (CCC). During the follow-up period, we recorded anthropometric, clinical, microbiological, and biumoral data. Liver events were defined as the development of hepatic encephalopathy (HE), variceal bleeding, ascites, acute kidney injury (AKI), hepatorenal syndrome (HRS), HCC, CCC, or portal vein thrombosis (PVT).

Bacterial infections were defined as:

1) spontaneous bacterial peritonitis (SBP) with ≥250 cells/mm3 polymorphonuclear (PMN) cell count on ascitic fluid

2) secondary bacterial peritonitis, ≥250 cells/mm3 PMN in the ascitic fluid and evidence of an infectious source

3) septic bacteremia, positive blood cultures for clinically relevant bacteria and a sequential organ failure assessment (SOFA) score ≥2 at diagnosis (in case of S. haemolyticus and S. epidermidis, when isolated in at least 2 consecutive sets of blood cultures)

4) urinary tract infection (UTI), >10 leukocytes/field in urinary analysis and/or positive urine culture with urinary symptoms (dysuria, pollakiuria, urgency, stranguria or a general increased urination frequency)

5) respiratory infections, clinical signs with a new onset chest X-ray consolidation and/or positive sputum culture

6) cholangitis, increased cholestasis, right hypochondrium pain, jaundice and/or fever, with/without evidence of biliary obstruction on imaging

7) skin and soft tissue infections, clinical signs of infection in association with signs of skin inflammation

8) any suspected bacterial infection, presence of fever (≥37.5˚C) and leukocytosis (>12,000 leukocytes/mm3) and abnormal levels of c-reactive protein/procalcitonin (CRP/PCT) without identification of the source

9) bacterial gastroenteritis, defined by acute diarrhea with a positive fecal culture.

Various conditions, such as bacteriascites (positive ascitic fluid culture with PMN <250 cells/mm^3), asymptomatic bacteriuria (positive urine culture and bacterial count ≥10^5 CFU/ml without urinary symptoms), and enteric colonization by multidrug-resistant bacteria (isolation of VRE and CRE at surveillance rectal swab), were considered and recorded.

Isolated microorganisms were defined as MDR gram-negative bacteria in the case of resistance to at least one drug in at least three different classes of antibiotics. For gram-positive bacteria, oxacillin-resistance or vancomycin-resistance were considered for Staphylococci or Enterococci, respectively.

Community-acquired infections were those acquired outside of a healthcare facility or within 48 hours of hospital admission without a recent hospitalization. Nosocomial infections are clinically documented after 48 hours of hospitalization, and healthcare-associated infections (HAIs) are acquired within 90 days prior to hospital admission or during a hospitalization in another healthcare setting. [42, 51]

Statistical Analysis

Continuous variables are presented as mean ± standard deviation (SD) or median with interquartile range (IQR) based on data distribution. Categorical variables are expressed as frequency numbers and percentages. Either one-way ANOVA or Kruskal Wallis one-way ANOVA were used to compare continuous variables according to the data distribution pattern (normal or not normal). Categorical variables were compared using the Chi-square test. Both logistic and linear multivariate regression analyses were performed to determine if anamnestic or clinical variables could be independently

associated with liver decompensation phases. The variable selection was done through sequential replacement (a stepwise method) which consists of a combination of backward and forward techniques. If the p-value was less than 0.05 or above 0.1 the covariates were respectively included and excluded from the regression model. Statistical Package for Social Science (SPSS) version 22 and Jamovi version 2.2.2 was used for all data analysis. All tests were 2-sided, and p-values<0.05 were considered statistically significant.

Results

1) First phase of the study: microbiological background and impact of multidrug resistant organisms

In the first phase of our research study, 229 patients were included, with 68.1% males with a mean age of 64.4 ± 11.2 years. During the follow-up, 101 infectious events were recorded in 72 patients (31.5% of the study population), with 31.7% of the infections occurring in patients who had previously experienced at least one infectious episode. Of the total infections, 48% were community-acquired, 25% were nosocomial, and 27% were healthcare-associated. No differences in the number of infections were documented between the years of follow up (about 9.5% of infections per single year).

Sepsis was the most common infection (24.7%), followed by pneumonia (19.8%) and SBP (17.8%). UTI accounted for 11.9% of the infections, while other recorded infections included cholangitis (3%), gastroenteritis (2%), and SSTI (3%). Additionally, 15 cases of bacterascites and 25 cases of asymptomatic bacteriuria were identified but not included in the further analysis. Microbiological isolation was achieved in 41 out of 101 total infective episodes (40.6%) through blood cultures. The most frequently isolated microorganism in blood cultures was S. aureus, followed by E. faecalis, S. haemolyticus, S. epidermidis, P. aeruginosa, and Salmonella spp. Staphylococcus spp., E. coli, and Enterococcus spp. were the most commonly isolated organisms in ascitic cultures, while E. coli, P. mirabilis, and Enterococcus spp. were the most frequently isolated organisms in urine. Of interest, no fungi or anaerobic microorganisms were detected.

When comparing infected patients with no-infection patients, antibiotic prophylaxis (27% vs 18.4%, p = 0.004), MELD score (11.8 ± 5.9 vs 9.3 ± 2.9, p = 0.002) and CP B-C (36% vs 9.7%, p < .001), resulted significantly different. Regarding blood tests, platelets count was significantly higher ($147x10^3$ ± 112 vs $116x10^3$ ± 55, p = .003), while hemoglobin levels were significantly lower (12.1 ± 2.2 vs 13.3 ± 2.1, p = .006) in patients with infections. *Table 1*

Table 1 Baseline characteristics in infected and not infected patients

Total cohort (n = 229)	Infected (n = 72)	Not infected (n = 157)	p-value
Age (years), mean ± SD	66.2 ± 12.9	63.6 ± 10.2	0.100
Male sex, n (%)	46 (63.9)	110 (70.1)	0.350
Comorbidities			
Diabetes, n (%)	28 (38.9)	43 (27.4)	0.080
CKD, n (%)	9 (12.5)	12 (7.6)	0.230
Hypertension, n (%)	34 (47.2)	67 (42.7)	0.520
Dyslipidaemia, n (%)	14 (19.4)	14 (8.9)	**0.020**
COPD, n (%)	2 (2.8)	6 (3.8)	0.690
Antibiotic prophylaxis, n (%)	27 (27.0)	29 (18.4)	**0.004**
Liver Functional scores			
MELD score, mean ± SD	11.8 ± 5.9	9.3 ± 2.9	**0.002**
Child-Pugh score B and C, (%)	26 (36)	15 (9.7)	**<.001**
Blood tests			
WBC (x 10^9/L), mean ± SD	5.96 ± 2.5	5.13 ± 2.3	0.080
Neutrophiles (x 10^9/L), mean ± SD	3.59 ± 2.0	3.12 ± 1.7	0.200
Lymphocytes (x 10^9/L), mean ± SD	1.35 ± 0.5	1.39 ± 0.6	0.810
Platelets (x 10^9/L), mean ± SD	147 ± 112	116 ± 55	**0.030**
Hemoglobin (g/dL), mean ± SD	12.1 ± 2.2	13.3 ± 2.1	**0.006**
CRP (mg/dL), mean ±SD	45 ± 10.0	2.5 ± 0.6	**0.001**
Complications			
PVT, n (%)	17 (23.6)	22 (14.0)	**0.050**
Bleeding, n (%)	14 (19.4)	18 (11.5)	0.100
HRS, n (%)	11 (15.3)	5 (3.2)	**<.001**
Ascites, n (%)	53 (73.6)	60 (38.2)	**<.001**
HE, n (%)	22 (30.6)	19 (12.1)	**<.001**
Death, n (%)	35 (48)	28 (17)	**0.004**
MDROs colonization, n (%)	8 (11.1)	8 (5.1)	0.030

CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; MELD, model for end-stage liver disease; WBC, white blood cells; CRP, c-reactive protein; PVT, portal vein thrombosis; HRS, hepatorenal syndrome; HE, hepatic encephalopathy; MDROs, multi-drug resistant microrganisms.

a) MDROs infections

15 patients (20.8%) experienced infections caused by MDROs, with an annual incidence rate of 3.5%. The most commonly detected MDROs in blood cultures were methicillin-resistant (MR) coagulase-negative Staphylococci, MDR Pseudomonas spp., and VRE in the bloodstream, while MDR Proteus, MDR Pseudomonas spp., MDR K. pneumoniae, and VRE in urine. Additionally, ascitic fluid samples

revealed the presence of ESBL-producing E. coli and MR coagulase-negative Staphylococci. No CRE, VRE and MR Staphylococcus aureus (MRSA) were isolated in our ascitic samples. 42.1% of these infections were sepsis, followed by SBP (26.3%), pneumonia (15.8%), and UTI (15.8%).

Furthermore, 16 patients (6.9%) were carriers of MDROs, as indicated by rectal swabs.

No significant differences were found between MDROs-infected and non-infected patients, except for COPD, platelet value, and antibiotic prophylaxis use. MELD and CP score resulted significantly higher in the MDROs infected subgroups. *Table 2*

Table 2 Comparison of baseline characteristics in MDROs infected patients and no-MDROs infected patients

Variable	MDROs infections (n = 15)	No MDROs infections (n = 57)	p-value
Age (years), mean ± SD	65.9 ± 12.7	66.3 ± 13.2	0.748
Male sex, n (%)	13 (86.7)	33 (57.9)	0.060
Comorbidities			
Diabetes, n (%)	4 (26.7)	24 (42.1)	0.282
CKD, n (%)	2 (13.3)	7 (12.3)	0.914
Hypertension, n (%)	6 (40)	28 (49.1)	0.536
COPD, n (%)	2 (13.3)	0 (0)	**0.005**
Antibiotic prophylaxis, n (%)	5 (33)	9 (15)	**0.005**
Liver Functional scores			
MELD score, mean ± SD	12.6 ± 6.1	8.7 ± 2.4	**0.050**
Child-Pugh score B and C, (%)	41	12	**< .001**
Blood tests			
WBC (x 10^9/L), median (IQR)	6.34 (3.4)	5.82 (2.2)	0.080
Neutrophiles (x 10^9/L), median (IQR)	4.20 (23.6)	3.44 (1.4)	0.200
Lymphocytes (x 10^9/L), median (IQR)	1.49 (0.5)	1.34 (0.6)	0.810
Platelets (x 10^9/L), mean ± SD	111 ± 58	161 ± 125	**0.023**
Complications			
PVT, n (%)	3 (20)	14 (24.6)	0.716
Bleeding, n (%)	3 (20)	11 (19.3)	0.952
HRS, n (%)	4 (26.7)	7 (12.3)	0.173
Death, n (%)	6 (40)	28 (49.1)	0.060
MDROs colonization, n (%)	3 (20)	6 (10.5)	0.167

MDROs, multi-drug resistant microrganisms; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; MELD, model for end-stage liver disease; WBC, white blood cells; CRP, c-reactive protein; PVT, portal vein thrombosis; HRS, hepatorenal syndrome.

2) Second phase of the study: different phases of liver decompensation

The patient cohort was expanded over subsequent years, encompassing a total of 456 individuals enrolled between January 2017 and December 2022, with a median follow-up period of 43.31 months (IQR 24-72). Follow-up was discontinued in the event of liver transplant, mortality, or an episode of ACLF as defined by the EASL criteria. Of the patients, 70.6% were male, with a mean age of 64 ± 11 years. *Table 3*

Cirrhosis etiology was predominantly viral (44.3%), followed by alcoholic (23.7%), metabolic (20.2%), autoimmune (7.5%), or other etiologies (4.3%). The most prevalent comorbidities included hypertension (48.5%), type II diabetes (34.9%), heart failure (15.4%), dyslipidemia (15.1%), and chronic kidney disease (CKD) (6.8%). At the time of enrolment until the occurrence of a liver-related event, the current therapy for each patient was recorded. 51.3% of patients were on β-blockers, 51.3% on diuretics, 13.2% on statins, and 11.8% on rifaximin. Additionally, nearly one-third of patients were on angiotensin-converting enzyme inhibitors or calcium channel blockers (ACE-i/CCBs), and 19.3% were on direct-acting oral anticoagulants (DOACs).

Table 3 Main Characteristics of the whole cohort at enrolment

Variable	**Total (n=459)**
Age, mean ± SD	63.7 ± 11.2
Male, n (%)	322 (70.6)
Death, n (%)	199 (43.3)
Etiology, n (%)	
Metabolic	92 (20.2)
ALD	108 (23.7)
Viral	202 (44.3)
Autoimmune	34 (7.5)

Others	23 (4.3)
Comorbidities, n (%)	
Type II Diabetes	159 (34.9)
COPD	19 (4.2)
CKD	31 (6.8)
Heart failure	70 (15.4)
Hypertension	221 (48.5)
Dyslipidemia	69 (15.1)
Therapy at enrolment, n (%)	
Diuretics	234 (51.3)
β-blockers	234 (51.3)
Statins	60 (13.2)
Rifaximin	54 (11.8)
ACE-i/CCBs	139 (30.5)
Antidiabetics	142 (31.1)
DOACs	88 (19.3)
Corticosteroids	45 (9.69)
Antibiotics	88 (19.3)

SD, standard deviation; ALD, alcoholic liver disease; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; ACE-i, ACE-inhibitors; CCBS, Calcium channel blockers; DOACs, direct acting anticoagulants

During the follow-up period, all liver-related events were recorded, allowing for the subclassification of the entire cohort into four main subgroups:

- The NAD subgroup (70 patients) exhibited a progressive development of ascites, hepatic encephalopathy grade 1-2, and jaundice in the case of non-cholestatic liver cirrhosis. These patients were primarily managed as outpatients.
- The AD subgroup (151 patients) was characterized by the acute onset of ascites or recurrent ascites grade 2-3 within 2 weeks, the first episode of hepatic encephalopathy grade 3-4 or recurrent episodes, acute gastrointestinal bleeding, and bacterial infections.
- The ACLF subgroup (81 patients) experienced the development of AD and multiorgan failure as classified by the CLIF-C ACLF score.

- The Compensated subgroup (154 patients) consisted of individuals who did not experience any liver events during the follow-up period.

As per the definition, bacterial infections were only observed in the AD and ACLF subgroups. Out of the total cohort, 142 patients (31.1%) experienced at least one infectious episode during the study period. *Table 4*

A positive colonization of MDROs was detected in 12.7% of the entire cohort, with approximately 20% of ACLF patients, 16.6% of AD patients, 10% of NAD patients, and 6.5% of compensated subgroup patients showing colonization. Among the infectious episodes, sepsis was the most frequently recorded (30.3%), followed by pneumonia (26.7%) and UTI (20.4%). UTI and MDROs were statistically more prevalent in the ACLF subgroup. Spontaneous bacterial peritonitis accounted for 26 cases (18.3% of all recorded infections). In 4.2% of cases, the infectious source remained unidentified, while MDROs infections constituted over 20% of cases.

Table 4 Bacterial infections prevalence in AD and ACLF subgroups

	TOTAL n = 456	**AD n = 151**	**ACLF n = 81**	**p-value**
Patients with infections, n (%)	142 (31.1)	81 (53.6)	61 (75.3)	**<.001**
Infectious episodes per single patient, median (IQR)	0.5 (1.1)	0.9 (1)	1.4 (1.6)	**<.001**
MDROs infections, n (%)	30 (6.6)	15 (9.9)	15 (18.5)	**0.046**
MDROs colonization, n (%)	58 (12.7)	25 (16.6)	16 (19.8)	NS
Sepsis, n (%)	43 (9.4)	20 (13.2)	23 (28.4)	NS
Pneumonia, n (%)	38 (8.3)	23 (15.2)	15 (18.5)	NS
UTI, n (%)	29 (6.4)	13 (8.6)	16 (19.8)	**0.003**
SBP, n (%)	26 (5.7)	14 (9.3)	12 (14.8)	NS

AD, acute decompensation; ACLF, acute-on-chronic liver failure; MDROs, multi-drug resistant organisms; UTI, urinary tract infections; SBP, spontaneous bacterial peritonitis.

Liver events are detailed in *Table 5*, categorized by all subgroups. As anticipated, as per the definition, the compensated group did not experience any liver events, whereas the percentages of

liver complications increased in parallel with the progression of liver decompensation. Of note, the occurrence of AKI or AKI- HRS was significantly more common in ACLF patients compared to AD patients, indicating the influence of renal function on the development of ACLF.

Table 5 Prevalence of liver events in the whole cohort during the study period

Liver event	Compensated (n=154)	NAD (n=70)	AD (n=151)	ACLF (n=81)	p-value
HCC, n (%)	59 (38.3)	44 (62.9)	71 (47)	40 (49.4)	[b]**0.004**
CCC, n (%)	2 (1.3)	0 (0)	7 (4.6)	0 (0)	NS
Ascites, n (%)	0 (0)	30 (42.9)	107 (70.9)	61 (75.3)	[e]**<0.001**
Variceal bleeding, n (%)	0 (0)	3 (4.3)	41 (27.2)	24 (29.6)	[e]**<0.001**
AKI or AKI-HRS, n (%)	0 (0)	0 (0)	25 (16.6)	46 (56.8)	[f]**<0.001**
HE, n (%)	0 (0)	11 (15.7)	49 (32.5)	25 (30.9)	[d]**0.009** [e]**0.056**
PVT, n (%)	6 (3.9)	7 (10)	30 (19.9)	20 (24.7)	[a]**<0.001** [c]**<0.001** [e]**0.039**

NAD, non-acute decompensation; AD, acute decompensation; ACLF, acute-on-chronic liver failure; HCC, hepatocarcinoma; CCC, cholangiocarcinoma; AKI: Acute-Kidney Injury; HRS: Hepatorenal Syndrome; HE, hepatic encephalopathy; PVT, portal vein thrombosis

[a]compensated-AD; [b]compensated-NAD; [c]compensated-ACLF; [d]NAD-AD; [e]NAD-ACLF; [f]AD-ACLF.

a) Compensated subgroup

Out of the total, 154 patients did not experience liver events that would categorize them into the NAD, AD, or ACLF subgroups.

Factors significantly associated with the compensated condition in the univariate analysis included viral etiology and current therapy with ACE-i/CCBs, β-blockers, statins, corticosteroids, and DOACs. Conversely, type II diabetes, CKD, heart failure, HCC, PVT, MDROs colonization, alcoholic etiology, and diuretics were found to be significantly unrelated to the compensated subgroup. *Table 6*

In the multivariate analysis, type II diabetes, CKD, diuretics, HCC, PVT, and MDROs colonization remained significant for liver decompensation. Conversely, current therapy with ACE-i/CCBs, β-blockers, corticosteroids, and DOACs were confirmed as significant factors for compensation. *Figure 2*

Table 6 Univariate and multivariate analysis of the compensated subgroup

	UNIVARIATE		MULTIVARIATE	
Variable	**p-value**	**OR CI (95%)**	**p-value**	**OR CI (95%)**
Type II Diabetes	0.027	0.62 (0.40-0.94)	**0.040**	0.58 (0.34-0.97)
CKD	0.039	0.36 (0.12-0.87)	**0.053**	0.32 (0.09-0.96)
Heart failure	0.038	0.53 (0.29-0.95)		
Metabolic cirrhosis	NS			
Alcoholic cirrhosis	0.002	0.45 (0.26-0.74)	0.355	0.73 (0.37-1.42)
Viral cirrhosis	0.002	1.87 (1.27-2.78)	0.060	1.71 (0.98-3.02)
Diuretics	0.006	0.57 (0.39-0.85)	**0.044**	0.61 (0.38-0.98)
ACE-i/CCBs	<0.001	2.10 (1.27-3.47)	**0.004**	2.10 (1.27-3.47)
β-blockers	0.030	1.54 (1.04-2.29)	**0.002**	2.14 (1.33-3.49)
Statins	0.095	1.60 (0.92-2.78)	0.295	1.44 (0.72-2.87)
Corticosteroids	0.004	2.47 (1.33-4.63)	**0.041**	2.15 (1.03-4.50)
DOACs	<0.001	2.48 (1.54-3.99)	**<0.001**	3.28 (1.76-6.23)
Rifaximin	NS			

HCC	0.009	0.59 (0.40-0.87)	**0.003**	0.48 (0.30-0.78)
PVT	<.001	0.17 (0.07-0.38)	**<0.001**	0.14 (0.05-0.35)
MDROs colonization	0.008	0.37 (0.17-0.72)	**0.008**	0.33 (0.14-0.72)

CKD, chronic kidney disease; ACE-i/CA, ace-inhibitors/calcium antagonists; DOAC, direct-acting oral anticoagulants; HCC, hepatocarcinoma; HE, hepatic encephalopathy; PVT, portal vein thrombosis; MDROs, multi-drug resistant microrganisms.

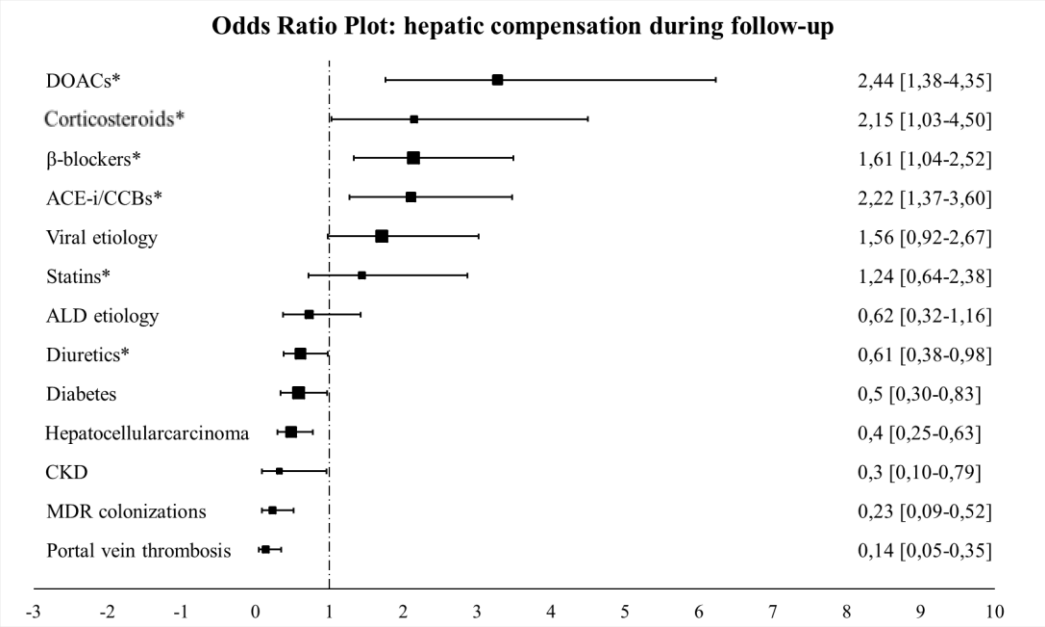

Figure 2 Forest plot for the compensated subgroup

b) NAD subgroup

The NAD subgroup comprises 70 patients who progressively (unlike the AD subgroup) developed ascites, hepatic encephalopathy grade 1-2, and jaundice in case of non-cholestatic liver cirrhosis (in the absence of ACLF episodes).

Regarding therapies, in the univariate analysis, current therapy with ACE-i/CCBs, β-blockers, statins, and DOACs showed protective effects against the development of NAD. *Table 7*

Factors associated with the NAD subgroup included the historical presence of HCC, while viral etiology and current therapy with DOACs were significantly more related to the compensated status in the multivariate analysis. *Figure 3*

Table 7 Univariate and multivariate analysis of the NAD subgroup

	UNIVARIATE		MULTIVARIATE	
Variable	**p-value**	**OR CI (95%)**	**p-value**	**OR CI (95%)**
Type II Diabetes	NS			
CKD	NS			
Metabolic cirrhosis	NS			
Alcoholic cirrhosis	NS			
Viral cirrhosis	0.160	0.75 (0.50-1.12)	**0.057**	0.66 (0.43-1.01)
ACE-i/CCBs	0.069	0.66 (0.42-1.03)	0.263	0.76 (0.47-1.22)
β-blockers	0.197	0.77 (0.52-1.14)	0.386	0.83 (0.55-1.26)
Statins	0.101	0.59 (0.30-1.08)	0.469	0.77 (0.36-1.55)
DOACs	0.008	0.47 (0.26-0.81)	**0.033**	0.52 (0.28-0.93)
Rifaximin	NS			
HCC	0.044	1.50 (1.01-2.22)	**0.004**	1.85 (1.22-2.81)
PVT	NS			
MDROs colonization	NS			

CKD, chronic kidney disease; ACE-i/CCBs, ace-inhibitors/calcium channel blockers; DOACs, direct-acting oral anticoagulants; HCC, hepatocarcinoma; PVT, portal vein thrombosis; MDROs, multi-drug resistant microrganisms.

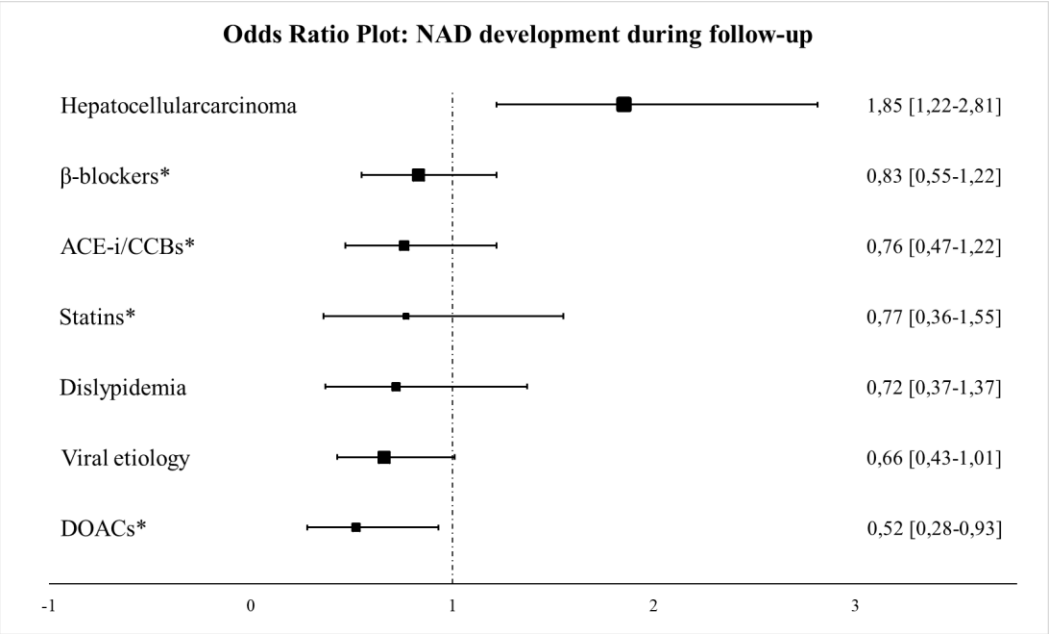

Figura 3 Forest plot of NAD subgroup

c) AD subgroup

The AD subgroup comprises 151 patients. When compared with the compensated subgroup, the univariate analysis revealed a significant association between the development of AD and the presence of type II diabetes, chronic renal failure, and heart failure, as well as current therapy with diuretics, hypertensive medications, β-blockers, corticosteroids, diabetic medications, and antidiabetics. No differences were found regarding different liver etiologies.

A positive MDROs colonization was significantly correlated with AD (p-value 0.001) with an Odds Ratio of 3.20 in the multivariate analysis, as well as the presence of PVT (p-value <.001, OR 5.69), CKD (p-value 0.003, OR 4.42) and type II diabetes (p-value 0.006, OR 1.97). *Table 8*

In terms of current therapy, β-blockers, DOACs, and corticosteroids were found to be protective against the development of AD, while statins and rifaximin did not show significance. *Figure 4*

Table 8 Univariate and multivariate analysis of the AD subgroups

	UNIVARIATE		MULTIVARIATE	
Variable	p-value	OR CI (95%)	p-value	OR CI (95%)
Type II Diabetes	0.003	1.80 (1.22-2.68)	**0.006**	1.97 (1.21-3.22)
CKD	0.004	3.58 (1.59-9.15)	**0.003**	4.42 (1.75-12.40)
Heart failure	0.008	2.06 (1.22-3.56)		
Metabolic cirrhosis	0.094	1.48 (0.94-2.37)	0.762	1.14 (0.50-2.58)
Alcoholic cirrhosis	0.015	1.72 (1.11-2.69)	0.385	1.40 (0.65-3.01)
Viral cirrhosis	0.003	0.57 (0.39-0.82)	0.288	0.68 (0.33-1.38)
Autoimmune cirrhosis	0.063	0.50 (0.23-1.02)		
Diuretics	0.009	1.64 (1.13-2.37)		
ACE-i/CCBs	0.049	0.67 (0.45-1.00)	0.110	0.68 (0.43-1.09)
β-blockers	0.039	0.68 (0.47-0.98)	**0.003**	0.52 (0.34-0.79)
Statins	NS		0.802	1.08 (0.58-2.02)
Antidiabetics/insulin	0.010	1.69 (1.14-2.54)		
Corticosteroids	0.003	0.36 (0.18-0.68)	**0.011**	0.37 (0.17-0.78)
DOACs	0.011	0.54 (0.33-0.86)	**0.016**	0.49 (0.27-0.87)
Rifaximin	NS			
HCC	NS			
PVT	<.001	4.46 (2.42-8.80)	**<.001**	5.69 (2.92-11.84)
MDROs colonizations	0.002	2.61 (1.46-4.87)	**0.001**	3.20 (1.65-6.48)

CKD, chronic kidney disease; ACE-i/CCBs, ace-inhibitors/calcium channel blockers; DOACs, direct-acting oral anticoagulants; HCC, hepatocarcinoma; PVT, portal vein thrombosis; MDROs, multi-drug resistant microrganisms

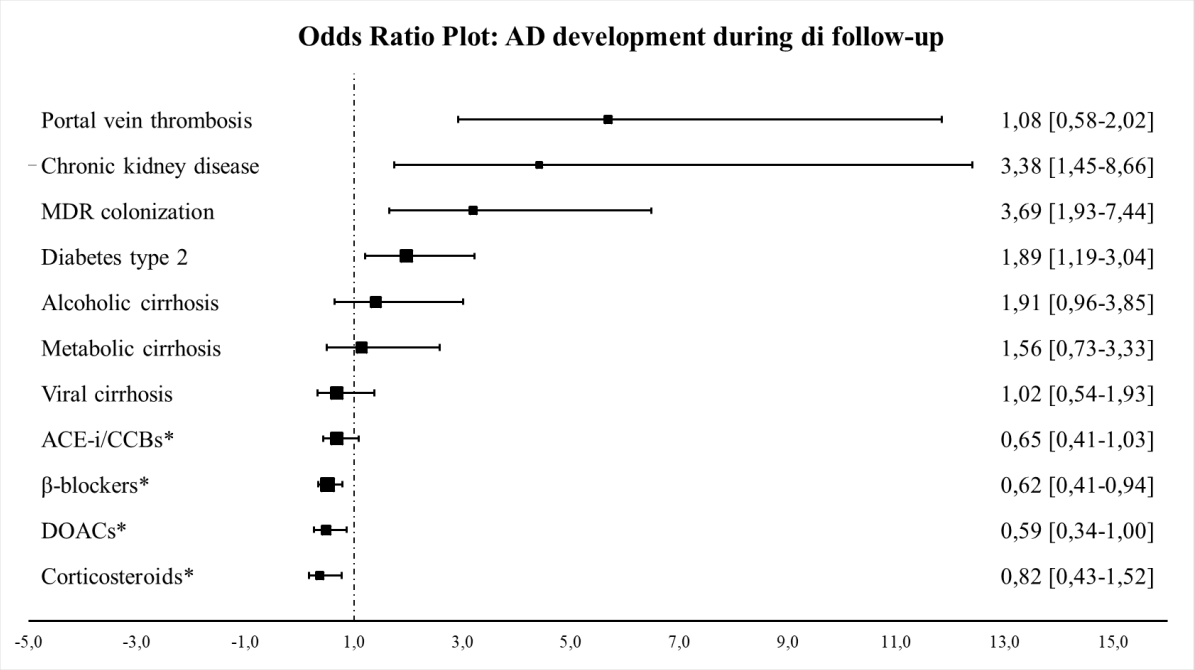

Figure 4 Forest plot for the development of AD

d) ACLF subgroup

During the study period, 81 patients experienced an episode of ACLF.

ACLF patients were found to be most frequently affected by CKD and heart failure. In multivariate analysis, current therapy with β-blockers was not significantly associated with the presence of ACLF. However, the presence of positive colonization with multidrug-resistant organisms (MDROs) (p-value 0.014, OR 2.55), portal vein thrombosis (PVT) (p-value <.001, OR 3.58), and a creatinine level higher than 1.15 mg/dl (p-value 0.001, OR 3.21) showed a strong association with the ACLF condition. *Table 9, Figure 5*

Table 9 Univariate and multivariate analysis of the ACLF subgroup

	UNIVARIATE		**MULTIVARIATE**	
Variable	**p-value**	**OR CI (95%)**	**p-value**	**OR CI (95%)**
Type II Diabetes	NS			
CKD	<0.001	4.40 (2.04-9.35)	0.151	2.09 (0.76-5.74)
Heart failure	0.004	2.33 (1.28-4.12)	0.180	1.66 (0.78-3.43)

Metabolic cirrhosis	NS			
Alcoholic cirrhosis	NS			
Viral cirrhosis	0.091	0.65 (0.39-1.06)	0.242	0.71 (0.40-1.25)
Diuretics	0.070	1.57 (0.97-2.58)		
ACE-i/CCBs	NS			
β-blockers	NS		**0.037**	0.54 (0.30-0.96)
Statins	NS	0.79 (0.35-1.61)		
DOACs	0.409	0.76 (0.38-1.41)	0.282	0.67 (0.31-1.36)
Rifaximin	NS			
HCC	NS			
PVT	0.002	2.53 (1.37-4.55)	**<.001**	3.58 (1.80-7.03)
MDROs colonization	0.039	1.95 (1.01-3.62)	**0.014**	2.55 (1.19-5.32)
Creatinine >1.15	<.001	4.08 (2.30-7.20)	**0.001**	3.21 (1.57-6.44)

CKD, chronic kidney disease; ACE-i/CCBs, ace-inhibitors/calcium channel blockers; DOACs, direct-acting oral anticoagulants; HCC, hepatocarcinoma; PVT, portal vein thrombosis; MDROs, multi-drug resistant microrganisms; SBP, spontaneous bacterial peritonitis; UTI, urinary tract infection.

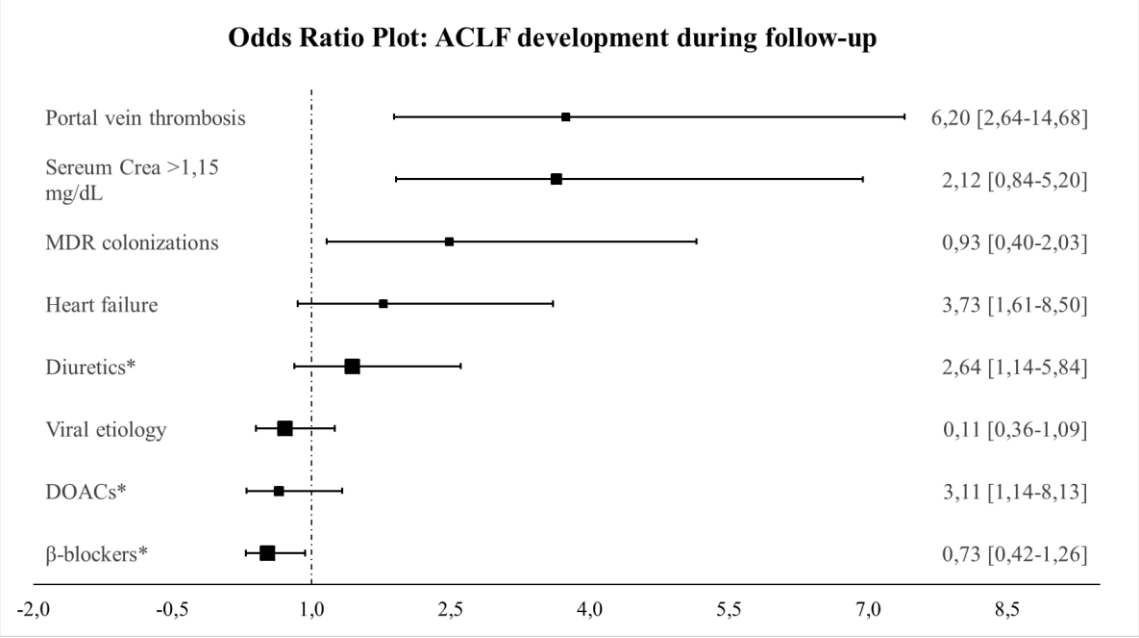

Figure 5 Forest plot for the development of ACLF

A comparison between AD and ACLF subgroups showed at univariate analysis a significant association of ACLF with UTI, sepsis and a positive MDROs infections. Conversely, in the multivariate

analysis only UTI and sepsis remained significant (p-value=0.048, OR 2.30 and p-value 0.033, OR 2.26 respectively). *Table 10*

Table 10 Univariate and multivariate analysis comparing AD and ACLF subgroups

Variable	UNIVARIATE p-value (95%)	OR CI	MULTIVARIATE p-value	OR CI (95%)
MDROs infections	0.067	2.06 (0.95-4.50)	0.646	1.23 (0.49-3.01)
MDROs colonization	NS			
SBP	NS			
Pneumonia	NS			
UTI	0.017	2.61 (1.19-5.84)	**0.048**	2.30 (1.01-5.33)
Sepsis	0.006	2.60 (1.33-5.14)	**0.033**	2.26 (1.06-4.83)
Abdominal infections	NS			

CKD, chronic kidney disease; AKI, acute kidney injury; HRS, hepatorenal syndrome; MDROs, multi-drug resistant microrganisms; SBP, spontaneous bacterial peritonitis; UTI, urinary tract infection

e) Survival

The 12-month survival rate of NAD patients is significantly lower compared to compensated patients (90% vs 97.9%, respectively). Moreover, when considering the 36 and 60-month survival rates, they are 72.9% and 58.8% for NAD patients versus 92.3% and 79.9% for compensated patients, respectively.

In terms of AD compared to the compensated subgroup, there is a significant difference in the survival rate at 24 months: 74.7% versus 94.6%. This difference continues to escalate exponentially at 60 and 72 months, with rates of 42.8% and 38.7% for AD patients versus 79.9% and 76.2% for compensated patients, respectively. Regarding the ACLF subgroup, as expected based on definition, there is a significant decrease in the survival rate: at 12 months, it is 97.9% for compensated patients versus 79.8% for the ACLF subgroup; at 3 years, it is 92.3% versus 44.9%; and at 5 years, it is 79.9% versus 14.2%. *Table 11*

Table 11 Survival rate in months during the study period

	12	24	36	48	60	72
Compensated	97.9%	94.6%	92.3%	84.9%	79.9%	76.2%
NAD	90%	80%	72.9%	63%	58.8%	58.8%
AD	91.1%	74.7%	61.5%	53.8%	42.8%	38.7%
ACLF	79.8%	54.4%	44.9%	29.9%	14.2%	9.5%

The comparison between compensated patients and the other subgroups (NAD, AD and ACLF) in terms of mortality showed a significant difference in all cases. Moreover, ACLF subgroups compared with AD and NAD subgroups, resulted significantly different (p-value <.001), while the comparison NAD-AD resulted still significant but with a lower p-value (p-value 0.040) underling the higher impact of ACLF on mortality. *Figure 6*

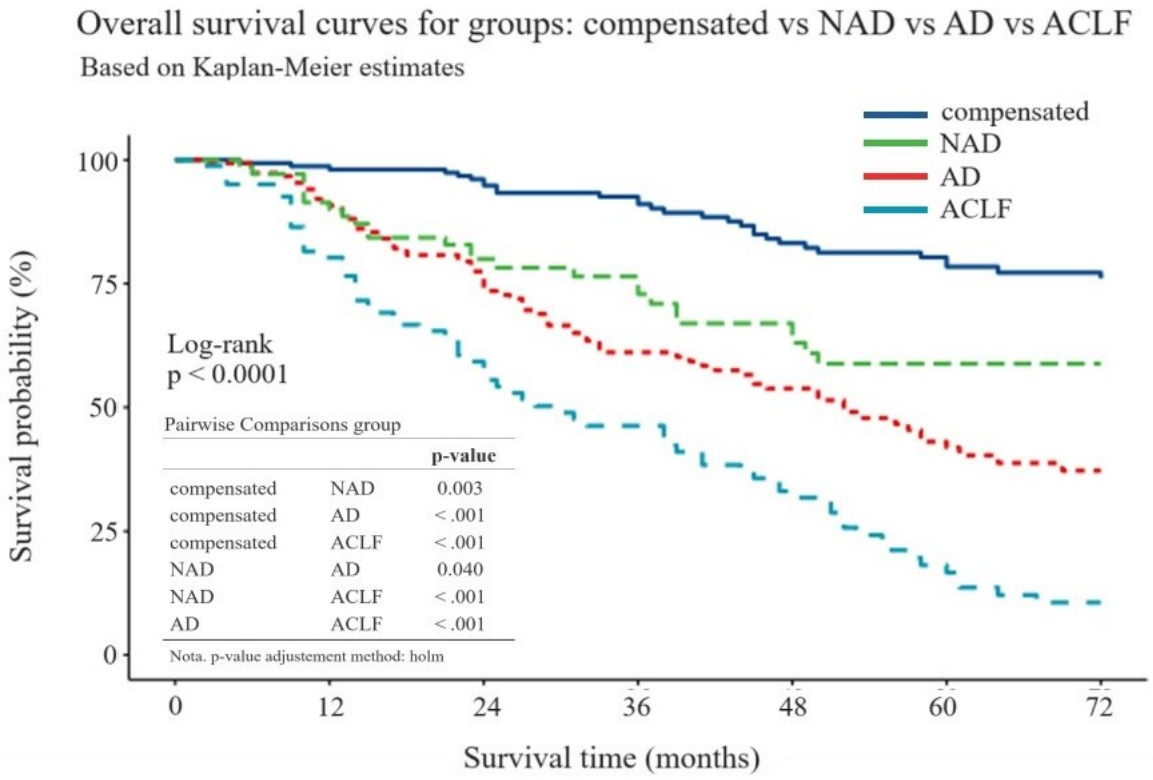

Figure 6 Survival considering different subgroups

Discussion

Bacterial infections represent a significant hazard to the health and well-being of patients with cirrhosis, particularly when they involve MDROs. Adhering to current guidelines, a thorough evaluation of the local and regional microbiological background is essential for optimizing antibiotic therapy through effective stewardship practices, including the selection of appropriate antibiotics, timing of administration, and de-escalation strategies. [47]

Our primary aim was to examine the prevalence of infections among cirrhotic patients followed at our centre, with a specific emphasis on those resulting from MDROs, and to analyse their microbiological profile. We discovered that 31% of our patients developed bacterial infections, aligning with existing literature. [34, 37, 42] Additionally, 30% of these infections occurred in patients with a history of previous infectious episodes, indicating a propensity for recurrent infectious complications in cirrhotic patients. [36, 38]

Throughout our study period, sepsis emerged as the most prevalent type of bacterial infection, accounting for 30% of cases, followed by pneumonia (25%), SBP (18%), and UTIs (20%). Notably, we observed an upward trend in pneumonia cases (19.8% vs 26.7%), likely influenced by the COVID-19 pandemic from 2020 to 2022. We also noted an increased incidence of UTIs (11.9% vs 20.12%). One potential explanation could be the notably higher occurrence of UTIs in patients with ACLF compared to those with AD. This suggests that more critically ill patients, who are more likely to require urinary catheterization, face an elevated risk of urinary tract infections.

Our research findings diverge from those of previous studies, particularly regarding the prevalence rates of sepsis (30% compared to 8–15%) and SBP (18% compared to 20–30%). [35, 38, 52] However, the heightened incidence of sepsis in our population may be attributed to the extensive blood culture testing conducted as part of our antimicrobial stewardship program's protocols. Additionally, unlike the study by Lingiah et al., which reported higher infection rates, we excluded asymptomatic bacteriuria and bacterascites from our primary analysis in alignment with our SAVE guidelines. These guidelines reserve antibiotic treatment only for cases with clinical indications of infection, consistent with similar guidelines. [53, 54] Piano et al., in a recent multicentre study, reported that MDROs infections affected 34% of cirrhotic patients overall, with notable regional disparities, particularly in India where the prevalence reached 73%. [36] Similarly, Fernandez et al. observed a rise in the

overall occurrence of MDROs infections from 29% in 2011 to 38% in 2018. [37, 48] In our investigation, the comparatively lower frequency of MDROs infections, at nearly 20%, could be attributed to our antibiotic stewardship measures. These practices emphasize the judicious use of antibiotics, de-escalation therapy, and meticulous management of colonized patients to mitigate MDROs transmission. Our findings mirror the rigorous contact isolation protocols enforced for colonized or infected patients in our ward, with the aim of contain the spread of MDROs as much as possible. The implementation of SAVE has facilitated an improvement in the screening and management of colonized patients, as demonstrated by the increase in the prevalence of colonized patients from the initial to the extended cohort (6.9% vs 12.7%), while concurrently maintaining the overall prevalence of MDROs infections (20.8 vs 21.1%).

Furthermore, we conducted an examination of the local microbial epidemiology, particularly comparing it with other studies conducted in Italy, where the approach to antibiotic therapy must account for a high incidence of MDROs. [52] However, in our local context, such a strategy might inadvertently contribute to an escalation in local antibiotic resistance and the prevalence of MDROs. To substantiate this concern, we documented an elevated occurrence of MDROs among patients with a history of antibiotic prophylaxis. In our investigation, MR coagulase-negative Staphylococci were the most frequently isolated MDROs (30%), while gram-negative bacteria, including Escherichia coli producing ESBLs, multidrug-resistant Pseudomonas spp., Klebsiella pneumoniae, and Proteus mirabilis, constituted 43% of the total isolated microorganisms. These findings align with the observed epidemiological shift in recent years, characterized by an increase in gram-positive infections, possibly influenced by the prophylactic use of norfloxacin for SBP in high-risk cirrhotic patients. [55] Conversely, ESBL-producing E. coli was infrequently identified (8.5% of MDR infections). [35, 36, 55, 56]

As mentioned in the introduction paragraph, liver cirrhosis requires a better prognostic definition due to the identification of different phases characterized by a significant difference in mortality rates, primarily with the transition from compensated to non-acute and acute decompensation, and secondarily with ACLF, characterized by the highest mortality.

The secondary focus of our research was to discern variations in prognosis among different subgroups and to identify factors associated with their development, with particular emphasis on the role of infections and pharmacological therapy.

The association between colonization by MDROs and hepatic decompensation was found to be significant, as evidenced by the odds ratio (OR) of 0.33 (95% CI 0.14-0.72) for compensated patients, while colonized patients faced a threefold higher risk of developing AD (OR 3.20; 95% CI 1.65-6.48). Additionally, an association between the development of ACLF and MDROs colonization was observed (OR 2.55; 95% CI 1.19-5.32), underscoring the impact of this condition on the deterioration of liver function. Although it is well established how MDROs infections can affect mortality and liver function, there is a dearth of literature regarding the influence of MDROs colonization on liver decompensation. A recent multicentre study conducted on patients in Barcelona and Frankfurt revealed that MDROs colonization was linked to a higher risk of infection by the colonizing bacteria in the short term. [57] Verma et al. focused solely on critically ill patients admitted to the intensive care unit (ICU) in their study, while others examined liver transplant recipients or patients with variceal bleeding. [58-60] Notably, Mucke et al. found that MDROs colonized cirrhotic patients admitted for variceal bleeding did not exhibit an increased risk of de novo infections or rebleeding compared to non-colonized patients. [61] To our knowledge, this is the first study to evaluate the impact of MDROs colonization on the development of liver decompensation, comparing compensated patients with those experiencing AD and ACLF. The robustness of this association may be considered particularly convincing, given the lower prevalence of MDROs colonization in our cohort compared to other studies.

Another factor not significantly associated with liver compensation but strongly linked to the development of both AD (OR 5.69; 95% CI 2.92-11.84) and ACLF (OR 3.58; 95% CI 1.80-7.03) was PVT.

The pathogenesis of non-neoplastic PVT remains unclear. A previous study conducted at our centre revealed, for the first time, a correlation between the development of PVT and prior infectious episodes. [89] It was hypothesized that systemic inflammation triggered by infections could initiate thrombotic events by inducing endothelial dysfunction and injury, reducing blood flow velocity, and increasing hypercoagulability. In particular, the local elevation of inflammatory markers might facilitate the development of PVT. Turon et al. in fact did not find an association between interleukin-6 (IL-6) or other markers of inflammation and acquired or hereditary hemostatic parameters and PVT. [62] Persistent local inflammation may be exacerbated by dysbiosis and altered intestinal mucosal junctions, leading to bacterial translocation and activation of innate immunity, resulting in the production of inflammatory cytokines. These mechanisms, as outlined in the introduction

paragraph, have been implicated in the development of ACLF and AD. This could elucidate the strong correlation observed in our study between ACLF/AD and PVT.

Regarding the role of pharmacological therapy in predisposing different phases of liver decompensation, we found some interesting discoveries. Over the past few years, several studies have suggested a potential benefits of statins for patients with liver cirrhosis. These benefits include lowering portal pressure, improving the function of blood vessels in the liver, reducing fibrogenesis, protecting against damage from ischaemic or re-perfusion damage, decreasing sensitivity to liver damage caused by toxins, and potentially preventing the development of ACLF and slowing down the progression of cirrhosis from various causes. [32, 63-67] However, despite these suggestions, there isn't enough solid evidence to definitively recommend the use of statins in patients with cirrhosis, as highlighted by the Baveno VII consensus. [11, 68]

In our analysis, we didn't find a significant association between prior statin therapy and any specific subgroup, indicating that statins neither significantly protect nor worsen the condition. This lack of association could be due to the relatively small number of patients in our study who were treated with statins, which was about 15%, homogenously distributed along the subgroups.

Rifaximin has been recognized for its usefulness in preventing HE by reducing bacterial overgrowth, which is present in 30-70% of patients with cirrhosis. Additionally, recent research by Kang et al. has suggested that rifaximin may also help prevent complications related to portal hypertension, such as SBP, bleeding, and death, leading to improved survival rates. [69-72] Furthermore, a recent study revealed that low-dose rifaximin can prevent various liver complications, including exacerbation of ascites, HE, and variceal bleeding, compared to a control group. However, it's important to note that there wasn't a significant difference in liver transplantation-free survival rates between the groups. [73] Interestingly, our data didn't show a protective effect of rifaximin during the decompensated phases of liver cirrhosis. Similar to the findings with statins, this could be due to the limited number of patients receiving rifaximin in our current therapy cohort.

Another class of drugs being studied are anticoagulants, which are mainly used in our cohort for conditions like venous thromboembolism, including PVT, or atrial fibrillation. According to the latest guidelines from the European Association for the Study of the Liver, cirrhotic patients have a thrombosis risk at least as high as the general population, if not higher. [74] Indeed, a recent systematic review and meta-analysis demonstrated a 1.7-fold increased risk of venous thromboembolism in cirrhotic patients compared to those without cirrhosis. [75] The previous

notion of a state of anticoagulation in liver cirrhosis has been superseded by newer studies indicating a balance between pro and anticoagulant factors due to decreased liver function. [74, 76] From heparin, which in a 2012 study showed a role in preventing hepatic decompensation and improving survival, to DOACs, there's been a progression in treatment options. [77] However, the safety of DOACs in cirrhotic patients remains a topic of debate, with a recent meta-analysis highlighting a lack of data and the need for further study to better understand their pharmacokinetic and pharmacodynamic profiles in this specific population. [78] Nonetheless, a metanalysis involving 3,111 cirrhotic patients with atrial fibrillation found that DOACs were associated with a reduced risk of major bleeding, gastrointestinal bleeding, and intracranial hemorrhage compared to warfarin. [79] Interestingly, our data revealed an association between DOACs and the compensated state of cirrhosis, with an Odds Ratio of 3.28 (95% CI 1.76-6.23), suggesting a protective role against decompensation. Similarly, neither NAD nor AD subgroups showed a significant association with DOACs, with Odds Ratios of 0.52 (95% CI 0.28-0.93) and 0.49 (95% CI 0.27-0.87) respectively. This suggests that DOACs could play a role in influencing the natural progression of liver cirrhosis, particularly in the development of NAD and AD.

Non-selective β-blockers are increasingly recognized for their role in preventing variceal bleeding and mitigating the worsening of portal hypertension. [3, 11, 80, 81] Recently, their impact has expanded to include decreasing bacterial translocation, reducing the risk of SBP, and improving overall survival independently of bleeding events, potentially even lowering the risk of HCC. However, a recent metanalysis suggests that further studies are needed to confirm these effects. [82-84] In the Predesci trial, long-term β-blocker treatment indicated a possible increase in decompensation-free survival among patients with compensated cirrhosis and clinically significant portal hypertension, primarily by reducing the incidence of ascites. [31] Our study supports a protective role of β-blockers in the compensated state (OR 2.14, 95% CI 1.33-3.49), while we found no significant association with AD or ACLF (OR 0.52, 95% CI 0.34-0.79; OR 0.54, 95% CI 0.30-0.96, respectively).

Medications that target the renin-angiotensin-aldosterone system appear to have a beneficial effect on reducing fibrosis in various organs, including the liver. In a recent meta-analysis, these drugs were found to decrease portal hypertension in Child-Pugh class A patients, while Zhang et al. suggested a potential role for them in reducing liver-related events and HCC in patients with non-alcoholic fatty liver disease. [85] Moreover, recent research indicated a potential role for amlodipine in murine

models of steatohepatitis, demonstrating its ability to reduce hepatic steatosis, intestinal dysbiosis, and bacterial overgrowth, while improving levels of inflammatory markers such as IL-6 and IL-10, and even regressing atherosclerosis through its anti-inflammatory and anti-oxidative stress properties. [86-88] However, literature on the effects of ACE-i/CCBs in compensated cirrhotic patients is currently lacking. [85] For the first time, we demonstrate the effectiveness of these drugs in preventing hepatic decompensation. In the compensated subgroup, the odds ratio was 2.10 (95% CI 1.27-3.47) with a p-value of .002, while in the NAD, AD and ACLF subgroups, these drugs did not show significant effects.

Our study has several limitations. Firstly, it is retrospective and conducted at a single centre, using prospectively collected data. Unfortunately, some microbiological results are missing from the dataset. Additionally, given that the study period coincided with the COVID-19 pandemic, there may be some missing admissions.

However, our study's strength lies in its sample size and the long follow-up period. Furthermore, we analysed our cohort using the latest classification for decompensation, comparing compensated patients with those experiencing AD and ACLF. We paid particular attention to infections and the role of MDROs colonization, as well as examining the effects of pharmacological therapy.

Conclusion

In conclusion, our study reaffirms the high incidence of bacterial infections in the medical history of cirrhotic patients, particularly in cases involving MDROs, and underscores the frequency of complications associated with these infections. It is crucial to promptly define local epidemiology regarding the most common microorganisms and their antibiotic resistance profiles to initiate empiric therapy promptly and subsequently transition to targeted treatment, aligning with antibiotic stewardship guidelines.

Furthermore, our study enabled the identification of specific factors associated with the development of various phases of liver decompensation. In general, we can conclude that therapy with DOACs, ACE-i/CCBs, and β-blockers appears to be protective against liver decompensation.

Conversely, statins and rifaximin in our cohort did not exhibit a protective correlation. On another note, for the first time, our study demonstrated that a history of PVT and colonization with MDROs may predispose individuals to AD and ACLF. Regarding infections and their role in liver

decompensation, urinary tract infections UTIs were found to pose a higher risk for the development of ACLF compared to AD alone. Additionally, MDROs colonization was associated with an increased risk of progressing to liver decompensation and AD. A clinical implication of our study could be to prioritize monitoring of PVT and MDRO carriers to prevent and promptly diagnose any potential infections or liver-related events, thereby mitigating the risk of liver decompensation to AD or, in the worst-case scenario, ACLF. Moreover, given that MDROs-colonized patients are at a higher risk of developing AD and ACLF, efforts to enhance screening and isolation protocols for these patients on hospital wards to reduce the spread of MDROs are strongly recommended. Future prospective and randomized controlled studies could provide further insights into the pharmacological effects and the impact of infections on the progression of cirrhosis.

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