Serum and Ascitic Fluid Bacterial DNA: A New Independent Prognostic Factor in Noninfected Patients with Cirrhosis

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We tested the hypothesis that the presence of bacterial DNA (bactDNA) in ascitic fluid and serum is associated with decreased survival in patients with cirrhosis. In a prospective, multicenter study, we analyzed the clinical evolution of 156 patients with cirrhosis and ascites (first or recurrence) with lower than 250 polymorphonuclear cells (PMN)/μL, negative ascites bacteriological culture, and absence of other bacterial infections being admitted for evaluation of large-volume paracentesis, according to the presence of bactDNA at admission. Survival, causes of death, and successive hospital admissions were determined during a 12-month follow-up period. BactDNA was detected in 48 patients. The most prevalent identified bactDNA corresponded to *Escherichia coli* (n = 32/48 patients, 66.6%). Patients were followed for 12 months after inclusion and in this period 34 patients died: 16 of 108 (15%) bactDNA negative versus 18 of 48 (38%) bactDNA positive ($P = 0.003$). The most frequent cause of death was acute-on-chronic liver failure in both groups (7/16 and 9/18 in patients without or with bactDNA, respectively), although more prevalent in the first month of follow-up in patients with presence of bactDNA (0 versus 4/7). When considering patients with model for end-stage liver disease (MELD) score less than 15, mortality was significantly higher in those with presence of bactDNA. Spontaneous bacterial peritonitis developed similarly in patients with or without bactDNA at admission. *Conclusion*: The presence of bactDNA in a patient with cirrhosis during an ascitic episode is an indicator of poor prognosis. This fact may be related to the development of acute-on-chronic liver failure at short term and does not predict the development of spontaneous bacterial peritonitis. (HEPATOLOGY 2008;48:1924-1931.)

Spontaneous bacterial peritonitis (SBP) is a life-threatening complication in patients with cirrhosis. It has been defined as an ascitic fluid (AF) infection in the absence of any intraabdominal, surgically treatable source of infection.[1] The accepted pathogenic theory of SBP postulates that bacteria of enteric origin cross the intestinal wall in a process that has been called bacterial translocation, reaching mes-

Abbreviations: AF, ascitic fluid; AOCLF, acute-on-chronic liver failure; bactDNA, bacterial DNA; MELD, model for end-stage liver disease; NO, nitric oxide; PMN, polymorphonuclear cells; SBP, spontaneous bacterial peritonitis; TIPS: transjugular intrahepatic portosystemic shunt.

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enteric lymph nodes and from there the systemic circulation and other organs.[2] Once bacteria reach AF, an SBP episode may develop in the context of a poor AF bactericidal activity.[3,4] To assess the hypothetical bacterial movement at a molecular level in patients with cirrhosis and noninfected AF, we developed a polymerase chain reaction–based method to detect bacterial DNA (bactDNA) in blood and AF in patients with cirrhosis and noninfected AF.[5]

We then reported that roughly 40% of patients with cirrhosis and ascites show the presence of bactDNA, being mostly from gram-negative bacteria.[5] Moreover, bacteria identified from blood samples taken every 8 hours, consecutively obtained during 3 days, were identical to those detected in the first sample.[6] Further studies reported a positive correlation between presence of bactDNA and a higher synthesis of proinflammatory cytokines and nitric oxide (NO), related to the inducible form of NO synthase, in the supernatant of the cultured peritoneal cell content.[7] There is now experimental evidence to suggest that the presence of bactDNA constitutes a surrogated marker of bacterial translocation in experimental cirrhosis.[8,9]

We have previously reported that the presence of bactDNA is associated with increased levels of interferon gamma and NO in both serum and ascitic fluid[7] in patients with noninfected AF. Increased levels of interferon gamma have been shown to first facilitate bacterial translocation through the induction of an abnormal intestinal permeability[10] and then to exacerbate liver damage and fibrogenesis.[11] These data, together with the fact that NO is a key agent in the development of hemodynamic abnormalities usually found in patients with advanced cirrhosis,[12] caused us to hypothesize that the detection of bactDNA might represent an independent marker of poor prognosis in patients admitted with AF. A pilot study supported this assumption.[13] To completely investigate this hypothesis, we designed a prospective, multicenter study in which a series of consecutively admitted patients with cirrhosis and noninfected AF were followed to assess mortality and incidence of SBP during a period of 12 months after first bactDNA detection.

Patients and Methods

Study Design. We conducted a prospective trial at five academic hospitals in Spain to evaluate the prognostic value of bactDNA detection in serum and AF of patients with cirrhosis and sterile AF. Only inpatients being admitted with an ascitic episode for consideration of large-volume paracentesis were considered for inclusion in this study. The inclusion criteria were cirrhosis and presence of noninfected AF [<250 polymorphonuclear (PMN) cells per microliter], regardless of whether it was the first episode of ascites. Cirrhosis was diagnosed by histology or by clinical, laboratory, or ultrasonographic findings. Exclusion criteria were the presence of a culture-positive blood or AF, patients showing two or more of criteria of systemic inflammatory response syndrome according to previously published criteria (temperature >38°C or <36°C, heart rate >90 beats/minute, respiratory rate >20 breaths/minute, blood white blood cells <4000 or >12,000/mm^3),[14] upper gastrointestinal bleeding or intake of antibiotics in the preceding 2 weeks including norfloxacin as primary or secondary prophylaxis of SBP, hepatocellular carcinoma, or portal thrombosis, previous liver transplantation, transjugular intrahepatic portosystemic shunt (TIPS), alcoholic hepatitis, and refusal to participate in the study. None of the patients had previously developed an episode of SBP. The Institutional Review Board at each center approved the study protocol, and all patients provided informed consent for inclusion in the study. Surviving patients were followed up at their respective hospitals according to their intrinsic protocols, and readmitted when clinically indicated.

SBP was defined as the presence of a PMN count in AF equal to or greater than 250/μL irrespective of the result of the microbiological culture.[1] Acute-on-chronic liver failure (AOCLF) was defined as an acute deterioration in liver function over 2 to 4 weeks leading to severe progressive clinical deterioration despite supportive care (over 48 hours) with increasing jaundice (serum bilirubin >5 mg/dL) and either encephalopathy (grade 2) or hepatorenal syndrome,[9,15,16] and bacterial infection–induced hepatorenal syndrome as renal failure in the context of bacterial infection in the absence of septic shock.[17] Primary prophylaxis of bacterial infections with norfloxacin was considered only in patients during an episode of upper gastrointestinal bleeding (400 mg twice daily during 7 days),[18] and secondary prophylaxis in all patients surviving an episode of SBP in the course of the investigation.[19]

Blood was obtained for routine hematological, biochemical, and coagulation studies. Simultaneously, a paracentesis was performed in all patients at admission in aseptic conditions following the usual procedures,[20] and samples for routine biochemical study and PMN count were obtained. Measurement of total protein, albumin, leukocyte count, and PMN count were performed. Blood and AF were inoculated at bedside in aerobic and anaerobic blood culture bottles, 10 mL each.[21] A sample of blood and AF were inoculated in rubber-sealed pyrogen-free tubes (Endo Tube ET, Chromogenix AB) for molecular studies. Tubes were centrifuged at 2000*g* for 10

minutes and stored at −20°C at each hospital. Samples were shipped in dry ice and labeled with consecutive numbers.

Detection of BactDNA. All laboratory procedures for bactDNA detection and species identification were performed at one central laboratory (CIBERehd, Hospital General Universitario de Alicante). Specimens were processed in airflow chambers, and tubes were never exposed to free air. Detection of bactDNA was performed as previously described.[5] Briefly, 200 μL serum or AF were incubated in a lysozyme-proteinase K buffer for 2 hours and placed into QIAamp Spin Columns (Qiagen, Hilden, Germany). A broad-range polymerase chain reaction reaction for the conserved region of the 16S ribosomal RNA prokaryote gene was carried out using the following universal primers: AGAGTTTGATCATGGCTCAG-3′ and 5′-ACCGCGACTGCTGCTGGCAC-3′. Total polymerase chain reaction volume was filtered with QIAquick Spin Columns (Qiagen, Hilden, Germany) before nucleotide sequencing with ABI-Prism Dye Terminator Cycle Sequencing v2.0 Ready Reaction Kit and ABI-Prism 310 automated sequencer (Applied Biosystems, Foster City, CA), according to manufacturers' indications. The identification of sequences was carried out by BLAST at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). Technical details of the method, including accuracy, precision, linearity, and reproducibility, are described elsewhere.[5]

Follow-up of Patients. Clinical and analytical data of all patients were recorded on inclusion in the study together with previous clinical history. Successive hospitalizations including reasons for admission, applied therapy, and diagnosis at discharge in any of the participating hospitals during follow-up were recorded. Participating investigators were unaware of the result of bactDNA detection in the collected samples, and management and follow-up of patients was the usual according to the patient's clinical requirements. Follow-up was finalized if the patient was submitted to TIPS or liver transplantation. The causes of death were recorded for final analysis.

Statistical Analysis. Continuous variables are reported as mean ± standard deviation and categorical variables as frequency or percentages. Statistical differences of basal characteristics between groups were analyzed using the Fisher's test for categorical data and the Mann-Whitney *U* test for quantitative data. Bivariate correlations between continuous variables were calculated using the Spearman test.

Variables listed in Table 1 were analyzed as possible predictors of bactDNA presence using logistic regression analysis. Variables reaching statistical significance ($P < 0.05$) in previous univariate analysis were entered into a forward stepwise conditioned, multiple logistic-regression analysis.

Table 1. Clinical and Analytical Characteristics of Patients with Presence in Serum and AF of bactDNA [bactDNA(+)] and Patients Without bactDNA [bactDNA(-)]

Variable	bactDNA(-) (n = 108)	bactDNA(+) (n = 48)
Age (years) mean ± SD (range)	60 ± 12 (38-83)	64 ± 12 (36-83)*
Male sex N (%)	78 (72)	26 (54)
Etiology N (%)		
Alcohol	63 (58)	20 (42)
HCV	25 (23)	14 (27)
Alcohol + HCV	9 (8)	7 (15)
Other	11 (10)	7 (15)
Active alcohol consumption (%)	48 (67)	17 (63)
Child-Pugh mean score	9.2 ± 1.7	9.4 ± 1.6
Child-Pugh (B/C), N	66/42	29/19
Meld score, N (%)	12.4 ± 4.8	13.1 ± 5.8
<15 (%)	81 (75)	32 (67)
15-24 (%)	25 (23)	16 (33)
24 (%)	2 (2)	0 (0)
Previous episodes of ascites(%)	59 (55)	19 (40)
Patients with refractory ascites† (%)	9 (8)	3 (6)
Previous episodes of hepatic encephalopathy (%)	8 (7)	9 (19)*
Previous episodes of upper gastrointestinal bleeding (%)	23 (21)	11 (23)
Infections other than SBP in the previous 6 months‡ (%)	13 (12)	5 (10)
Patients receiving nonselective beta blockers (%)	14 (12.9)	6 (12.5)
Mean arterial pressure (mmHg)	87 ± 14	83 ± 9*
Heart rate (beats/minute)	80 ± 13	79 ± 12
Temperature (&dg;C)	36.5 ± 0.5	36.5 ± 0.5
Bilirubin (mg/dL)	3.4 ± 3.7	3.1 ± 2.4
Albumin (g/dL)	2.8 ± 0.5	2.8 ± 0.5
Quick (%)	63 ± 14	60 ± 15
INR	1.5 ± 0.4	1.6 ± 0.4
Serum creatinine (mg/dL)	1.0 ± 0.4	0.9 ± 0.3
Serum sodium (mEq/L)	134 ± 6	135 ± 4
Serum potassium (mEq/L)	4.3 ± 0.6	4.2 ± 0.5
Platelets/mm^3	124,289 ± 65,485	104,309 ± 56,707
Blood WBC/mm^3	6049 ± 2630	6174 ± 3459
AF WBC/mm^3	191 ± 178	167 ± 174
% AF PMNs	22 ± 21	27 ± 27
AF Total protein (g/dL)	1.5 ± 0.8	1.5 ± 0.6
AF albumin (g/dL)	0.7 ± 0.5	0.7 ± 0.4

▲AF, ascitic fluid; INR, International normalized ratio; N, number; SD, standard deviation; WBC, white blood cells.

*$P < 0.05$.

†According to Ascitic Club Criteria.

‡None of those infections developed in a 30-day period before inclusion.

The primary outcome was overall survival, defined as the timeframe between study entry and death or end of follow-up. The Kaplan-Meier life-table analysis was used because precise dates of death or termination of follow-up were known. Data were censored at 365 days. Patients subjected to liver transplantation or TIPS procedures were censored at time of procedure. Log-rank test was used to compare sur-

vival curves. Variables included in Table 1 were analyzed as possible predictors of overall survival using the Kaplan and Meier method and the log-rank test. For continuous variables, the cutoff levels chosen were their median values. Etiology was stratified in alcoholic origin or other causes, and model for end-stage liver disease (MELD) score above or below 15 points. Intervals of confidence were calculated using the standard error of survival calculated according to the Greenwood method.

Variables that achieved statistical significance ($P < 0.05$) in previous univariate analysis were entered into a forward stepwise conditioned Cox proportional-hazards regression and hazard ratios, and 95% confidence intervals for overall survival were computed. All reported P values are two-sided, and P values of less than 0.05 were considered to indicate significance. All calculations were performed using the SPSS 14.0 software.

Sample size was estimated according to previous data. In a pilot study investigating the relationship between presence of bactDNA and survival in a group of 34 patients with cirrhosis and ascites, the 1-year survival probability in the group of patients without bactDNA (n = 20) was 0.85 and 0.64 in the group of patients with bactDNA.[13] Then, to detect differences between survival curves of both groups using a bilateral log-rank test with a one-sided type I error of 0.05 and 80% power, a minimum of 142 patients should be included.

Results

Patient Characteristics. A total of 198 consecutively admitted patients were enrolled between December 2003 and December 2006. Forty-two patients were excluded for the following reasons: 13 had an SBP in the baseline paracentesis, five patients showed signs or symptoms of systemic inflammatory response syndrome, in eight patients samples could not be appropriately collected or stored, five patients showed a hepatocellular carcinoma, eight had an upper gastrointestinal bleeding, one had an alcoholic hepatitis episode at admission, one had previously received a liver graft, and one patient had received neomycin in the previous week. Finally, 156 patients were included in the study.

The average (± standard deviation) age of patients was 61 ± 12 years, and 67% were male. The cause of cirrhosis was alcohol in 83 patients, chronic infection by hepatitis C virus in 39 patients, simultaneous chronic hepatitis C infection and alcohol in 16 patients, and other causes in 18 patients. According to the Child-Pugh classification of cirrhosis, 61% were Child-Pugh B and 39% were Child-Pugh C. Seventy-two percent of patients had a MELD score lower than 15, and only two patients (1%) showed a MELD score higher than 24. Fifty percent (n = 78) of patients were included in the study during their first episode of ascites. Among the rest, 36% of patients presented their first episode in the previous year, 6% in the previous 3 years, and 8% of patients more than 3 years before inclusion in this investigation. Twelve patients fulfilled diagnostic criteria of refractory ascites[17] at admission without significant differences between groups (Table 1). Twenty-two percent of patients previously had an episode of upper gastrointestinal bleeding and 11% had previous episodes of hepatic encephalopathy.

Thirteen episodes of SBP were detected in all patients included in this investigation during follow-up. Table 4 shows the bactDNA status of these patients at inclusion in the study. Three patients died as a direct consequence of the episode. Norfloxacin was initiated as secondary prophylaxis of SBP in the surviving cases. Three patients of this subgroup died during follow-up, at 100, 35, and 33 days after resolving SBP, and the causes of deaths were pneumonia, hepatorenal syndrome, and liver insufficiency, respectively.

Bacterial DNA Fragments in Serum and AF. Forty-eight patients (31%) showed the simultaneous presence of bactDNA at the time of inclusion in the study in blood and AF. BactDNA was never detected only in one of these biological samples in a given patient. The identified bacteria were *Escherichia coli* in 32 (66.6%), *Klebsiella spp.* in four (8.3%), *Enterococcus spp.* in three (6.3%), and *Staphylococcus aureus* in nine patients (18.8%). The baseline clinical, basic hemodynamic, and serum and AF analytical characteristics of patients with bactDNA [bactDNA(+)] compared with patients without bactDNA [bactDNA(−)]are shown in Table 1. Mean age was significantly higher, mean arterial blood pressure significantly lower, and previous antecedent of hepatic encephalopathy more frequently observed in bactDNA(+) patients. No other statistical significant differences between groups were found. Clinical and analytical parameters described in Table 1 were included in a logistic regression analysis to evaluate predictive factors associated with bactDNA presence. Age, mean arterial pressure, and a previous episode of hepatic encephalopathy showed a significant relationship with presence of bactDNA ($P = 0.012$, 0.02, and 0.03, respectively).

Survival According to bactDNA in Serum and AF. During a 12-month period after inclusion in the study, 18 of 48 bactDNA(+) patients died as compared with 16 of 108 bactDNA(−) patients (Fisher's exact test; $P = 0.003$) (Fig. 1). Three bactDNA(+) patients, and none of the bactDNA(−) group, died during the baseline hospital admission. Seven of 18 deaths among bactDNA(+) patients occurred over a 30-day period after bactDNA de-

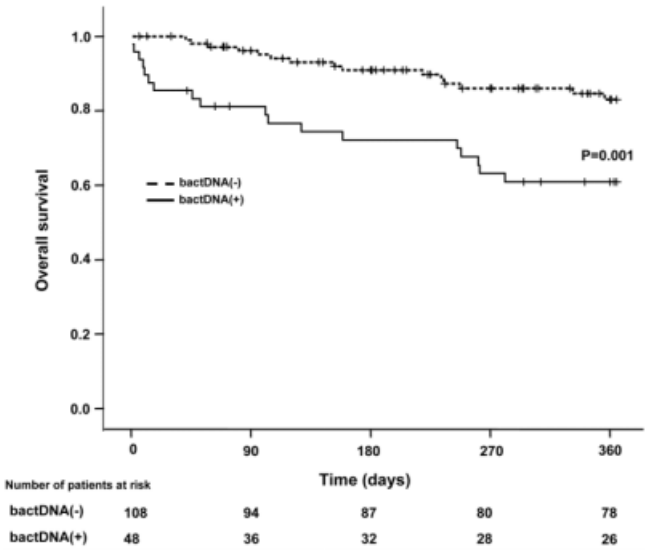

Fig. 1. Overall survival Kaplan-Meier curves in patients with cirrhosis and ascitic fluid according to bactDNA status at 365 days. BactDNA(−) and bactDNA(+) represent patients with absence or presence of bactDNA at baseline respectively.

termination, compared with none among bactDNA(−) patients in the same period, and causes of death included four cases of AOCLF not induced by any infectious complication, one upper gastrointestinal bleeding, one myocardial infarction, and one sepsis of biliary origin. Table 2 details all causes of death of the overall series of patients during the study period.

Figure 1 shows the probability of overall survival for both groups of patients. Two bactDNA(+) patients (4.2%) and seven bactDNA(−) patients (6.5%) did not complete the 12-month follow-up period and were censored from analysis in the last recorded visit. Two bactDNA(+) patients and four bactDNA(−) patients were censored because they underwent TIPS procedures. Three bactDNA(−) patients were censored because of a liver transplantation.

When comparing baseline characteristics of patients who died or survived during the study period, only the presence of bactDNA, male sex, and previous episodes of encephalopathy reached significance (Table 3A). Basal Child-Pugh score was not different in surviving or dying patients during follow-up (Child-Pugh 9.2 ± 1.6 versus 9.6 ± 1.9, respectively, $P = 0.295$). Overall survival Kaplan-Meier curves of patients diverged according to the presence or absence of bactDNA. MELD score at admission was closely correlated to survival, although it did not reach significance ($P = 0.09$), probably because of the short number of mortal events. Notably, the probabilitiy of surviving at the end of the study period in bactDNA(+) patients with MELD < 15 was 0.62, whereas this figure was of 0.89 in this same subgroup of patients without presence of bactDNA ($P = 0.01$) (Fig. 2).

In the Cox proportional hazards multiple regression analysis, the only significant relationship was established between bactDNA status and death [hazard risk of death: 2.6, 95% confidence interval: 1.3-5.2, $P = 0.008$] (Table 3B). Meld score, serum creatinine, and sodium were not statistically significant in the univariate analysis. Because these parameters have been shown to be repeatedly associated with survival in the literature, all three were therefore included in a second multivariate analysis together with all values that showed statistical significance. Again, the only significant relationship was established between bactDNA status and death (hazard risk of death: 2.8, 95% confidence interval: 1.2-6.3, $P = 0.01$).

A significant proportion of deaths were observed in the first month since inclusion in the group of patients with presence of bactDNA (Fig. 1) (7 of 48 versus 0 of 108 according to the presence or absence of bactDNA, respectively, $P = 0.000$). The main cause of death in this period was AOCLF.

Seventy-eight patients were included in this investigation during their first episode of ascites, and bactDNA was detected in 29 patients (37%). One-year mortality was higher in this subset of patients, although values did not reach significance (10 of 29 with presence of bactDNA versus 8 of 49 without bactDNA, $P = 0.069$).

Clinical Evolution of Patients According to bactDNA in Serum and AF. A total of 90 patients of 153 discharged from hospital after their first admission were readmitted during the 12-month follow-up period (58.8%). Twenty-three of 45 bactDNA(+) patients (51%) and 67 of 108 bactDNA(−) patients (62%) (P = NS) required a total of 61 and 209 new hospital admissions during the follow-up period, respectively. The mean time to second hospital admis-

Table 2. Causes of Death in Both Groups of Patients

Cause of Death	bactDNA(-) n = 16	bactDNA(+) n = 18
Acute-on-chronic liver failure	5	7
Upper gastrointestinal bleeding	3	3
Spontaneous bacterial peritonitis (SBP) and	3	3
Liver insufficiency*	1	1
AOCLF	2	2
Type I hepatorenal syndrome	2	1
Infectious complications other than SBP	3	3
Pneumonia	1	¾
Urinary sepsis	1	2
Clostridium difficile cholitis	1	¾
Biliary sepsis		1
Cardiac ischemic disease		1

*Not reaching diagnostic criteria of acute-on-chronic liver failure.

Table 3. Univariate and Multivariate Analysis of Survival

Variable	Dead Patients	Alive Patients	*P*	Categories According to Cutoff Level*	Probability of Survival (%)(CI 95%)†	Log-Rank Test *P* Value
(A) Univariate Survival Analysis at 12 Months						
BactDNA (yes/no) (n)	18/16	30/92	0.001	Yes No	62.5 (48.5-76.5) 85.2 (79.4-92.8)	0.001
Sex (male/female) (n)	16/18	88/34	0.02	Male Female	84.6 (77.5-91.7) 67.3 (54.3-80.3)	0.010
Previous hepatic encephalopathy (Yes/No) (n)	8/26	9/113	0.015	Yes No	52.9 (28.7-77.1) 81.3 (74.7-87.9)	0.015

	Overall Survival (Hazard Ratio [95% CI])	*P* Value
(B) Multivariate Survival Analysis at 12 Months		
BactDNA (yes/no)	2.58 (1.28- 5.19)	0.008
Sex (male/female)	1.99 (0.99-4.00)	0.054
Previous hepatic encephalopathy (yes/no)	1.98 (0.86-4.54)	0.107

N, number; CI 95%, confidence interval 95%.

*For continuous variables, the cutoff level chosen was its median value.

†Intervals of confidence were calculated using the standard error of survival calculated according

sion was 83 days (range, 6-245 days) in bactDNA(+) and 46 days (range, 5-301 days) in bactDNA(−) patients ($P = 0.32$). The causes of new admissions are detailed in Table 4.

Discussion

This multicenter, prospective, and observational investigation shows that the presence of bactDNA in blood

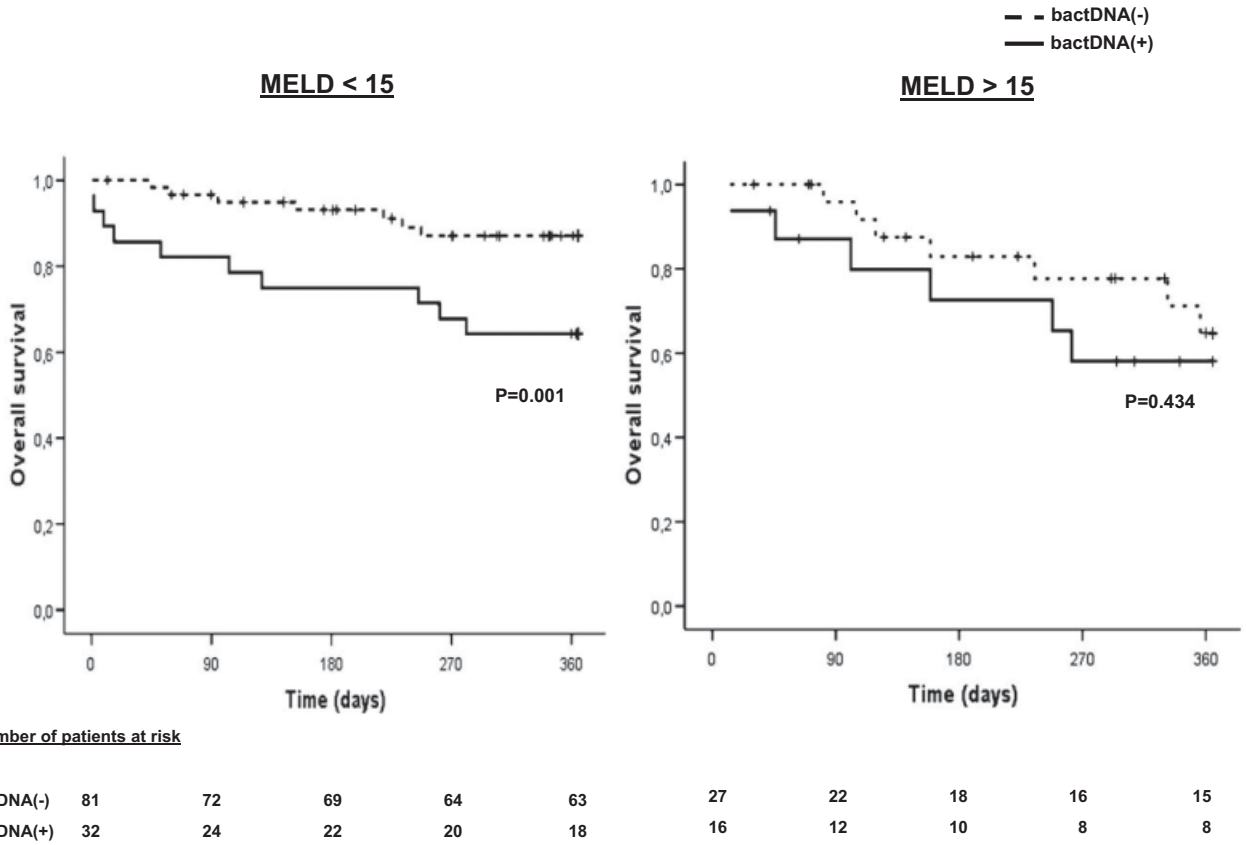

Fig. 2. Overall survival Kaplan-Meier curves in patients with cirrhosis and ascitic fluid according to bactDNA status and Meld score (> or < 15)

Table 4. Causes and Number of New Hospital Admissions in Patients According to Presence of bactDNA

Cause	bactDNA(-) (n = 209)	bactDNA(+) (n = 61)
Ascites	126 (60.3%)	32 (52.4%)
Upper gastrointestinal bleeding	10 (4.8%)	6 (9.8%)
Spontaneous bacterial peritonitis (SBP)	10 (4.8%)	3 (4.9%)
Encephalopathy	33 (15.8%)	5 (8.2%)
Hepatorenal syndrome	6 (2.9%)	4 (6.6%)
Liver transplantation	3 (1.4%)	0 (0.0%)
Infections other than SBP	4 (2.0%)	2 (3.2%)
Abdominal pain unrelated to SBP	4 (1.9%)	2 (3.3%)
Lower gastrointestinal bleeding	2 (0.9%)	5 (8.2%)
Hepatocellular carcinoma	1 (0.5%)	0 (0.0%)
Other*	10 (4.8%)	2 (3.2%)

*Includes tumors other than hepatocellular carcinoma, neurological, cerebrovascular, and peripheral vascular diseases.

and AF constitutes an independent predictor of mortality in patients with cirrhosis. The mortality rate significantly increases in bactDNA(+) patients (Fig. 1), and AOCLF is the most frequent cause of death related to bactDNA presence in the first month after inclusion.

Previous investigations showed that approximately 30% to 40% of patients with cirrhosis and AF show episodes of bactDNA translocation.[5,6] Preliminary data from our group pointed to a higher mortality rate in patients with bactDNA, unrelated to the development of infectious episodes and SBP.[13] Then, to ascertain whether the presence of bactDNA in blood and AF constitutes a new prognostic factor of early mortality, a multicenter, prospective investigation was designed.

There are two main findings of this investigation: first, the risk of mortality at short-term is increased in patients with the presence of bactDNA, and second, AOCLF is the main cause of death in this period. Information already published provides a rationale to explain the prevalence of AOCLF as the most frequent cause of death at short-term in patients with presence of bactDNA. BactDNA activates immune cells through joining toll-like receptor 9 and this in turn induces release of NO and a powerful inflammatory response at both the cellular[7,22] and systemic[23] levels. Increased serum tumor necrosis factor alpha and interferon gamma, which are some of the factors related to the innate immune response activation, up-regulate intestinal permeability and bacterial translocation.[10,24] As a consequence, liver damage and fibrogenesis are exacerbated,[11] and the baseline impaired liver function become further compromised.

As reported here, AOCLF also develops during the clinical evolution of patients without bactDNA on admission, and the development of this complication also may be related to translocation of bacterial fragments. This is a known transient event that may develop in the clinical course of patients.[6] The fact that a series of patients showed bactDNA at inclusion likely indicates that episodes of translocation are more frequent in a subgroup of patients but does not exclude the possibility that episodes of bactDNA translocation occur during follow-up. The fact that a consecutive series of patients included in this investigation (not an inception cohort) have been analyzed when presenting a first, subsequent, or refractory episode of ascites suggests that the clinical status regarding ascites does not indicate the likelihood of showing the presence of bactDNA. Because this investigation was not designed to detect bactDNA translocation in all admissions, we cannot rule out the possibility that patients without bactDNA at the time of inclusion change their molecular bacterial translocation status in subsequent admissions.

The presence of bactDNA in serum and AF is associated with increased levels of NO.[23] NO is a powerful vasodilator agent that plays a key role in the pathogenesis of the peripheral and splanchnic vasodilatation found in cirrhosis.[25] Furthermore, NO levels are significantly increased in the subset of patients with more advanced renal dysfunction.[12] Taking this information into account, we consider that bactDNA translocation increases NO release, further impairing splanchnic hemodynamics. The finding of a significantly lower mean arterial pressure in patients with the presence of bactDNA supports this hypothesis. In this regard, too, it is worthy to consider the use of norfloxacin to revert this situation. In fact, a recently published investigation in patients with advanced cirrhosis who are at high risk of developing SBP has shown a significant reduction in mortality when given primary prophylaxis with norfloxacin.[26]

It may seem counterintuitive that the presence of bactDNA does not precede the development of SBP episodes in patients included in this investigation. Two reasons may explain this: first, the development of SBP requires a viable microorganism reaching AF, and our methodology only detects bacterial fragments that are not necessarily equivalent to bacterial viability; second, it has been shown that the presence of bactDNA may increase the bactericidal defense ability and even immune response in both experimental[27] and clinical settings,[7,28,29] providing protection against bacterial infections when bacterial DNA fragments are repeatedly administered.

Whether bactDNA may act solely as an indicator of poor prognosis or may influence mortality *per se* is not clear from these results. Neither Child-Pugh nor MELD scores take into consideration certain clinical conditions in patients with advanced cirrhosis, such as the degree of portal hypertension, intestinal motility, permeability, and bacterial overgrowth. These phenomena have been usually considered for research purposes, but they might af-

fect survival. BactDNA translocation from the intestinal lumen to blood and AF may be facilitated in these circumstances. In our series, patients with a MELD score lower than 15 and the presence of bactDNA showed a significantly higher mortality rate than that observed in patients with similar MELD in the absence of bactDNA. A large proportion of patients from the bactDNA(+) showed liver insufficiency or AOCLF (Table 2) as the cause of death, suggesting that the presence of bactDNA may be associated with hemodynamic and clinical deterioration of the overall clinical situation of patients.

In summary, we here report evidence that the presence of bactDNA in serum and AF in the patient with cirrhosis and noninfected ascites constitutes a reliable marker of poor prognosis in the short and medium terms. BactDNA positivity is not recognized by other commonly used prognostic scoring systems.

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