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Genetic variants of innate immunity receptors are associated with mortality in cirrhotic patients with bacterial infection

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Abstract

Background & Aims: Acute-on-chronic liver failure (ACLF) is characterized by acute decompensation of cirrhosis (AD), organ failure(s) and high risk of short-term mortality with bacterial infection frequently as precipitating event. Innate immune pattern recognition receptors and members of the lectin pathway of complement activation are crucial to the innate immune response to pathogens. The aim of this study was to investigate whether single nucleotide polymorphisms (SNPs) of innate immune components are associated with the occurrence of bacterial infections or mortality in patients with cirrhosis hospitalized for AD or ACLF.

Methods: Twenty-one innate immunity SNPs with known functional implications were genotyped in 826 AD/ACLF patients included in the CANONIC study. Associations between baseline characteristics of the patients, the occurrence of bacterial infections and survival rate at 90 days of follow-up in relation to the innate immunity genetic variants were analysed.

Results: The NOD2-G908R genetic variant was associated with mortality (HR 2.25, $P = .004$) independently of age and MELD Score. This association was also found in a predefined subgroup analysis in patients with bacterial infections (HR 2.78, $P < .001$) along with MBL_Yx (HR 1.72, $P = .008$) and MASP2_371 (HR 1.67, $P = .012$) genetic

Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; CANONIC, CLIF acute-on-chronic liver failure in cirrhosis; CRP, C-reactive protein; HRMA, high-resolution melting analysis; INR, international normalized ratio; MASPs, MBL-associated serine proteases; MBL, mannan-binding lectin; MELD score, model for end-stage liver disease; MYD88, myeloid differentiation factor 88; NOD2, nucleotide-binding oligomerization domain 2; SBP, spontaneous bacterial peritonitis; SNP, single nucleotide polymorphisms; TLR2, toll-like receptor 2; TLR4, toll-like receptor 4; WBC, white blood cell.

variants. None of the analysed SNPs were significantly associated with the occurrence of acute bacterial infections or spontaneous bacterial peritonitis in particular.
Conclusions: Innate immune system-specific NOD2-G908R, MBL_Yx and MASP2_371 genetic variants were independently associated with increased risk of short-term mortality in AD/ACLF patients with bacterial infection.

KEYWORDS
acute-on-chronic liver failure, bacterial infection, end-stage liver disease, innate immunity, single nucleotide polymorphism

1 | INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a syndrome characterized by acute decompensation (AD) of a patient with cirrhosis, combined with the development of multi-organ failure and a high short-term mortality rate.[1] Bacterial infections are considered the most frequent potential precipitating events in ACLF along with active alcoholism within the last 3 months and gastrointestinal haemorrhage. Bacterial infections are more frequently present in patients with ACLF as compared to patients with 'mere' AD of chronic liver disease (32.6% vs 21.8%, $P < .001$).[1] This higher frequency of bacterial infections in patients with ACLF was because of a higher prevalence of spontaneous bacterial peritonitis (SBP) (10.6% vs 5.6%; $P < .01$) and pneumonia (6.1% vs 2.2%; $P < .01$). Bacterial infections are known to be associated with poorer clinical course and higher mortality in patients with ACLF.[2] Host defense mechanisms are impaired in patients with cirrhosis. Bacterial translocation, defined as the migration of bacteria or bacterial products from the intestinal lumen to mesenteric lymph nodes,[3] and the hyperdynamic circulatory state have been postulated to be key factors in the pathogenesis of infections in cirrhosis.[4] Gut microbiota, alterations in the intestinal mucosal barrier and innate immunity, the antigen nonspecific immune function, are all involved in bacterial translocation.[5,6]

The lectin pathway is part of the complement activation cascade in which molecules function as pattern recognition molecules to active processes of innate immunity. Lectins include ficolins (FNCs) and mannan-binding lectin (MBL) which are mainly produced by the liver.[7] FNCs are a group of oligomeric lectin proteins which recognize components of bacterial or fungal cell walls.[8,9] MBL-associated serine proteases (MASP) can form complexes with MBL and FNCs, leading to activation of the lectin pathway, involving C3b-mediated opsonization of the pathogen and formation of the membrane attack complex.[10] Various single nucleotide polymorphisms (SNPs) in innate immunity genes with functional implications on protein levels have been described. Polymorphisms in the MBL2 gene, for instance, are known to affect proper lectin composition and impair its activity.[11-13] In patients with cirrhosis, deficiency in MBL protein serum levels is reported to be associated with an increased risk of and shorter time to developing bacterial infection.[14] Low levels of serum ficolin were found to be associated with a higher risk of occurrence of cirrhosis-associated bacterial infections.[15]

Key points

- Patients with cirrhosis, a chronic liver disease, have frequent bacterial infections.
- MASP, MBL and NOD2 genes have an important function in the immune system.
- In this study we found that patients with an infection and specific genetic variants in those genes had a higher risk of dying after 90 days.

Multiple other components are also involved in innate immune system signalling against pathogens, such as Toll-like receptor 2 (TLR2), Toll-like receptor 4 (TLR4), myeloid differentiation factor 88 (MYD88) and nucleotide-binding oligomerization domain 2 (NOD2). NOD2 and TLR receptors are involved in the intracellular recognition of bacterial pathogens and therefore are of key importance in the innate immune system. It is yet undefined whether SNPs with known functional implications in these innate immunity components are associated with bacterial infections or mortality in patients with decompensated cirrhosis or ACLF. In this study, we aimed to assess if functionally relevant polymorphisms in the lectin complement pathway and innate immune signalling components for pathogen recognition contribute to the susceptibility to bacterial infections and risk of mortality in cirrhotic patients hospitalized for AD or ACLF.

2 | PATIENTS AND METHODS

2.1 | Study design

This study was performed as part of the CLIF Acute-on-chronic liver failure in cirrhosis (CANONIC) study, a prospective, observational, multicentre study aimed at identifying the diagnostic criteria of ACLF and to describe the development of this syndrome in European patients with AD.[1] Between February and September

2011, 1349 patients with cirrhosis hospitalized for AD were included in 29 hospitals in eight European countries. All 826 patients who gave informed consent for isolation and storage of DNA for future research with DNA available were included in this study. This study was performed in accordance with the Declaration of Helsinki and approved by the ethics committees of all participating centres.

Data from time of enrolment concerning demographic and clinicopathological characteristics of the patients, laboratory measurements (INR, white blood cell [WBC], CRP) and clinical scores (Child-Pugh score, MELD score, CLIF-organ failure score) were retrieved from the CANONIC study database. Patients were followed until death, liver transplantation or end-of-follow-up 3 months following the date of inclusion. AD was defined as an acute development of one or more complications of chronic liver disease. ACLF and individual organ failures were defined using the CLIF-Organ Failure score.[16] The definition of acute bacterial infection included SBP, spontaneous bacteraemia, urinary tract infection, pneumonia and cellulitis as well as every other type of acute bacterial infection diagnosed at baseline hospital admission or during the 3-month follow-up period.

2.2 | Genotype determination

Twenty-one genetic variants in the MBL2, FCN2, MASP2, TLR2, TLR4 and NOD2 genes with known functional implications on protein level were evaluated.[17-20] Genomic DNA was extracted from 10 mL EDTA peripheral blood samples. Genotype identification was performed at the Leiden University Medical Centre, Leiden, The Netherlands. All genotypes were analysed using High-Resolution Melting Analysis (HRMA) with oligonucleotide primers, deoxyribonucleotide triphosphates (dNTPs), polymerase chain reaction (PCR) buffer FS-Taq Polymerase and specific probe in the presence of fluorochrome LC-green Plus. Detailed description of genotyping and assessment has been published previously.[17,18] DNA fragments were visualized using a Idaho Technology Lightscanner. HRMA genotyping of NOD2 was not conclusive (data not shown), therefore it was performed with Biorad Realtime thermal cycler CFX96 with Precision Melt Analysis Software with oligonucleotide primers (Table S1) in the presence of SsoFast EvaGreen Supermix. All found genetic variants were validated by DNA sequencing. The investigators were blinded for clinical outcomes during genotype determination. European cohort minor allele frequencies of the 1000 Genome Project were used as reference of genetic variation.[21]

2.3 | Statistical analysis

Associations between baseline characteristics of the patients, follow-up data and tested genetic variants were analysed by using the Mann-Whitney U test, Student's *t* test or Chi-square test when appropriate. The chi-squared test for Hardy-Weinberg equilibrium was used to evaluate the deviation of equilibrium. Multivariate logistic regression was used to evaluate the association between genetic variants and the occurrence of bacterial infection. Univariate and multivariate competing risks proportional hazards regression analysis was performed for prognostic factors of mortality using the method of Fine and Gray[22] with liver transplantation as competing risk event. Univariate parameters were included at the $P = .20$ level and results with $P \leq .05$ were considered to be statistically significant. The probability of survival within 90 days of follow-up according to the genetic variants was determined with cumulative incidence curves.

3 | RESULTS

3.1 | Patient population

Clinical data and DNA samples were available for 826 patients with decompensated cirrhosis included in the CANONIC study. Within this group, 185 patients were diagnosed with ACLF at study enrolment and 78 patients developed ACLF during the follow-up period. Univariate analysis showed no association between individual SNPs and occurrence or grade of ACLF, therefore in the analyses no distinction was made between patients with ACLF or AD alone. Baseline clinical characteristics of all patients are shown in Table 1. Mean age was 57.6 years and gender was predominantly male (63.6%). Main aetiologies of cirrhosis were alcoholic liver disease (60%) and hepatitis C (32.4%). The majority of patients had ascites at the time of inclusion in this study (89.7%). Patients with acute bacterial infection had higher baseline levels of CRP, WBC and clinical disease score (MELD score 21.6 vs 16.6, $P < .001$ and CLIF-C OF score 8.5 vs 7.1, $P < .001$). Previous prophylactic antibiotic use for SBP was lower in patients with acute bacterial infection (15.6% vs 9.7%, $P = .016$). In Table S2, genotypes and allele frequencies are shown for all genotyped SNPs. Minor allele frequencies of all analysed genetic variants were comparable to previously reported frequencies of the European cohort in the 1000 Genome Project.[21] In some cases, available DNA quantity was not sufficient to obtain complete genotype for all polymorphisms. All allele frequencies were in Hardy-Weinberg equilibrium, except for MBL2_HL ($P = .008$), MASP2_371 ($P = .008$) and MD2_157 ($P < .001$). A homozygous NOD2 mutation was not detected in any patient. For 22 patients no information about the presence or absence of bacterial infection was available.

3.2 | Innate immunity genetic variants are associated with increased mortality

Univariate analysis between genetic variants and mortality showed that patients with a NOD2-G908R genetic variant had a significantly higher risk of mortality at 90 days of follow-up as compared to wildtype profile (5.7% vs 2.2%, $P = .028$), whereas patients with NOD2-R702W and NOD2-L1007FSINSC genetic variants showed no relation with decreased survival (Table 2). Multivariate analysis for mortality at 90 days in all patients (n = 812) showed that NOD2-G908R (OR 2.25, $P = .004$) was the strongest predictor of mortality

TABLE 1 Baseline characteristics of entire cohort of patients with AD or ACLF

Variable	Presence of bacterial infection		*P*-value
	No bacterial infection (N = 473)	Bacterial infection (N = 331)	
Age (y)	58.3 ± 11.79	56.7 ± 11.90	.072
Male gender	314/473 (66.4%)	199/331 (60.1%)	.069
Aetiology of cirrhosis			
Alcoholic liver disease	284/468 (60.7%)	191/327 (58.4%)	.520
HCV	135/447 (30.2%)	112/315 (35.6%)	.120
HBV	22/446 (4.9%)	17/313 (5.4%)	.759
Other	98/449 (21.8%)	57/319 (17.9%)	.178
Clinical features			
Alcoholic hepatitis	4/445 (0.9%)	6/322 (1.9%)	.245
Ascites	406/473 (85.8%)	314/331 (94.9%)	<.001
Hepatic encephalopathy	132/473 (27.9%)	134/331 (40.5%)	.002
Gastrointestinal bleeding	84/473 (17.8%)	42/331 (12.7%)	.052
Organ failures			
Liver	48/473 (10.1%)	63/331 (19.0%)	.003
Kidney	21/473 (4.4%)	80/331 (24.2%)	<.001
Cerebral	7/473 (1.5%)	40/331 (12.1%)	<.001
Coagulation	23/473 (4.9%)	38/331 (11.5%)	.005
Respiratory	4/473 (0.8%)	14/331 (4.2%)	.001
Circulatory	4/473 (0.8%)	28/331 (8.5%)	<.001
Treatments			
Nonselective betablockers	39/461 (8.5%)	38/319 (11.9%)	.112
Prophylactic antibiotic for SBP	72/461 (15.6%)	31/320 (9.7%)	.016
Rifaximin	12/460 (2.6%)	22/319 (6.9%)	.004
Laboratory data			
INR	1.41 (1.26-1.70)	1.60 (1.39-2.05)	<.001
CRP	13.00 (5.00-25.50)	30.40 (13.00-56.00)	<.001
WBC	5.49 (3.88-8.04)	6.94 (4.80-10.80)	<.001
Platelet count	92.00 (58.00-144.00)	77.00 (52.00- 119.00)	.001
Bilirubin	2.70 (1.50-5.60)	4.20 (1.90-9.06)	<.001
Creatinine	0.90 (0.70-1.20)	1.11 (0.80-1.87)	<.001
Clinical scores			
Child-Pugh	9.0 ± 1.93	10.3 ± 2.18	<.001
MELD	16.6 ± 5.84	21.6 ± 8.11	<.001
CLIF-C OF	7.1 ± 1.23	8.5 ± 2.37	<.001

Abbreviation: ACLF, acute-on-chronic liver failure; AD, acute decompensation; CLIF-C OF, chronic liver failure organ failure score; CRP, C-reactive protein; HCV, hepatitis C virus; HE, hepatic encephalopathy; INR, international normalized ratio; MAP, mean arterial pressure; MELD, model for end-stage liver disease; SBP, spontaneous bacterial peritonitis; WBC, white blood cell.

with liver transplantation as competing risk, along with age (HR 1.03, $P < .001$) and MELD score (HR 1.15, $P < .001$). In a predefined subgroup analysis in patients who developed acute bacterial infection at baseline or during 3 months after inclusion (n = 331), heterozygous genetic variants of NOD2-G908R (8.0% vs 0.9%, $P = .002$), MASP_371 (42% vs 25.8%, $P = .012$) and MBL_Yx (42.2% vs 31.8%, $P = .056$) were associated with increased mortality after 90 days of follow-up in comparison to the wildtype profile (Table 3). In a competing risk proportional hazards model, NOD2-G908R (HR 2.78, $P < .001$), MASP2_371 (HR 1.67, $P < .012$) and MBL_Yx (HR 1.72, $P < .008$) genetic variants were associated with worse survival at 90 days, independently of age and MELD score (Figures S1 and S2).

TABLE 2 Univariate and multivariate analysis - mortality of all patients after 90 days

Univariate analysis				Multivariate analysis		
	Alive	Dead	*P*-value		HR (95% CI)	*P*-value
MASP2_120 (TT)	618/652 (94.8%)	146/160 (91.3%)	.089	Age	1.03 (1.02-1.05)	<.001
MASP2_120 (TC)	34/652 (5.2%)	14/160 (8.8%)		MELD score	1.15 (1.12-1.17)	<.001
FCN2B_6359 (CC)	344/655 (52.5%)	69/160 (43.1%)	.103	NOD2_G908R (GC vs GG)	2.25 (1.30-3.91)	.004
FCN2B_6359 (CT)	256/655 (39.1%)	75/160 (46.9%)				
FCN2B_6359 (TT)	55/655 (8.4%)	16/160 (10.0%)				
MASP2_371 (AA)	424/651 (65.1%)	97/158 (61.4%)	.192			
MASP2_371 (AC)	186/651 (28.6%)	55/158 (34.8%)				
MASP2_371 (CC)	41/651 (6.3%)	6/158 (3.8%)				
MBL_haplotype (A)	561/649 (86.4%)	129/159 (81.1%)	.089			
MBL_haplotype (B)	88/649 (13.6%)	30/159 (18.9%)				
MYD_88 (AA)	444/658 (67.5%)	117/160 (73.1%)	.168			
MYD_88 (AG/GG)	214/658 (32.5%)	43/160 (26.9%)				
NOD2_R702W (CC)	582/651 (89.4%)	148/158 (93.7%)	.105			
NOD2_R702W (CT)	69/651 (10.6%)	10/158 (6.3%)				
NOD2_G908R (GG)	636/650 (97.8%)	149/158 (94.3%)	.028			
NOD2_G908R (GC)	14/650 (2.2%)	9/158 (5.7%)				
NOD2_L1007FSINSC (GG)	612/651 (94.0%)	153/158 (96.8%)	.160			
NOD2_L1007FSINSC (GC)	39/651 (6.0%)	5/158 (3.2%)				

Note: In univariate analysis only SNPs with *P*-value < .2 are presented, which were included in the multivariate analysis. Liver transplantation as competing risk in multivariate analysis of mortality. Age and MELD score were taken as independent variables.

3.3 | Acute bacterial infection

Two hundred patients (24.9%) were admitted to the hospital with bacterial infection at baseline and 131 additional patients were diagnosed with a bacterial infection during the 3-month follow-up period. Of all bacterial infections during the study period, 78 (23.6%) were SBP. None of the individual functional polymorphisms were significantly associated with the occurrence of acute bacterial infections or SBP in particular (Table S3). In Table 4, genetic variants in relation to bacterial infections with $P \leq .20$ in univariate analysis are presented. In multivariate analysis, CLIF-C OF score (OR 1.61, $P < .001$) and WBC (OR 1.07, $P < .001$) were significantly associated with bacterial infection. Prophylactic antibiotic use for SBP during the whole study period showed a significant reduced risk for bacterial infection (OR 0.45, $P = .004$).

3.4 | NOD2-G908R, MASP2_371 and MBL_Yx haplotype

Three hundred and fifteen patients (38.7%) had no genetic variant of NOD2-G908R, MASP2_371 or MBL_Yx. Three hundred and sixty-nine patients (45.3%) had one genetic variant present and 130 patients (16%) had two or more genetic variants. In univariate analysis, patients with a haplotype of one, or two or more genetic variants had a significantly increased mortality rate at 28 days (respectively 11% and 17% vs 7%, $P = .001$) and 90 days (21% and 25% vs 17%, $P = .038$) (Table S4). In a predefined subgroup analysis of patients with a bacterial infection (n = 326) and a haplotype of one, or two or more genetic variants, mortality was even higher as compared to patients without genetic variants at 28 days (respectively 24% and 33% vs 10%, $P < .001$) and 90 days (37% and 44% vs 20%, $P < .001$) (Table S5). In a competing risk proportional hazards model, a haplotype with one genetic variant (HR 2.27, CI 1.44-3.56, $P < .001$) and a haplotype of two or more genetic variants (HR 2.64, CI 1.51-4.62, $P < .001$) were associated with worse survival in patients with bacterial infection at 90 days, independently of age or MELD score (Figure 1).

In patients without bacterial infection with at least one genetic variant (n = 466), mortality was not increased as compared to patients without genetic variants at 28 days (3% vs 4%, $P = .289$) and 90 days (9% vs 14%, $P = .088$). There was no association between haplotype genetic variants and occurrence of bacterial infection in all patients (OR 1.12, CI 0.84-1.50, $P = .443$).

4 | DISCUSSION

Bacterial infections are among the most frequent precipitating events in patients with ACLF in Western countries and are associated with systemic inflammation, poor clinical course and high mortality rate.[2]

TABLE 3 Univariate and multivariate analysis - mortality in patients with bacterial infection after 90 days

Univariate analysis				Multivariate analysis		
	Alive	Dead	*P*-value		HR (95% CI)	*P*-value
MASP2_120 (TT)	209/221 (94.6%)	91/101 (90.1%)	.140	Age	1.03 (1.01-1.04)	.003
MASP2_120 (TC)	12/221 (5.4%)	10/101 (9.9%)		MELD score	1.11 (1.08-1.14)	<.001
FCN2B_6359 (CC)	123/226 (54.4%)	44/101 (43.6%)	.023	MBL_Yx (GC/CC vs GG)	1.72 (1.15-2.57)	.008
FCN2B_6359 (CT)	79/226 (35.0%)	51/101 (50.5%)		MASP2_371 (AC/CC vs AA)	1.67 (1.12-2.49)	.012
FCN2B_6359 (TT)	24/226 (10.6%)	6/101 (5.9%)		NOD2_G908R (GC vs GG)	2.78 (1.74-4.44)	<.001
FCN2_4 (AA)	118/223 (52.9%)	48/102 (47.1%)	.067			
FCN2_4 (AG)	84/223 (37.7%)	50/102 (49.0%)				
FCN2_4 (GG)	21/223 (9.4%)	4/102 (3.9%)				
MASP2_371 (AA)	148/221 (67.0%)	54/100 (54.0%)	.012			
MASP2_371 (AC)	57/221 (25.8%)	42/100 (42.0%)				
MASP2_371 (CC)	16/221 (7.2%)	4/100 (4.0%)				
MBL_Yx (GG)	142/223 (63.7%)	51/102 (50.0%)	.056			
MBL_Yx (GC)	71/223 (31.8%)	43/102 (42.2%)				
MBL_Yx (CC)	10/223 (4.5%)	8/102 (7.8%)				
NOD2_R702W (CC)	193/221 (87.3%)	95/100 (95.0%)	.036			
NOD2_R702W (CT)	28/221 (12.7%)	5/100 (5.0%)				
NOD2_G908R (GG)	219/221 (99.1%)	92/100 (92.0%)	.002			
NOD2_G908R (GC)	2/221 (0.9%)	8/100 (8.0%)				

Note: In univariate analysis only SNPs with *P*-value < .2 are presented, which were included in the multivariate analysis. Liver transplantation as competing risk in multivariate analysis of mortality. Age and MELD score were taken as independent variables.

TABLE 4 Univariate and multivariate analysis - risk for acute bacterial infection in all patients

Univariate analysis				Multivariate analysis		
	No bacterial infection	Bacterial infection	*P*-value		OR (95% CI)	*P*-value
FCN2B_6424 (GG)	387/471 (82.2%)	251/328 (76.5%)	.051	CLIF-C OF score	1.61 (1.44-1.81)	<.001
FCN2B_6424 (GT/TT)	84/471 (17.8%)	77/328 (23.5%)		WBC	1.07 (1.03-1.11)	<.001
MBL_HI (GG)	193/466 (41.4%)	141/325 (43.4%)	.187	Prophylactic antibiotic for SBP	0.45 (0.26-0.77)	.004
MBL_HI (GC)	222/466 (47.6%)	137/325 (42.2%)				
MBL_HI (CC)	51/466 (10.9%)	47/325 (14.5%)				

Note: In univariate analysis only SNPs with *P*-value < .2 are presented, which were included in the multivariate analysis. In the multivariate logistic regression model CLIF-C score, WBC and prophylactic use of AB were taken as independent variables.

Abbreviations: CLIF-C OF, organ failure score for acute-on-chronic liver failure; SBP, spontaneous bacterial peritonitis; WBC, white blood cell.

We hypothesized that identification of genetic factors predisposing to systemic infections or mortality in cirrhosis could contribute to better preventive strategies and potential improvement of outcome in cirrhotic patients hospitalized for AD or ACLF. Such an approach might in time contribute to personalized treatment protocols, aiming to reduce bacterial infections as potential precipitating events of AD or ACLF and ultimately improved survival.

In this study we assessed the association between single nucleotide polymorphisms (SNPs) in the lectin complement pathway and innate immune signalling components for pathogen recognition and the occurrence of bacterial infections or mortality in patients with cirrhosis hospitalized for AD or ACLF. We found that patients with bacterial infection and individually or combined NOD2-G908R, MASP2_371 and MBL_Yx genetic variants had a significantly higher risk of mortality during the study period as compared to patients with a wildtype profile. None of the analysed genetic variants were significantly associated with the occurrence of acute bacterial infections in general or SBP in particular.

Bacterial translocation and activation of the innate immune response play important roles in the development of AD in patients with cirrhosis.[5] Innate pattern recognition receptors such as NOD2 receptors are involved in the recognition and clearance of bacterial pathogens and the presence of genetic alterations in these receptors may cause impaired immune function and increased bacterial translocation.[23,24] The association between NOD2 genetic variants

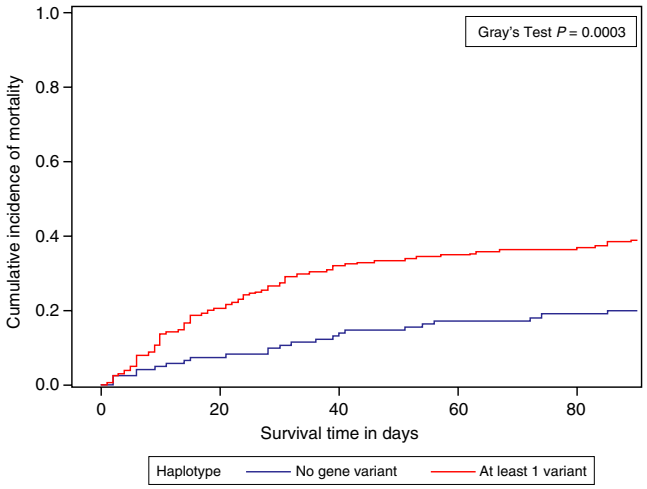

FIGURE 1 NOD2_G908R, MASP2_371 and MBL_Yx gene variants and cumulative incidence of mortality with liver transplantation as competing risk in cirrhotic patients with acute bacterial infection (n=326) at 90 days

and bacterial infection and mortality have been subject of previous studies, showing conflicting results. Appenrodt et al[20] found in 150 patients with cirrhosis and ascites that carriers of common NOD2 genetic variants had a significantly reduced survival in comparison to patients with wildtype genotypes. Bruns et al (n = 175)[25] and Dinya et al (n = 349)[26] subsequently studied this association, however no significant association between common NOD2 genetic variants and higher risk of mortality was observed. In our study, the sample size was sufficient to perform the survival analysis for each NOD2 genetic variant individually, whereas in other studies the carrier status for any NOD2 genetic variant was pooled in the analysis. Interestingly, we found that both NOD2-R702W and NOD2-L1007FSINSC were not associated with increased mortality whereas NOD2-G908R was associated with worse survival independently of age and MELD score. This finding may explain that no effect of NOD2 genetic variants on mortality was reported in the studies of Bruns and Dinya, since all NOD2 genetic variants were pooled together in those survival analyses.[25,26] Another explanation for the difference in findings regarding survival could be that Appenrodt et al and our study had a relatively short follow-up time, in contrast to the longer follow-up periods of the studies of Bruns and Dinya. It is likely that after a longer follow-up period other factors such as (de)compensated end-stage liver failure or other organ failures may have more impact on the survival, whereas in the short term the innate immunity genetic variant has a negative impact on survival, particularly in patients with an acute bacterial infection.

In patients after liver transplantation, our group[17] found that single nucleotide polymorphisms in the MBL, MASP and FNC2 genes were major determinants for the occurrence of clinically significant bacterial infection and mortality. Furthermore, various studies showed that functional deficiencies of MBL, FNC and MASP serum protein levels lead to increased risk of bacterial infection in patients with cirrhosis.[14,15] In our study we did not confirm that the genetic profile of the lectin pathway of complement activation had a major impact on the occurrence of bacterial infection in patients with decompensated cirrhosis. Serum protein levels of lectin pathway molecules were not determined in our study, therefore we could not assess if presence of a genetic variant correlated with clinically relevant deficiency of these molecules. However, NOD2-G908, MBL_Yx and MASP2_371 genetic variants were associated with a significantly higher short-term mortality in patients with an acute bacterial infection. The NOD2-G908 gene variant has previously been shown to be associated with increased translocation of bacterial DNA fragments in ascitic fluid in patients with culture-positive SBP.[24,27] These studies support the functional relevance of this single nucleotide polymorphism. It may be hypothesized that these genetic variants are associated with a more severe clinical course in the presence of bacterial infection, rather than affecting the susceptibility of developing a bacterial infection. However, our study does not permit a conclusion in that respect. There were many missing data with regard to the presence of sepsis and systemic inflammatory response syndrome, which may be considered as markers of more severe outcome. Moreover, there were no data available in the CANONIC database with regard to primary cause of death or the presence or absence of bacterial infection at time of death.

The studies of Appenrod et al[20] and Bruns et al[25] reported strong associations between pooled NOD2 genetic variants and the presence of SBP in patient cohorts with comparable disease severity as expressed by the Child-Pugh score and MELD score. In this study we could not confirm these findings, however there are several potential explanations for this discrepancy. Firstly, the prophylactic intake of antibiotics varied between the cohorts although to which extend this influenced the outcome cannot be determined. Secondly, no protocol-driven diagnostic paracentesis was performed for our study, therefore potentially some SBP cases may have been missed. Finally, the approach we used by analysing the different NOD2 single nucleotide polymorphisms separately may account for absence of association between SBP and NOD2 SNPs. In a recent study in patients with compensated cirrhosis, pooled NOD2 genetic variants were found to be a risk factor for the occurrence of bacterial infections but not in a subgroup of patients with decompensated cirrhosis.[28] This suggests that NOD2 genetic variants have an impact on susceptibility to bacterial infection in compensated cirrhosis and other factors may be more relevant in decompensated cirrhosis.

Strengths of this study were the large cohort of patients from different European centres which allowed us to study genetic variants individually and combined, the vigorous and uniform prospective collection of clinical data and blinding of the authors for clinical outcomes during genotyping of the SNPs. Limitations of this study were the testing of selected SNPs with known functional relevance, whereas an unbiased approach may provide new insights and the lack of information about bacterial infections occurring more than 3 months preceding inclusion in this study.

In conclusion, NOD2-G908R, MASP2_371 and MBL_Yx genetic variants were found to be independently associated with increased risk of short-term mortality in cirrhotic patients with decompensated cirrhosis or ACLF, particularly in those with bacterial infections. Functionally relevant polymorphisms in the lectin complement pathway and innate immune signalling components for pathogen recognition were not associated with

increased occurrence of a bacterial infection or SBP alone. Further studies are required to address whether a personalized approach based on innate immunity genetic variants could improve survival. A well-designed randomized clinical trial should be performed in order to define whether antibiotic prophylaxis would be beneficial for cirrhotic patients with genetic variants at risk for acute bacterial infection.

CONFLICT OF INTEREST

Prof. dr Jalan has a patent DIALIVE licensed to Yaqrit, a patent TLR 4 antagonist licensed to Akaza and Yaqrit, a patent MNK 5105 AND MNK 5106 licensed to Mallinckrodt, a patent AM-535 pending to Thoeris and a patent Alcochange issued to Cyberliver. All other authors declare no conflict of interest concerning this manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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