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Presence of *NOD2* mutations is not associated with hepatic or systemic hemodynamic abnormalities of cirrhosis

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ABSTRACT

Background: Patients with cirrhosis who carry *NOD2* mutations are susceptible to bacterial infections. The aim was to evaluate the association of *NOD2* mutations with hepatic and systemic hemodynamics in cirrhosis.

Patients and methods: This is a secondary analysis of a prospectively collected database in the context of the screening for the INCA trial (EudraCT 2013-001626-26). This cross-sectional study compared hemodynamic findings according to *NOD2* status in 215 patients. Patients were genotyped for *NOD2* variants (p.N289S, p.R702W, p.G908R, c.3020insC, rs72796367). Hepatic hemodynamic study and right heart catheterization were performed.

Results: Patients had a median age of 59 (IQR 53–66) years, and 144 (67%) were men. Most patients (64%) were Child-Pugh stage B. Sixty-six patients (31%) carried a *NOD2* mutation, which was slightly more common among Child-Pugh stage C ($p = 0.05$), without differences in MELD [wild-type: 13 (10–16); *NOD2* variants 13 (10–18)]. No differences in hepatic and systemic hemodynamics were observed according to *NOD2* status. If excluding patients on prophylactic or therapeutic antibiotics, again no association between hepatic or systemic hemodynamics and *NOD2* status could be observed.

Conclusion: *NOD2* mutations are not associated with hepatic or systemic hemodynamic abnormalities in patients with decompensated cirrhosis, suggesting that other mechanisms leading to bacterial translocation predominate.

1. Introduction

Cirrhosis is the end-stage of many chronic diseases of the liver. Portal hypertension is one of the main drivers of the complications of cirrhosis. The increase in intrahepatic vascular resistance leads to splanchnic vasodilation and the activation of vasoactive compensatory mechanisms, which in turn cause hyperdynamic circulation [1]. Furthermore, the release of damage-associated molecular patterns (DAMPs) due to inflammation of the liver and pathogen-associated molecular pattern (PAMPs) due to bacterial translocation leads to systemic inflammation, which can further impact systemic and hepatic hemodynamics [2–5].

Variants of the *NOD2* (nucleotide-binding oligomerization domain containing 2) gene are associated with impaired mucosal barrier function in chronic inflammatory bowel disease [6,7]. *NOD2* is an intracellular pattern recognition receptor expressed in macrophages and is involved in the intestinal recognition of bacteria and their products, shaping bacterial colonization [8]. Insufficient activation of NF-κB in carriers of *NOD2* risk variants may result in deficient anti-microbial activity, altered microbiome and enhanced bacterial translocation (BT) from the intestine [9]. Previous studies have associated *NOD2* gene variants with spontaneous bacterial peritonitis (SBP) and mortality of patients with decompensated cirrhosis [10,11].

If the presence of *NOD2* gene variants were to lead to an increase in bacterial translocation, we hypothesized that patients carrying these variants could show more marked hemodynamics abnormalities. The aim of this study was to evaluate the association of the presence of *NOD2* gene variants with hepatic and systemic hemodynamics in patients with cirrhosis.

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2. Patients and methods

2.1. Study population

This study is a retrospective analysis of a prospectively collected patient cohort including all consecutive Caucasian patients with cirrhosis in whom a determination of *NOD2* risk variants was performed in the context of screening for the *INCA* trial (EudraCT 2013-001626-26) between 02.2014 and 05.2019 [12] (n = 825). In order to increase recruitment in the INCA study, *NOD2* genotyping was offered to all patients with cirrhosis independent of the presence of ascites including both compensated and decompensated disease. Cirrhosis was defined by (i) biopsy, (ii) a combination of clinical, laboratory, ultrasound and endoscopy findings, or (iii) by transient elastography. Patients with severe comorbidities such as end-stage heart failure, HIV infection and non-resectable cancer except hepatocellular carcinoma BCLC stages A-C were excluded. Only patients who had undergone a hepatic venous pressure gradient (HVPG) measurement were included in the present study. This measurement is routinely performed in the context of cirrhosis including patients with refractory ascites for transjugular intrahepatic portosystemic shunt (TIPS) evaluation, in the context of transplant evaluation, or in the setting of transjugular liver biopsy among others.

2.2. Hemodynamic measurements

2.2.1. Hepatic vein catheterisation

Beta-blockers were routinely paused 2 days before performing the measurement. No hemodynamic measurement was performed in the context of a variceal bleeding episode under treatment with vasoactive drugs such as terlipressin. The hemodynamic study was performed after an overnight fast. After local anesthesia, a 9F vascular introducer sheath (Boston Scientific, Nanterre Cedex, France) was placed into the right internal jugular vein according to Seldingerś technique. Afterwards, a 7F balloon catheter (Cordis SA, Miami, FL, USA) was inserted into the right hepatic vein, approximately 2–3 cm from the vena cava, in order to assess free and wedged hepatic venous pressure (FHVP and WHVP). The zero-pressure level was set in the mid-axillary line. The hepatic venous pressure gradient (HVPG) was calculated as WHVP minus FHVP. Each measurement was performed in triplicate after at least one minute of stabilization.

2.2.2. Right heart catheterization

Besides the hepatic hemodynamic study, a Swan-Ganz catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted into the right pulmonary artery to determine mean pulmonary arterial pressure (MPAP; mmHg), pulmonary capillary wedge pressure (PCWP; mmHg) and right arterial pressure (RAP; mmHg). The cardiac output (CO; l/min) was measured by the thermodilution technique, with the average of at least three consecutive values, allowing a maximum difference of 0.5 l/min between them. The hemodynamic parameters were permanently recorded. Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated with the following formulae, respectively: SVR= (MAP-RAP)*80/CO; PVR= (MPAP-PCWP)*80/CO, in dyn.s.cm^{-5}.

2.2.3. NOD2 *genotyping*

After isolation of genomic DNA from EDTA-anticoagulated blood using a membrane-based extraction kit (Qiagen, Hilden, Germany), the NOD2 variants rs2066844 (p.R702W), rs2066845 (p.G908R), rs2066847 (c.3020insC), rs72796367 (c.−958T>C) and rs5743271 (p.N289S) were genotyped using Taqman PCR-based allelic discrimination assays (LifeTechnologies, Carlsbad, California, USA). The assays utilized were: p.R702W: C__11717468_20; p.G908R: C__11717466_20; c.−958T>C: C__97921071_10; p.N289S: C__26935007_10. For c.3020insC, the following specifically designed primer and probe sequences were MGB_F CCAGGTTGTC-CAATAACTGCATC; MGB_R CCTTACCAGA-CTTCCAGGATGGT; VIC TGCAGGCCCCTTG; FAM CTGCAGGCCCTTG. This determination was offered as part of the routine work-up after informed consent and was used in the context of pre-screening for the randomized controlled INCA trial [12]. All technicians performing the genotyping were blinded to clinical data.

2.3. Statistical analysis

Data are presented as median and interquartile range (IQR). Categorical data are presented as proportions, whereas continuous variables are presented with medians and interquartile ranges. Mann-Whitney-Wilcoxon test or ANOVA were used for comparison between groups. The association between continuous variables was assessed using the Spearman rank correlation. Hemodynamic characteristics of patients with and without *NOD2* variants were compared. Taking into account recent data suggesting that the presence of hyperdynamic circulation is defined by the presence of a shift in the cardiac output and systemic vascular resistance relationship instead of achieving determined cut-offs [13], we compared the CO-SVR relationship in patients with and without NOD2 risk variants. The study was conducted according to the Declaration of Helsinki and Good Clinical Practice (European guidelines). Institutional review board approval was obtained (Halle 2017–85; Homburg: 271/11). All participants provided written informed consent.

3. Results

A total of 215 patients with cirrhosis, genotyping of *NOD2* risk variants and hemodynamic evaluation were included in the study. Patients had a median age of 59 years (IQR 53–66 years) and were mainly men (n = 144, 67%). As expected, most patients had alcohol-associated liver disease (n = 161, 75%). Most patients were in Child-Pugh stages B and C (75%), and almost all patients were decompensated (87%). Table 1 lists the baseline characteristics of the total population.

A total of 66 patients (31%) carried at least one *NOD2* risk variant. Only two patients carried two *NOD2* variants, and these patients were grouped together with the 64 heterozygous carriers. Albeit there was an increase in the proportion of patients with *NOD2* risk variants in patients with Child-Pugh stage C cirrhosis (p = 0.05), no differences were observed in MELD score [wild-type: 13 (10–16); *NOD2* variants 13 (10–18)]. Furthermore, patients with and without *NOD2* variants had a similar age [wild type: 61 (IQR 53–66) vs. *NOD2* variants: 59 (IQR 53–65); p = 0.999]. Table 2 summarizes the baseline parameters according to the presence or absence of *NOD2* risk variants. There were no differences in markers of systemic inflammation such as CRP between groups.

Table 3 shows the comparison of hepatic and systemic hemodynamic parameters stratified according to the presence of *NOD2* risk variants. No significant differences could be observed in the hepatic hemodynamics or the systemic hemodynamics between patients with and without *NOD2* risk variants.

Given the fact that a subgroup of patients was on antibiotics (including prophylaxis in 18 patients), the analysis was repeated excluding these 22 patients, of which 4 had NOD2 risk variants (18%). Again, in this subgroup, no association between hepatic or systemic hemodynamic parameters and *NOD2* status could be observed (Suppl. Table 1.)

Stratified analysis was performed according to presence or absence of clinical decompensation and Child-Pugh stages. Although

Table 1
Baseline characteristics of the study population.

	All patients (n = 215)
Age	59 (53–66)
Sex (male)	144 (67%)
NOD2 risk variant	66 (31%)
Etiology of liver disease	
-Alcohol	161 (75%)
-NAFLD	22 (10%)
-Other	28 (15%)
Decompensated	186 (87%)
Child-Pugh Grade	
A	52 (24%)
B	135 (63%)
C	24 (11%)
Varices	172 (80%)
Previous variceal bleeding	44 (21%)
Ascites	
-no ascites	37 (17%)
-diuretic sensitive	79 (37%)
-diuretic refractory	95 (44%)
Prior Hepatic Encephalopathy	39 (18%)
Hepatocellular carcinoma	20 (10%)
Beta-blockers	92 (43%)
Prophylactic antibiotic	22 (10%)
MELD score	13 (10–16)
Leucocytes Gpt/L	6.2 (4.7–8.0)
Hemoglobin mmol/L	6.8 (5.7–7.8)
Thrombocytes Gpt/L	126.0 (79.5–181.0)
ASAT µkat/l	0.74 (0.56–1.10)
ALAT µkat/l	0.41 (0.31–0.58)
CRP mg/l	10.25 (4.7–25.8)
Bilirubin µmol/l	24.0 (14.2–41.3)
Albumin g/dL	31.4 (27.1–35.5)
INR	1.33 (1.21–1.51)
Creatinine µmol/l	83 (68–117)
HR (bpm)	75 (65–84)
MAP (mmHg)	83 (75–90)
CO (l/min)	5.6 (4.8–7.2)
CI (l/min/m2)	3.0 (2.6–3.7)
SVR (dyn·*sec*·cm^{-5})	1054 (815–1351)
SVRI (dyn·*sec*/cm^5/m^2)	1972 (1577–2485)
PAP (mmHg)	16 (13–20)
PCWP (mmHg)	11 (7–15)
PVR (dyn·*sec*·cm^{-5})	74 (41–112)
CVP (mmHg)	7 (5–10)
VCI (mmHg)	8 (5–11)
WHVP (mmHg)	29 (23–33)
FHVP (mmHg)	12 (8–15)
HVPG (mmHg)	19 (15–22)

HR: heart rate, MAP: mean arterial pressure, CO: cardiac output; CI: cardiac index; SVR: systemic vascular resistance, SVRI: systemic vascular resistance index, PAP: pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure, PVR: pulmonary vascular resistance; CVP: central venous pressure; VCI: Vena cava inferior; WHVP: wedged hepatic venous pressure; FHVP: free hepatic venous pressure, HVPG: hepatic venous pressure gradient.

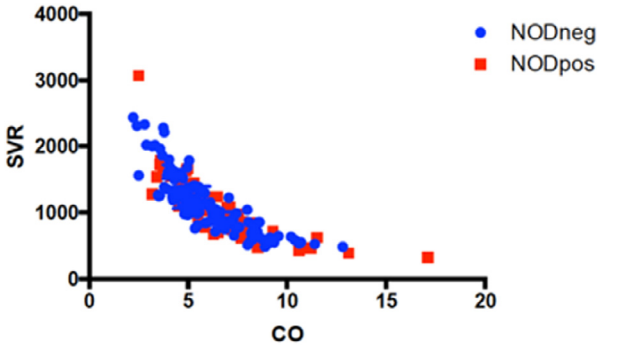

Fig. 1. Relationship between cardiac output (CO) and systemic vascular resistance (SVR) in patients according to the presence of *NOD2* risk variants.

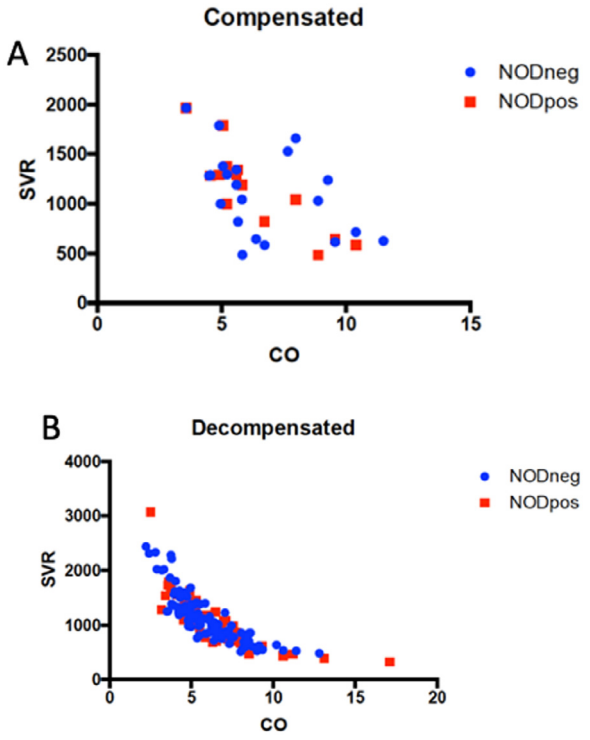

Fig. 2. A Relationship between cardiac output (CO) and systemic vascular resistance (SVR) in compensated patients according to the presence of *NOD2* risk variants. B Relationship between cardiac output (CO) and systemic vascular resistance (SVR) in decompensated patients according to the presence of *NOD2* risk variants.

patients with compensated cirrhosis and a *NOD2* risk variant presented with higher cardiac output and lower systemic vascular resistance, no statistical difference in hemodynamic parameters was observed according to clinical decompensation (Table 4). Patients with Child-Pugh stage A and presence of a *NOD2* risk variant had a significantly higher heart rate than patients with wild-type alleles [*NOD2* risk variant 82 (75–87) bpm vs. wild-type 69 (62–77) bpm (p = 0.01)]. Other than this observation, no differences could be identified between groups (Suppl. Table 2). Specifically in the Child Pugh stage C, although there was a trend to a lower MAP in the group with NOD2 risk variants, there were no differences in other hemodynamic parameters associated to hyperdynamic circulation such as HR, CO, CI, and SVR. Furthermore, there were no differences in baseline characteristics such as age, previous decompensations or OHE and infections at baseline between groups (Suppl. Table 3).

Further analyses were performed according to the degree of portal hypertension (Suppl Table 4). Again, no differences in the subgroups could be observed according to the presence or absence of *NOD2* risk variants.

Finally, the relationship between CO and SVR was evaluated according to the presence or absence of a *NOD2* variant (Fig. 1). Again, no difference was observed among the two groups when evaluating the whole patient population. Although the small sample size precluded further statistical analysis, no differences could be observed according to the presence of *NOD2* variant in patients with compensated or decompensated cirrhosis (Fig 2A and B).

4. Discussion

This study evaluates the association between the presence of *NOD2* risk variants and hemodynamic abnormalities of patients with cirrhosis. Contrary to our hypothesis, no association between the presence of *NOD2* risk variants and hepatic (HVPG) or systemic hemodynamics (CO and SVR) could be observed.

Table 2
Comparison of baseline characteristics according to the presence or absence of *NOD2* risk variants.

	NOD2 wild-type (n = 149)	*NOD2* variants (n = 66)	p value
Age	61 (53–66)	59 (53–65)	0.999
Sex (male)	100 (67%)	44 (67%)	0.949
Etiology of liver disease			0.237
-Alcohol	112 (77%)	49 (74%)	
-NAFLD	14 (10%)	8 (12%)	
-Other	19 (13%)	9 (14%)	
Child Pugh Grade			0.050
A	42 (29%)	10 (15%)	
B	88 (61%)	47 (71%)	
C	15 (10%)	9 (14%)	
Varices	121 (81%)	51(77%)	0.284
Previous variceal bleeding	27 (19%)	17 (26%)	0.237
Ascites			0.595
-none	26 (18%)	11 (17%)	
-diuretic responsive	51 (35%)	28 (42%)	
-diuretic refractory	68 (47%)	27 (41%)	
Prior Hepatic encephalopathy	24 (16%)	15 (23%)	0.316
Decompensation	127 (85%)	59 (89%)	0.177
Beta-blocker	62 (42%)	30 (46%)	0.763
MELD score	13 (10–16)	13 (10–18)	0.699
Leucocytes (Gpt/L)	6.23 (4.55–8.03)	6.17 (4.84–7.95)	0.965
Hemoglobin (mmol/L)	6.8 (5.7–7.9)	6.7 (5.4–7.4)	0.158
Thrombocytes (Gpt/L)	131 (80–190)	116 (77.3–167.5)	0.171
ASAT (µkat/l)	0.71 (0.51–1.11)	0.82 (0.61–1.06)	0.152
ALAT (µkat/l)	0.40 (0.30–0.56)	0.44 (0.35–0.64)	0.090
CRP (mg/l)	10.25 (3.95–26.95)	10.20 (4.98–24.13)	0.812
Bilirubin (µmol/l)	22.0 (14.2–36.9)	30.6 (13.2–46.0)	0.244
Albumin (g/dL)	32.1 (26.9–36.4)	30.4 (27.8–34.3)	0.070
INR	1.33 (1.21–1.49)	1.33 (1.18–1.64)	0.977
Creatinine (µmol/l)	87 (69–128)	80 (66–101)	0.066

Table 3
Comparison of hemodynamic parameters according to *NOD2* status.

	NOD2 wild-type	*NOD2* variants	p value
HR (bpm)	74 (65–83)	78 (65–87)	0.405
MAP (mmHg)	83 (76–89)	83 (75–91)	0.961
CO (l/min)	5.56 (4.67–7.08)	5.97 (4.98–7.64)	0.183
CI (l/min/m2)	2.93 (2.47–3.52)	3.31 (2.59–3.95)	0.075
SVR (dyn·*sec*·cm^{-5})	1069 (840–1376)	1042 (747–1273)	0.219
SVRI (dyn·*sec*/cm^5/m^2)	2016 (1641–2490)	1814 (1473–2399)	0.315
PAP (mmHg)	16 (13–20)	17 (14–20)	0.285
PCWP (mmHg)	11 (7–15)	12 (8–15)	0.237
PVR (dyn·*sec*·cm^{-5})	75 (43–112)	65 (32–112)	0.542
CVP (mmHg)	7 (5–10)	7 (4–10)	0.452
VCI (mmHg)	8 (6–12)	8 (5–11)	0.395
WHVP (mmHg)	29 (23–32)	28(23–35)	0.483
FHVP (mmHg)	12 (8–15)	12 (7–15)	0.982
HVPG (mmHg)	19 (15–22)	19 (15–23)	0.994

HR: heart rate, MAP: mean arterial pressure, CO: cardiac output; CI: cardiac index; SVR: systemic vascular resistance, SVRI: systemic vascular resistance index, PAP: pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure, PVR: pulmonary vascular resistance; CVP: central venous pressure; VCI: Vena cava inferior; WHVP: wedged hepatic venous pressure; FHVP: free hepatic venous pressure, HVPG: hepatic venous pressure gradient.

Table 4
Stratified analysis according to *NOD2* status stratified by the presence or absence of clinical decompensation.

	NOD2 wild-type	NOD2 variants	p value
Compensated patients (n = 22)			
HR (bpm)	70 (67–77)	82 (79–84)	0.130
MAP (mmHg)	85 (74–92)	93 (90–97)	0.535
CO (l/min)	5.5 (5.0–9.1)	7.7 (4.7–10.4)	0.535
CI (l/min/m2)	3.1 (2.8–4.4)	3.9 (2.6–4.6)	0.424
SVR (dyn·*sec*·cm^{-5})	1095 (629–1482)	716(621–1594)	0.689
SVRI (dyn·*sec*/cm^5/m^2)	1935 (1340–2739)	1568 (1352–2867)	0.964
PAP (mmHg)	16 (12–18)	17 (16–23)	0.581
PCWP (mmHg)	8 (5–13)	11 (8–15)	0.731
PVR (dyn·*sec*·cm^{-5})	95 (46–126)	90(55–120)	0.407
CVP (mmHg)	6 (2–9)	7(5–7)	0.837
VCI (mmHg)	7 (3–7)	8 (6–9)	0.416
WHVP (mmHg)	22 (16–31)	23 (21–30)	0.699
FHVP (mmHg)	9 (6–12)	12 (7–15)	0.447
HVPG (mmHg)	16 (12–21)	17 (14–21)	0.588
Decompensated patients (n = 186)			
HR (bpm)	74 (65–83)	74 (63–83)	0.527
MAP (mmHg)	82 (75–88)	81 (74–87)	0.615
CO (l/min)	5.5 (4.6 −7.1)	5.5 (5.0–7.2)	0.315
CI (l/min/m2)	3.0 (2.4 −3.4)	3.2 (2.6–3.8)	0.121
SVR (dyn·*sec*·cm^{-5})	1052 (826–1367)	1054 (779–1238)	0.299
SVRI (dyn·*sec*/cm^5/m^2)	2022 (1676–2547)	1814 (1486–2383)	0.320
PAP (mmHg)	16 (13–20)	17 (14–20)	0.326
PCWP (mmHg)	11 (7–15)	12 (8–16)	0.232
PVR (dyn·*sec*·cm^{-5})	75 (42–112)	63 (27–110)	0.354
CVP (mmHg)	7 (5–10)	7 (4–11)	0.472
VCI (mmHg)	8 (5–12)	9(5–12)	0.332
WHVP (mmHg)	29 (23–33)	29 (25–35)	0.313
FHVP (mmHg)	12 (8–15)	11 (7–15)	0.957
HVPG (mmHg)	20 (16–23)	20 (15–22)	0.910

HR: heart rate, MAP: mean arterial pressure, CO: cardiac output; CI: cardiac index; SVR: systemic vascular resistance, SVRI: systemic vascular resistance index, PAP: pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure, PVR: pulmonary vascular resistance; CVP: central venous pressure; VCI: Vena cava inferior; WHVP: wedged hepatic venous pressure; FHVP: free hepatic venous pressure, HVPG: hepatic venous pressure gradient.

The presence of *NOD2* variants has been shown to confer impaired mucosal barrier function and enhanced bacterial translocation from the intestine. In cirrhosis, the presence of *NOD2* gene variants has been associated with an increase in bacterial infections, mainly SBP in patients with ascites, and mortality in decompensated cirrhosis [10,11,14,15]. However, one study identified an association with mortality only in non-alcoholic cirrhosis and another study found no association with non-SBP infections [14,16]. We have previously observed an association between infections and the presence of *NOD2* risk variants in compensated patients only [17].

Portal hypertension is the consequence of the interaction between the flow in the portal vein and the resistance to this flow in the liver [1]. The intrahepatic vascular resistance has a structural but also a dynamic component, which is due to an imbalance between vasoconstrictors and vasodilators in favor of the former. This dynamic component is susceptible to modification and is one of the main targets in the treatment of portal hypertension [18,19].

Patients with cirrhosis have increased gut permeability, which is correlated to portal hypertension [20]. Indeed, patients with cirrhosis have a reduced expression of the tight junction proteins occluding and claudin-1 compared with healthy controls, with a greater reduction in patients with decompensated than compensated cirrhosis [21]. Similar findings have been observed in another study including patients with compensated cirrhosis, although this led only to an increased intestinal permeability in the large rather than the small intestine [22]. Indeed, increased intestinal permeability for macromolecules has been mainly observed in patients with decompensated cirrhosis rather than compensated cirrhosis [23].

Endotoxin derived from bacteria is hemodynamically active [24]. In the splanchnic circulation it can lead to vasodilation, while in the liver it leads to vasoconstriction [25]. Indeed, selective intestinal decontamination leads to a reduction in portal pressure [5,26], suggesting that bacterial and/or bacterial products could increase portal pressure by acting on the dynamic component of increased intrahepatic resistance. If the presence of *NOD2* risk variants were to lead to an increase in bacterial products in the splanchnic circulation, this would theoretically lead to an increase of intrahepatic vascular resistance. However, in the present study no differences in HVPG could be observed between patients who carried *NOD2* risk variants and those who did not.

According to the actual systemic inflammation hypothesis, bacterial translocation and systemic inflammation have a major impact in the natural history of cirrhosis, marking the development of multiple end-organ dysfunction [2]. Bacterial translocation and systemic inflammation have also been associated to the characteristic hyperdynamic circulation of cirrhosis [4,27,28]. Indeed, PAMPs lead to an increase in the release of nitric oxide and therefore splanchnic vasodilation, decrease of systemic vascular resistance, activation of vasoactive systems, and compensatory increase in cardiac output. Despite the predominant role of PAMPs in the pathophysiology of cirrhosis, no association between hepatic or systemic hemodynamic abnormalities and the presence of *NOD2* risk variants could be identified. One can speculate that this lack of association of hemodynamic abnormalities with the presence of *NOD2* variants is due to the fact that any effect of *NOD2* variants on the mucosa barrier function is overridden by the presence of other factors, which develop during cirrhosis progression. Indeed, we had previously observed an association between infections and the presence of *NOD2* variants in patients with compensated disease but not in decompensated disease [17]. On the other hand, one could argue that it is unlikely that only one gene mutation could lead to changes in the systemic circulation. However, previous studies have linked this mutation to an increase incidence of infections, suggesting that there is a greater translocation of PAMPS and therefore, it is plausible that it could be related to the characteristic hemodynamic changes of cirrhosis.

The limitations of the study are mainly the cross-sectional and retrospective design of this study. Indeed, the selection of patients according to the clinical indication of HVPG measurement, may just suggest that all patients are evaluated in a similar moment of the natural history of their disease. Nevertheless, there were no differences in the age of patients with and without *NOD2* risk variants, suggesting that the presence of *NOD2* risk variant did not accelerate the disease course. There were also no differences in previous decompensations among the two groups. Due to the retrospective nature of the study, no samples were available to evaluate markers of bacterial translocation. Thirdly, the small number of patients with compensated cirrhosis or in the subgroup with Child Pugh C cirrhosis precludes further analysis in these subpopulations. Furthermore, inclusion of mainly patients with decompensated cirrhosis, which is associated to more marked changes in systemic hemodynamics [26], may impact the results. Additionally, some patients were on antibiotics for prophylaxis of SBP or hepatic encephalopathy. Rifaximin has been shown to affect the gut mucosal barrier [29]. However, the exclusion of patients who were on antibiotics at the time of the hemodynamic study did not change the results. Lastly, no data was available regarding the intake of statins in this population.

In conclusion, the presence of *NOD2* risk variants has no effect on hepatic or systemic hemodynamics in patients with mainly decompensated cirrhosis, suggesting that at this stage of the disease other factors outweigh any effect on gut mucosal barrier as a consequence of these variants.

Conflict of interest and ethical standards

All authors have no conflicts of interest and ethical standards

The authors have no relevant financial or non-financial interests to disclose

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Author contributions

Cristina Ripoll and Alexander Zipprich conceived, designed and supervised the study. But all authors contributed to the study conception and design. Markus Casper, Matthias Christian Reichert, Robin Greinert selected patients' samples and generated data. *NOD2* genotyping was performed in the lab of the University of Saarland under the supervision of Frank Lammert. Cristina Ripoll, Alexander Zipprich and Robin Greinert analysed the data and wrote the original draft. All authors participated in review & editing the manuscript to the final version.

Informed consent

Informed consent: Informed consent was obtained from all individual participants included in the study.

Human rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study

Conflict of interest

None declared.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2023.05.016.

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