



Lectin complement pathway molecules are decreased in patients with cirrhosis and constitute the risk of bacterial infections

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Complete List of Authors:	Foldi, Ildiko; University of Debrecen, Department of Internal Medicine, Division of Gastroenterology Tornai, Tamás; University of Debrecen, Department of Internal Medicine, Division of Gastroenterology Tornai, David; University of Debrecen, Department of Laboratory Medicine Sipeki, Nora; University of Debrecen, Department of Internal Medicine, Division of Gastroenterology Vitalis, Zsuzsanna; University of Debrecen, Department of Internal Medicine, Division of Gastroenterology Tornai, Istvan; University of Debrecen, Department of Internal Medicine, Division of Gastroenterology Dinya, Tamas; University of Debrecen, Institute of Surgery Antal-Szalmas, Peter; University of Debrecen, Department of Laboratory Medicine Papp, Maria; University of Debrecen, Department of Internal Medicine, Division of Gastroenterology
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Lectin complement pathway molecules are decreased in patients with cirrhosis and constitute the risk of bacterial infections

Ildiko Foldi^{1#}, Tamas Tornai^{1#}, David Tornai², Nora Sipeki², Zsuzsanna Vitalis¹, Istvan Tornai¹, Tamas Dinya³, Peter Antal-Szalmas², Maria Papp¹

¹Division of Gastroenterology, Department of Internal Medicine, ²Department of Laboratory Medicine, ³Institute of Surgery, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

[#]These authors contributed equally to the work and both should be considered as first authors.

Corresponding author: Maria Papp, MD, PhD, Department of Internal Medicine, Division of Gastroenterology, Faculty of Medicine, University of Debrecen, Nagyerdei krt. 98, H-4032 Debrecen, Hungary, Phone/Fax: 36-52-255-152, e-mail: papp.maria@med.unideb.hu

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3 **List of abbreviations:** AD: acute decompensation, ACLF: acute-on chronic
4 liver failure, CSI: clinically significant bacterial infections, FCN: ficolin, MASP:
5 mannan-binding lectin serine proteases, MBL: mannan-binding lectin, MELD:
6 model for end-stage liver disease, HBV: hepatitis B virus, HCV: hepatitis C
7 virus, SBP: spontaneous bacterial peritonitis, SNP: single nucleotide
8 polymorphism, sPRM: soluble pattern recognition molecule
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ABSTRACT

Background&Aims: Lectin pathway molecules of the complement system are synthesized by hepatocytes and have pivotal role in innate host defense against infectious organisms. Ficolins(FCNs) act as soluble pattern recognition molecules, while mannan-binding lectin serine proteases(MASPs) do as effector molecules in elimination of pathogens. We aimed to study the significance of low level of these molecules in the development of cirrhosis-associated bacterial infections, which has not been elucidated so far.

Methods: Sera of 266 stable outpatients with cirrhosis and 160 healthy subjects were assayed for a panel of lectin molecules (FCN-2, FCN-3 and MASP-2) by ELISA. In cirrhosis, a 5-year follow-up observational study was conducted to assess a possible association between lectin levels and development of clinically significant bacterial infections(CSI). **Results:** FCN-2, FCN-3 and MASP-2 levels were significantly lower in cirrhosis compared to healthy subjects and decreased according to disease severity ($p<0.001$ for all molecules). In Kaplan-Meier analysis, development of CSI was associated with low level of FCN-2 ($<427\text{ng/ml}$, $p\text{LogRank}=0.047$) and FCN-3 ($<4857\text{ng/ml}$, $p\text{LogRank}=0.029$), but not with MASP-2 deficiency ($<100\text{ng/ml}$, $p\text{LogRank}=0.306$). Combined FCN deficiency was associated with increased risk of development of bacterial infections in a step-wise manner. Patients with low level of both FCNs had higher cumulative probability of CSI (63.8%) compared to those with low level of one or normal FCN (52.7% and 45.7%, $p\text{LogRank}=0.016$). Neither FCN serum profile, nor MASP-2 deficiency were associated with infection-related mortality. **Conclusions:** Low level of FCNs associated with hepatic insufficiency might be considered as an additional

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3 constituent of cirrhosis-associated immune dysfunction.
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7 **Word count for abstract:** 248
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9 **Key words:** ficolin, mannan-binding lectin serine protease, cirrhosis, bacterial
10 infection, mortality
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14 15 **Key Points**

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17 • The present 5-year follow-up cohort study comprehensively evaluated
18 associations of serum level of lectin pathway molecules with disease
19 specific characteristics of cirrhosis and also their role in the
20 development of bacterial infections, that has not been assessed
21 previously.
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- 23 • Ficolins(FCN-2, FCN-3) and mannan-binding lectin serine protease-
24 2(MASP-2) serum levels were decreased in cirrhosis and were
25 associated with disease severity.
26
- 27 • Low level of individual or combined FCNs, but not MASP-2 deficiency
28 constituted higher risk for the development of cirrhosis-associated
29 bacterial infections.
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- 31 • These results highlight deleterious effect of decreased level of soluble
32 pattern recognition molecules in cirrhosis, and might be considered
33 additional constituents of cirrhosis-associated immune dysfunction.
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INTRODUCTION

Multiple levels of immune dysfunction have been found in patients with cirrhosis rendering them susceptible to various bacterial infections [1]. Infectious episodes are common causes of clinical deterioration in this patient group [2] as they play a substantial role in the development of disease specific complications and mortality [3,4]. Prevention of bacterial infections in cirrhosis is of paramount importance, which requires early identification of patients at high risk for the development of these episodes. Only some clinical predictors of bacterial infections, such as advanced disease stage [5] and the presence of gastrointestinal hemorrhage [6] have been known for a long while. Recently, various genetic polymorphisms affecting different functions of innate immune system were identified to have a substantial impact on the development of bacterial infections in cirrhosis. These functional variations, assessed either by serologic [7,8] or single nucleotide polymorphism(SNP) based genetic methods [9–12], are considered to further abolish the pre-existing immune dysfunction in cirrhosis. Most of these studies, however focused on one or two single markers and primarily assessed the risk for the development of spontaneous bacterial peritonitis(SBP) and not for other type of bacterial infections. Nonetheless, in cirrhosis, SBP comprises only up to 25% of the infectious episodes.

Lectin pathway molecules of the complement system are synthesized in the liver and have pivotal roles in the innate host defense against infectious organisms. Mannose-binding lectin(MBL) and ficolins(FCNs) act as soluble pattern recognition molecules(sPRM), while mannan-binding lectin serine proteases(MASPs) do as effector molecules in elimination of the pathogens

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3 [13]. Low levels of the functional proteins increase the risk of various
4 infectious diseases mostly in immune-deficient conditions [14–16].
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6 Association of deficiencies in lectin pathway molecules with the development
7 of infectious episodes in patients with cirrhosis is quite reasonable, but has
8 scarcely been investigated. Our group has previously demonstrated, that the
9 absolute MBL deficiency is a risk for the development of clinically significant
10 bacterial infections (CSI) independently from both the disease severity and
11 the presence of co-morbidity. Furthermore, in a small study of *Chong et al.*,
12 the *MBL2* deficient genotype predisposed patients with hepatitis B virus (HBV)
13 induced liver cirrhosis to develop SBP [17].
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25 In the present study, we aimed to assess the serum levels of various
26 lectin pathway molecules in a large cohort of stable outpatients with cirrhosis
27 and also their association with the disease specific characteristics.
28 Additionally, in a 5-year follow-up observational study we aimed to evaluate
29 whether serum levels of various lectin pathway molecules constitute a risk for
30 the development of cirrhosis-associated bacterial infections.
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41 PATIENTS AND METHODS

42 Patient population

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44 We performed a cohort study among adult patients with established diagnosis
45 of cirrhosis of different etiologies in a tertiary care referral center of Hungary
46 (Division of Gastroenterology Department of Internal Medicine, Clinical
47 Center, University of Debrecen). The present study population is a part of our
48 entire patient cohort comprising a total of 404 patients with cirrhosis and
49 recruited consecutively between May 1, 2006 and December 31, 2010 from
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3 the outpatient clinic during regular or extraordinary follow-up visits and also
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5 from the inpatient ward owing to hospitalization with an acute decompensation
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7 (AD) episode [18]. In this study only stable outpatients with available serum
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9 samples (n=266) were included.
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11 Clinical characteristics of patients at inclusion are presented in **Table 1**.
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13 Blood samples, routine laboratory data and detailed clinical phenotype were
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15 captured at inclusion. Clinical data were determined by in-depth review of the
16
17 patients' medical records using a structured interview. Medical records that
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19 documented age at diagnosis, etiology, presence of hepatocellular carcinoma,
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21 esophageal varices, extrahepatic co-morbidities, history of previous AD
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23 episode(s), and cirrhosis-related medication were retrospectively analyzed for
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25 the period prior to the observational follow-up study. At enrolment, disease
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27 severity assessed by liver-oriented scores (Child-Pugh and MELD) was
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29 determined.
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36 **Phenotypical characterization of patients during follow-up**

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38 Patients were enrolled into an observational follow-up study, where the
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40 attending gastroenterologist registered date and type of clinically significant
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42 bacterial infections (CSI) warranted hospital admission. Follow-up period
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44 lasted 5 years, or until death/loss of follow-up. The median follow-up was 979
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46 days (IQR: 331-1825). Collected data were transferred and stored in a
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48 database. At the end of the study period, December 31, 2013, all clinical data
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50 were extracted for further analysis.
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54 Development of CSI was carefully established by compatible clinical
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56 symptoms and findings, laboratory data (leucocyte count, high-sensitivity C-
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3 reactive protein and procalcitonin, results of urine analysis (sediment) and
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5 imaging findings (abdominal ultrasound and chest X-ray) and if ascites was
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7 present the result of diagnostic tap (neutrophil count and ascites culture) was
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9 considered. Based on the results of this procedure, cultures from specific sites
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11 (sputum, urine, wound discharge, etc.) were obtained according to location of
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13 infection, though blood cultures were obtained in sepsis, or if the location of
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15 the infection could not be clearly identified. Regarding laboratory results,
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17 elevated leucocyte count (absolute >10.8 G/L or relative [in patients with
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19 leukopenia]: double of count at former visits) with an elevated neutrophil rate
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21 ($>76\%$) and increased serum levels of CRP (>10.0 mg/L) and/or PCT (>0.15
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23 $\mu\text{g/L}$) [19] were considered to support the diagnosis of infection. Based on
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25 specific clinical symptoms and findings the following infections were
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27 diagnosed. Further characterization of bacterial infections was done on the
28
29 basis of conventional criteria [20–23]. (1) Spontaneous bacterial peritonitis:
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31 neutrophil cell count $>250/\text{mm}^3$ and/or positive culture of ascitic fluid, in the
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33 absence of intra-abdominal source of infection. (2) Urinary tract infection:
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35 presence of dysuria, pyuria (leucocyte $>10/\text{mm}^3$) and positive urine culture.
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37 (3) Pneumonia: presence of cough and expectoration, positive chest X-ray,
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39 positive sputum culture. (4) Miscellaneous: skin and soft tissue, biliary tract,
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41 orocavital, intestinal tract infection, osteomyelitis, endocarditis. (5) Bacterial
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43 infection with unknown origin: positive blood culture in the absence of site-
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45 specific infection.
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52 The healthy control group consisted of 160 age- and gender-matched
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54 individuals (male/female: 72/88, age: 51.5 ± 16.9 years) selected from
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56 consecutive blood donors in Debrecen. The control subjects did not have any
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3 known gastrointestinal or liver diseases.
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7 8 **Serological analysis**

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10 Blood samples were obtained at enrolment from each patient and were frozen
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12 at -70°C until testing collectively after end of the study period.
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14 Serum level of MASP-2 was determined by a commercially available
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16 solid-phase enzyme-linked immunoassay(ELISA), according to the
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18 manufacturer's instructions (Hycult Biotechnology, Uden, Netherlands).
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20 MASP-2 deficiency was defined a level of <100ng/ml in agreement with
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22 literature data [24].
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25 Serum level of L-ficolin (Liver ficolin, ficolin-2 [FCN-2]) and H-ficolin,
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27 Hakata ficolin, ficolin-3 [FCN-3]) were determined by an in-house developed
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29 double-antibody sandwich ELISA. Since no definition exists for FCN
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31 deficiency, low level of FCN-2 (<427ng/ml) and FCN-3 (<4857ng/ml) were
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33 determined arbitrarily corresponding to the 25th serum level percentile of the
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35 patients.
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38 Detailed description of these experiments such as analytical properties
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40 are presented in the **Supplementary Material**.
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45 46 **Ethical considerations**

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48 The study protocol was approved by Regional and Institutional Research
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50 Ethics Committee of University of Debrecen and the National Scientific and
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52 Research Ethics Committee (DEOEC-RKEB/IKEB 5306-9/2011,
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54 3885/2012/EKU [60/PI/2012]). Each patient or legal surrogate was informed of
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56 the nature of the study and signed an informed consent form.
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Statistical analysis

Variables were tested for normality using Shapiro Wilk's W test. Continuous variables were summarized as means (standard deviation [SD]) or as medians (interquartile range [IQR, lowest 25%- highest 25%]) according to their homogeneity. Categorical variables were compared with Fisher's exact test or χ^2 test with Yates correction, as appropriate. Continuous variables were compared with Mann-Whitney U test or Kruskal-Wallis H test with Dunn's multiple comparison *post hoc* analysis. Paired samples were analyzed by Wilcoxon signed rank test. The Spearman's nonparametric rank correlation test was used to determine correlations. Kaplan–Meier analysis was used to calculate the cumulative probability of adverse outcome (CSI and mortality). Differences in observed probabilities were assessed by the log-rank test. The association between categorical clinical variables or serum levels of lectin pathway molecules and adverse disease outcomes during follow-up was assessed by univariate Cox-regression analysis. Multivariate analyses were performed with backward elimination procedure and likelihood ratio test to identify independent predictors. Associations are given as hazard ratio [HR] with 95% confidence intervals [CI]. For statistical analysis and graphical presentation, the SPSS 22.0 [SPSS, Chicago, IL], and GraphPad Prism 6 [San Diego, CA] programs were used. A 2-sided probability value of <0.05 was considered to be statistically significant.

RESULTS

Serum levels of lectin pathway molecules in cirrhosis

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3 Serum levels of lectin pathway molecules in patients with cirrhosis and the
4 control group are presented in **Table 2**. FCN-2, FCN-3 and MASP-2 levels
5 were significantly lower in patients with cirrhosis compared to healthy subjects.
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7 Similarly, MASP-2 deficiency occurred more often in patients with cirrhosis.
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11 In cirrhosis, levels of all three lectin pathway molecules decreased
12 gradually according to disease severity, as rated by the Child-Pugh stage.
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14 Furthermore, both types of the FCNs but not the MASP-2 levels were
15 significantly lower in patients with ascites as compared to those without
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21 **(Table 2)**.

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23 FCN levels were also associated with disease etiology to some degree.
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25 FCN-2 and 3 levels were significantly lower in alcoholic as compared to non-
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28 alcoholic patients, but only in patients with Child A stage (FCN-2: 505 [440 –
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30 562] vs. 548 [489 – 679] ng/ml, $p=0.001$ and FCN-3: 7509 [5508 – 10897] vs.
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32 10596 [7065 – 15379] ng/ml, $p<0.001$, respectively), or in those without
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34 ascites (data not shown). No similar association was found in the case of
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36 MASP-2 **(Table 2)**.

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38 Significant correlation was found between levels of lectin pathway
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40 molecules and laboratory markers of impaired renal and liver function and
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42 accordingly with liver-oriented scores (Child-Pugh and MELD). Non-
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44 parametric correlations are summarized in **Table 3**.
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49 **Significance of serum levels of lectin pathway molecules in the risk of** 50 **clinically significant bacterial infections**

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54 Ninety-five (35.7%) of involved patients encountered at least one episode of
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57 CSI during the follow-up period. The median time to the development of first
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3 infectious episode was 626 [169-799] days. Urinary tract infection was the
4 most commonly diagnosed CSI and accounted for 33.7% of events, followed
5 by SBP (24.2%) and pneumonia (12.6%). 5.3% of the cases were multifocal.
6
7 Other sites of bacterial infections were as follows: erysipelas (4.2%),
8 cholangitis (2.1%), bacteremia (2.1%), acute bronchitis (3.2%) and
9 unidentified in 12 (12.6%) cases.
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16 Low FCN-2 and FCN-3 levels were associated with an increased
17 cumulative probability of CSI compared to normal level of the molecules
18 (62.6% vs. 46.7% for FCN-2 with HR: 1.55, 95%CI: 1.00-2.39, p=0.047 and
19 59.3% vs. 48.2% for FCN-3 with HR: 1.61, 95%CI: 1.05-2.47, p=0.029) (**Table**
20 **4**). FCN serum profile, considering both levels of FCN molecules, showed an
21 ever-increasing cumulative risk for development of CSI with increasing
22 number of low FCN levels. Patients with normal level of both FCNs had a
23 cumulative probability of 45.7% to develop a CSI, while those with low level of
24 one or two FCNs had 57.2% and 63.8%, respectively (HR [95%CI]: 1.39
25 [0.87-2.22], p=0.164 and 2.00 [1.15-3.47], p=0.016, respectively) (**Table 4** and
26 **Figure 1A**). No similar association was found, however, in the case of MASP-
27 2 deficiency either individually (**Table 4** and **Figure 1B**) or in combination with
28 FCN levels (data not shown).
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47 *Covariates*

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49 Analysis of clinical factors associated with development of CSI, using
50 univariate Cox regression analysis, is shown in **Table 4**. Alcoholic disease
51 etiology (HR: 1.68, 95%CI: 1.07-2.66, p=0.024), advanced disease stage
52 (Child-Pugh stage B/C [HR: 2.42, 95%CI: 1.61-3.64, p<0.001] or presence of
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3 ascites [HR: 2.31, 95%CI: 1.54-3.46, $p < 0.001$]) and prior CSI episode (HR:
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5 2.64, 95%CI: 1.76-3.96, $p < 0.001$) were significantly associated with the
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7 increased risk for the development of CSI.
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10 11 *Multivariate analysis*

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14 Cox regression analysis and the backward elimination procedure, taking
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16 serum levels of the lectin pathway molecules and all clinical covariates into
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18 account, indicated that only clinical factor, such as advanced disease stage
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20 and prior CSI episode, but neither individual FCNs nor FCN serum profile
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22 were independently associated with the risk of CSI development (**Table 4**).
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27 **Mortality**

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29 Potential impact of FCN serum profile and MASP-2 deficiency on
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31 infection-related mortality was also evaluated ($n=95$). During the course of the
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33 first bacterial infection, 19 patients died (20%). Neither FCN serum profile nor
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35 MASP-2 deficiency at enrolment was associated with the risk of mortality
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37 during a subsequent bacterial infection in Kaplan-Meier survival analysis
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39 (**Figure 1C and D**).
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44 In the total cohort liver-related mortality occurred in 85 (32.0%)
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46 subjects. Kaplan-Meier survival analysis demonstrated a significantly worse
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48 survival in patients with advanced disease according to Child-Pugh stage
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50 ($p < 0.001$) or ascites ($p < 0.001$) or prior CSI episode ($p = 0.035$). MASP-2
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52 deficiency ($p = 0.387$) or FCN serum profile ($p = 0.093$), however, were not
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54 associated with overall survival (**Figure 1E and F**).
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DISCUSSION

To our knowledge, this is, to date the largest study to investigate the complex association between serum levels of the lectin pathway molecules and the disease etiology along with severity in a large cohort of patients with cirrhosis. Of the lectin pathway molecules of the complement system, two sPRMs (FCN-2 and FCN-3) and one effector molecule were evaluated (MASP-2).

Serum concentrations of the lectin pathway components were found to show large inter-individual variations in healthy adults [25]. In the present study MASP-2 levels [26] and occurrence of MASP-2 deficiency [24] corresponded to those reported previously, while FCN-2 and FCN-3 levels were lower. In the case of MASP-2, we applied a commercially available assay that is widely used in complement studies [24]. The disparity for FCNs is supposedly attributable to the variations in the applied methodology and research materials of different source. Since various methodologies are available, we adjusted an own in-house double-antibody sandwich ELISA system. Another complicating consideration for FCN could be the handling and source of protein standard. It is known, that values obtained from the calibration curve are different by virtue of whether recently isolated FCN preparation or stocked one was used [27].

In a large group of stable outpatients with cirrhosis (n=266), we found that serum levels of all three molecules were significantly lower compared to healthy controls. Two previous reports verify our findings. In a study of *Laurson et al.* [28], low levels of FCN-2 and FCN-3 were found in a small group of patients (n=20) with stable alcoholic cirrhosis compared with healthy controls. Likewise, significantly lower FCN-2 levels were reported in patients

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3 (n=120) with chronic HBV infection related cirrhosis by *Hoang et al.* [29].
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5 Contrary, in a single study of patients with chronic HCV infection [30], serum
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7 FCN-2 levels were significantly higher in patients with cirrhosis (n=21) than
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9 those in the control and other patient groups including chronic inactive or
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11 active HCV patients without cirrhosis. The reason, why serum levels of lectin
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13 pathway molecules are decreased in most of the patients with cirrhosis is yet
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15 to be elucidated. One reasonable explanation could be that in cirrhosis,
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17 synthetic capacity of hepatocytes is damaged, that results in a decreased
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19 production of these proteins. This assumption is supported by our findings
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21 that FCN-2, FCN-3 and MASP-2 levels showed gradual decrease according
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23 to disease severity.
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28 When the adaptive immune response is immature or compromised, the
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30 innate immune system constitutes the principle defense against infection.
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32 Clinical studies have shown that common functional gen polymorphisms in
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34 *FNC2* [27], *FCN3* [16] and *MASP2* [15], which affect the composition,
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36 structure and function of respective proteins, confer an increased risk for
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38 various bacterial infections particularly in immunocompromised patients.
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40 Cirrhosis has been characterized as an acquired immunodeficiency syndrome
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42 [31]. Thus, it is reasonable to assume that serum levels of the lectin pathway
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44 molecules might be related to the bacterial infectious episodes among
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46 patients with cirrhosis; however, it has not been evaluated so far. Accordingly,
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48 we conducted a follow-up observational study to assess whether the low level
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50 of FCNs or MASP-2 deficiency constitutes a risk for the development of CSI in
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52 patients with cirrhosis.
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57 Several SNPs in the promoter and the encoding region of *FCN2* and
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3 *MASP2* gene have been implicated in influencing protein concentrations but
4 relatively few SNP has been identified in *FCN3* gene [15,16]. Considerable
5 linkage disequilibrium occurs between pairs of promoter and structural gene
6 dimorphisms, confounding the investigation of the relationship between allele
7 expression and protein concentration [27]. Thus it has been clearly proved,
8 that genotypes have very modest influence on average protein concentrations,
9 each being associated with large and overlapping ranges [32]. Besides gene
10 polymorphisms of the lectin pathway of complement system, up- and
11 downregulation of these genes and increase or decrease of serum proteins
12 also appears to be associated with various infectious and inflammatory
13 diseases [16]. Due to these limitations we evaluated serum level of the
14 molecules instead of genotyping.

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30 Regarding serological markers, however one could take into
31 consideration various factors, that might influence stability of the serum level
32 of molecule. Acute phase reaction might be such a factor, thus to avoid
33 interfering effect of acute complication of the disease on the synthesis of the
34 molecules, only stable outpatients were involved in the present study.
35 Moreover, in a subgroup of patients (n=32) we carefully evaluated whether
36 the level of FCN-2, FCN-3 and MASP-2 are stable over time. They have a
37 subsequent sample from a later outpatient visit with time-lag of 655 [IQR: 246-
38 1090] days. Median time between sample procurements was comparable with
39 the time to first CSI episode. None of the serum level of these molecules
40 showed significant changes (FCN-2: 504 [458-600] vs. 479 [423-543],
41 p=0.092; FCN-3: 9449 [5934-13070] vs. 8814 [5999-11006], p=0.837; MASP-
42 2: 221 [129-369] vs. 208 [119-324], p=0.313). No significant change in
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3 patients' Child-Pugh category was observed during this time period ($p=1.000$
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5 with Wilcoxon signed ranks test).
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8 Low level of sPRMs can lead to insufficient recognition of microbes and
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10 decreased complement activation, opsonization and inflammatory cytokine
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12 secretion, which results in increased susceptibility to infections [33]. In
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14 Kaplan-Meier analysis, in patients with low level of either FCN-2 or FCN-3 the
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16 cumulative probability of CSI was significantly higher, compared to those with
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18 normal level. Moreover, considering both levels of FCN molecules a step-wise
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20 increase was observed in the risk of the CSI development with increasing
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22 number of low FCN levels. In the multivariate Cox-regression analysis,
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24 adjusting for Child-Pugh category, however, these associations were not
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26 found to be independent from diseases severity. This might support the notion,
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28 that decreased level of FCNs are related to deterioration of synthetic capacity
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30 of hepatocytes. And also suggests that low level of FCNs is an additional
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32 constituent of cirrhosis-associated immune deficiency and increased risk of
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34 CSI in this patient population.
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39 MASP-2 is the central but not a single activator of the lectin pathway.
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41 After lectin type sPRMs bind their targets, MASPs are cleaved and activated
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43 and set in motion a proteolytic cascade, which culminates in formation of the
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45 membrane attack complex and pathogen lysis [15]. In the present study,
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47 serum MASP-2 level was not associated with the risk of developing a CSI.
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49 However, we did not perform functional analysis that is an indisputable
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51 limitation of our study. MASP-2 antigen level not necessarily indicates the
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53 function of the molecule and scarcely assessed in previous studies. One
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55 cannot exclude that MASP-2 activity could have been associated with this
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3 outcome.

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5 Finally, serum level of lectin pathway components at enrolment was
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7 also not associated with the infection-related mortality. Since complement
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9 system has a dual role in disease susceptibility, one can expect that during an
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11 on-going inflammatory process rather its excessive activation with subsequent
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13 tissue damage than its lack of activity poses a risk of harm to the host.
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15 Another important consideration is that serum level of these molecules might
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17 change during acute phase reactions like bacterial infection. We did not re-
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19 assess serum samples during the first CSI episodes, which is evidently
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21 another limitation of this analysis. These issues warrant further evaluation in
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23 future studies of the lectin pathway in cirrhosis.
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28 In conclusion, findings of our large referral cohort study indicate that
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30 serum level of the lectin complement pathway molecules are decreased in
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32 cirrhosis and are related to the disease severity verifying importance of the
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34 liver in the production of FCNs and MASP-2. Moreover, low levels of soluble
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36 pattern recognition FCN molecules might be considered as an additional
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38 constituent of cirrhosis-associated immune dysfunction resulting in an
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40 increased risk of bacterial infections in this patient population.
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Figure Legends

Fig. 1. Development of bacterial infection (A,B) (n=266), infection-related mortality (C,D) (n=95) and liver-related mortality (E,F) (n=266) in cirrhosis according to serum level of ficolins (FCNs) or mannan-binding lectin serine protease-2 (MASP-2).

Patients with low level of both FCNs have higher cumulative probability of CSI compared to those with normal or low level of one FCN. MASP-2 deficiency was not associated with the risk of CSI development. Neither FCN serum profile and nor MASP-2 deficiency were associated with the infection-related or liver-related mortality.

MASP-2 deficiency:<100ng/ml, low level of FCN-2:<427ng/ml and FCN-3:<4857ng/ml

* Log-Rank test

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Tables

Table 1. Clinical characteristics of patients with cirrhosis.

	TOTAL	Patients with alcoholic cirrhosis	Patients with non-alcoholic cirrhosis
Number	266	170	96
Gender (male/ female)	133/133	91/79	42/54
Age (years) ^a	56 (49-64)	53 (47-64)	57 (51-63)
Child-Pugh stage, n(%)			
A	148 (55.6)	83 (48.8)	65 (67.7)
B	101 (38.0)	75 (44.1)	26 (27.1)
C	17 (6.4)	12 (7.1)	5 (5.2) [#]
MELD score ^a	11 (8-14)	12 (9-14)	10 (7-13) [#]
Serum bilirubin (µmol/L) ^a	27 (16-42)	28 (18-44)	22 (13-37) [#]
Serum albumin (g/L) ^a	38 (33-42)	37 (32-42)	39 (34-44) [#]
INR ^a	1.2 (1.1-1.3)	1.2 (1.1-1.3)	1.1 (1.1-1.3) [#]
Ascites, n(%)	96 (38.1)	74 (43.5)	22 (22.9) [#]
Co-morbidity, n(%)	136 (51.1)	91 (53.5)	45 (46.9)
HCC, n(%)	26 (9.8)	16 (9.4)	10 (10.4)
Prior CSI, n(%)	97 (36.5)	71 (41.8)	26 (27.1) [#]

CSI: clinically significant bacterial infection; HCC: hepatocellular carcinoma; INR: international normalized ratio; ^amedian, IQR (lowest 25% - highest 25%)

[#]p<0.01 between patients with alcoholic vs. non-alcoholic cirrhosis

p values were calculated by Mann-Whitney U-test, Fisher's exact test or χ^2 -test with Yates correction as appropriate

Table 2. Levels of lectin pathway molecules in patients with cirrhosis and healthy controls.

	Number	FCN-2 level (ng/ml) ^a	FCN-3 level (ng/ml) ^a	MASP-2 level (ng/ml) ^a	MASP-2 deficiency (< 100 ng/ml), n(%)
Healthy controls	160	769 (629 – 1145)	10797 (9017 – 13867)	412 (285 – 586)	3 (2.0)
Cirrhosis with various etiology	266	505 (426 – 596) [#]	7301 (4857 – 10601) [#]	212 (126 – 359) [#]	52 (19.5) [#]
Alcoholic	170	492 (398 – 564)	6593 (4532 – 9569)	206 (127 – 357)	35 (20.6)
Non-alcoholic	96	536 (470 – 652) [×]	9498 (6318 – 13614) ⁺	234 (123 – 363)	17 (17.7)
Child-Pugh stage					
A	148	527 (455 – 618)	8962 (6064 – 12872)	250 (159 – 372)	22 (14.9)
B	101	486 (391 – 583)	6032 (4416 – 8714)	194 (106 – 320)	23 (22.8)
C	17	427 (314 – 499) [⊙]	5487 (3986 – 8139) [□]	134 (56 – 295) [§]	7 (41.2) [§]
Ascites					
No	170	528 (453 – 625)	8110 (5764 – 12006)	239 (133 – 370)	29 (17.1)
Yes	96	472 (392 – 550) [±]	6314 (4094 – 8917) [±]	195 (103 – 335)	23 (24.0)

FCN: ficolin; MASP: mannan-binding lectin serine protease; ^amedian, IQR (lowest 25% - highest 25%)

[#]p < 0.001 between cirrhosis and healthy controls;

⁺p < 0.001 and [×]p < 0.01 between non-alcoholics and alcoholics

[□]p < 0.001, [⊙]p < 0.01 and [§]p < 0.05 between three different Child groups

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[‡]p < 0.01 between no-ascites and ascites

p values were calculated by Mann-Whitney U-test, Fisher's exact test or χ^2 -test with Yates correction as appropriate

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Table 3. Nonparametric correlation between serum levels of lectin pathway molecules and laboratory characteristic of patients with cirrhosis

	FCN-2 level		FCN-3 level		MASP-2 level	
	Spearman's rho	p-value	Spearman's rho	p-value	Spearman's rho	p-value
Age	-0.014	0.815	0.007	0.915	0.05	0.415
Creatinine	0.087	0.168	0.066	0.293	0.094	0.137
Bilirubin	-0.292	<0.001	-0.441	<0.001	-0.249	<0.001
Albumin	0.184	0.003	0.317	<0.001	-0.300	<0.001
INR	-0.300	<0.001	-0.442	<0.001	-0.240	<0.001
CPS	-0.263	<0.001	-0.390	<0.001	-0.205	0.001
MELD	-0.193	0.002	-0.404	<0.001	-0.256	<0.001
AST	0.143	0.020	0.177	0.004	0.031	0.620
ALT	0.200	0.001	0.299	<0.001	0.104	0.089
Platelet	0.086	0.168	0.127	0.038	0.090	0.143

FCN: ficolin; MASP: mannan-binding lectin serine protease; CPS: Child-Pugh score;

INR: international normalized ratio, AST: aspartate aminotransferase; ALT: alanine aminotransferase

Table 4. Univariate and multivariate Cox-regression analysis for the association of serum levels of lectin pathway molecules and clinical factors with the cumulative incidence of clinically significant bacterial infection in patients with cirrhosis.

		CSI development		CP of CSI %	p-value*	Univariate analysis	p-value	Multivariate analysis	p-value
		n of subject	n of event			HR (95% CI)		HR (95% CI)	
Total cohort		266	95	50.6%	-				
Age	<65	216	74	47.9%					
	>65	50	21	64.2%	0.173	1.40 (0.86-2.28)	0.175		
Gender	male	133	48	48.7%					
	female	133	47	53.0%	0.676	1.09 (0.73-1.63)	0.676		
Co-morbidity	absent	130	42	44.5%					
	present	136	53	57.4%	0.132	1.37 (0.91-2.05)	0.136		
Etiology	other	96	25	41.6%					
	alcoholic	170	70	55.5%	0.024	1.68 (1.07-2.66)	0.025	1.36 (0.85-2.15)	0.196
Prior CSI	absent	169	42	38.8%					
	present	97	53	68.7%	<0.001	2.64 (1.76-3.96)	<0.001	2.48 (1.65-3.73)	<0.001
Child-Pugh stage	Child A	148	43	40.1%					
	Child B/C	118	52	66.8%	<0.001	2.42 (1.61-3.64)	<0.001	2.27 (1.51-3.41)	<0.001
PPI use	no	148	43	41.8%					
	yes	118	52	61.3%	0.005	1.76 (1.18-2.64)	0.006	1.25 (0.81-1.91)	0.317
NSBB use	no	139	42	43.1%					
	yes	127	53	58.7%	0.033	1.55 (1.03-2.32)	0.034	1.08 (0.67-1.74)	0.753
Ascites	absent	170	52	42.3%					
	present	96	43	67.9%	<0.001	2.31 (1.54-3.46)	<0.001		
MASP-2	normal	214	74	48.6%					
	deficient	52	21	59.6%	0.306	1.29 (0.79-2.09)	0.307		
FCN-2	normal	200	65	46.7%					
	low level	66	30	62.6%	0.047	1.55 (1.00-2.39)	0.049	1.09 (0.69-1.72)	0.715
FCN-3	normal	200	64	48.2%					
	low level	66	31	59.3%	0.029	1.61 (1.05-2.47)	0.030	1.00 (0.63-1.58)	0.985
Combined FCNs	normal	167	51	45.7%	ref.				
	low level of one FCN	66	27	57.2%	0.160	1.39 (0.87-2.22)	0.164	0.97 (0.59-1.59)	0.908
	low level of both FCNs	33	17	63.8%	0.016	2.00 (1.15-3.47)	0.014	1.09 (0.60-1.99)	0.770

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3 * p-values of the log-rank tests;
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5 CP: cumulative probability; CI: confidence interval; CSI: clinically significant bacterial
6 infections; FCN: ficolin; HR: hazard ratio; MASP: mannan-binding lectin serine
7 protease; NSBB: non-selective beta blocker; PPI: proton-pump inhibitor; ref.:
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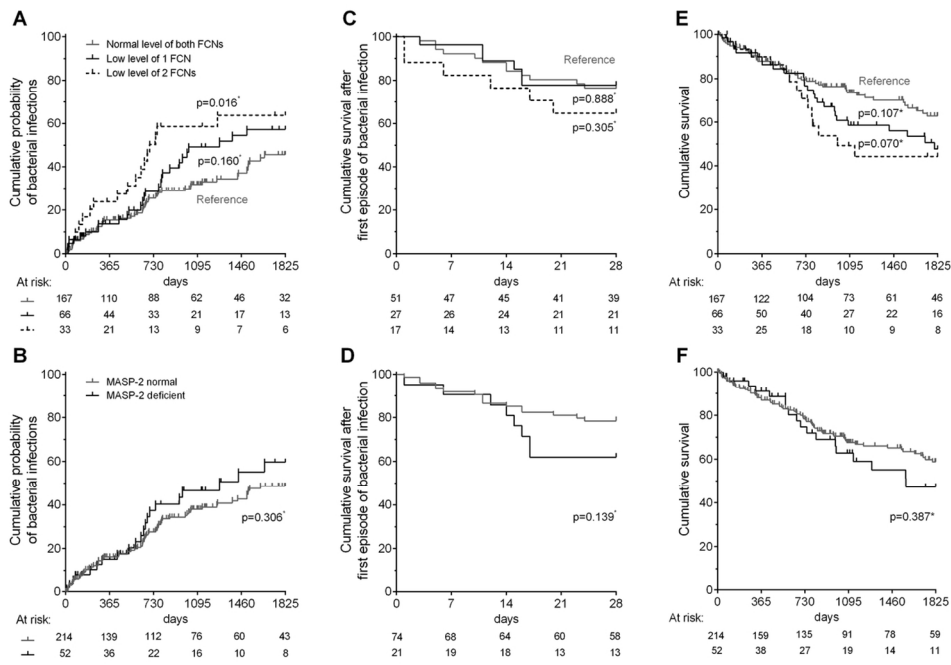


Figure 1
120x82mm (300 x 300 DPI)

Review